Expression and biological roles of FABPs in macroglia: implications for molecular and regenerative medicine

（グリア細胞における脂肪酸結合タンパク質の発現と機能：分子再生医学への応用について）

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Summary

Fatty acid binding proteins (FABPs) are a multigene family of intracellular lipid chaperones with distinct expression patterns, which regulate cellular functions through controlling lipid homeostasis. An increasing body of research, some of which are reviewed in the first chapter, have shown therapeutic and diagnostic potentials of FABPs, making them as important targets in cellular and molecular medicine and addressing the importance of studying on these molecules. Several evidences support the association of FABPs and their ligands especially polyunsaturated fatty acids (PUFAs) with the normal development of the brain and pathophysiology of major CNS diseases including neuropsychiatric and neurodegenerative disorders and cancers. However, still expression pattern and biological significance of FABPs in the brain are poorly understood. FABP7, the most specific FABP of the brain, is abundantly expressed in neural stem cells and radial glia of the developing brain and is involved in neurogenesis. In the second chapter, we examine the expression pattern of FABP7 in the adult brain and its involvement in the reactive astrogliosis, a component of brain's regenerative program and a common hallmark of CNS pathologies, using cortical stab injury model in wild type (WT) and FABP7-knockout (KO) mice. We immunohistochamically show that FABP7 is expressed in the astrocytes and oligodendrocytes progenitor cells (OPCs) in the normal cortex; is upregulated in reactive astrocytes of the injured cortex; and positively regulates the proliferation of reactive astrocytes. In addition, by using primary culture of astrocytes from WT and FABP7-KO mice, we show that FABP7 promotes the proliferation and n-3 PUFA uptake in astrocytes. In the third chapter, we examine the involvement of FABPs in oligodendrogenesis, another component of brain's regenerative program. By immunohistochemical evaluation, we show the in vivo and in vitro reciprocal expression of FABP7 and FABP5 in mouse OPCs and oligodendrocytes respectively. Furthermore, using embryonic stem cell derived culture of oligodendrocyte lineage from WT and FABP-KO mice, we show that FABP7 regulates the proliferation of OPCs and their differentiation to oligodendrocytes and FABP5 regulates the maturation of oligodendrocytes. Our novel findings highlight the biological roles of FABPs in macroglia and provide new hypotheses regarding the significance of FABPs in brain regeneration and their diagnostic and therapeutic potentials in CNS diseases.

CHAPTER 1: INTRODUCTION

The increasing significance of FABPs in cellular and molecular medicine; need for further studies

Lipids are one of major macromolecules which are essential for normal function of living organisms and involved in the pathophysiology of various diseases. Fatty acids (FA), major components of lipids, have crucial structural, metabolic and signaling roles. They are incorporated in to membrane phospholipid and serve as source of energy. In addition, fatty acids exert signaling roles through enzymatic and transcriptional networks to modulate various pathways. Therefore, intracellular trafficking of fatty acids has critical regulatory impacts on normal cell function through balancing different FAs at different times and locations [2].

FABPs: the multigene family of intracellular lipid chaperones with distinct expression patterns

Fatty acid binding proteins (FABPs) are a multigene family of intracellular lipid chaperones that mediate the uptake, transportation, storage and signal transduction of long-chain fatty acids. FABPs are a family of small low molecular weight (14-15 KDa) proteins which are highly conserved across species and show relatively high tissue/cell type specificity and different ligand affinities. Through reversible binding to hydrophobic long chain fatty acids, FABPs are thought to control the availability and distribution of FAs as ligands to several signaling processes and thereby mediating FA functions [1-3].

At least nine members of FABP family have been identified so far in mammals. Different FABPs have distinct patterns of tissue/cell type expression and are abundant in tissues with active lipid metabolism. Historically, FABPs have been named after the organ where they had been originally detected. However, later it was revealed that none of the FABPs are exclusively specific for any organ and also most organs and tissues express more than one FABP. Accordingly, an alternative numerical nomenclature was invented. Major members of FABP family are: liver-FABP (LFABP or FABP1), intestinal-FABP (IFABP or FABP2), heart-FABP (HFABP or FABP3), adipocyte-FABP (AFABP or FABP4), epidermal (EFABP or FABP5), ileal-FABP (IlFABP or FABP6), brain-FABP (BFABP or FABP7), myelin-FABP (MFABP or FABP8) and testis-FABP (TFABP or FABP9) [1-4].

The underlying regulatory mechanism for tissue/cell type specific expression and function of FABPs is not well known. Abundance of FABPs (up to 1-5% of soluble cytosolic proteins) in cells with high lipid metabolism (such as adipocytes, hepatocytes, and cardiomyocytes) as well as upregulation of FABPs in response to increased exposure of many cell types to fatty acids, support the notion that FABP expression pattern indicates the requirement and/or capacity of various tissues/cell types for lipid metabolism and FABPs are key players in controlling lipid homeostasis [1-2, 5].

Similar tertiary structure of FABPs despite their diverse primary structure points to differential ligand affinities and functions

All FABPs bind a single long chain fatty acid (LCFA) except FABP1 which can bind to two FAs [2, 6-7]. The binding affinity increases with the hydrophobicity of the ligand [8]. Despite the moderate diversity in amino acid sequence (~15-70% homology), which can be responsible for difference in ligand affinities, FABPs share similar tertiary structures. A "10-stranded antiparallel β-barrel structure" is common in all FABPs. The fatty acids bind to the interior cavity of the β-barrel. The barrel is mostly filled with water molecules lined with polar and hydrophobic amino acid residues and only a small volume is occupied by the ligands lined form hydrocarbon tail by hydrophobic amino acid residues. The opening of the barrel includes an "N-terminal helix-turn-helix domain" [2, 4, 7] (Fig. 1.1).

FABPs have been categorized into 3 groups according to their amino acid sequence and consequently their binding abilities: group 1 consists of FABP1 and FABP6 which in addition to FAs can bind to bulky ligands like bile salts and cholesterol . Group 2 consists of FABP3, FABP4, FABP5, FABP7, FABP8 and FABP9 which in addition to FAs can bind to retinoids and eicosanoids. Group 3 consists of FABP2 which only binds to FAs [4]

While other lipid binding proteins are coded by a single gene, diversity of FABPs despite their similar 3D structure, indicates the cell / tissue specific functions of FABPs and their ligands [1].

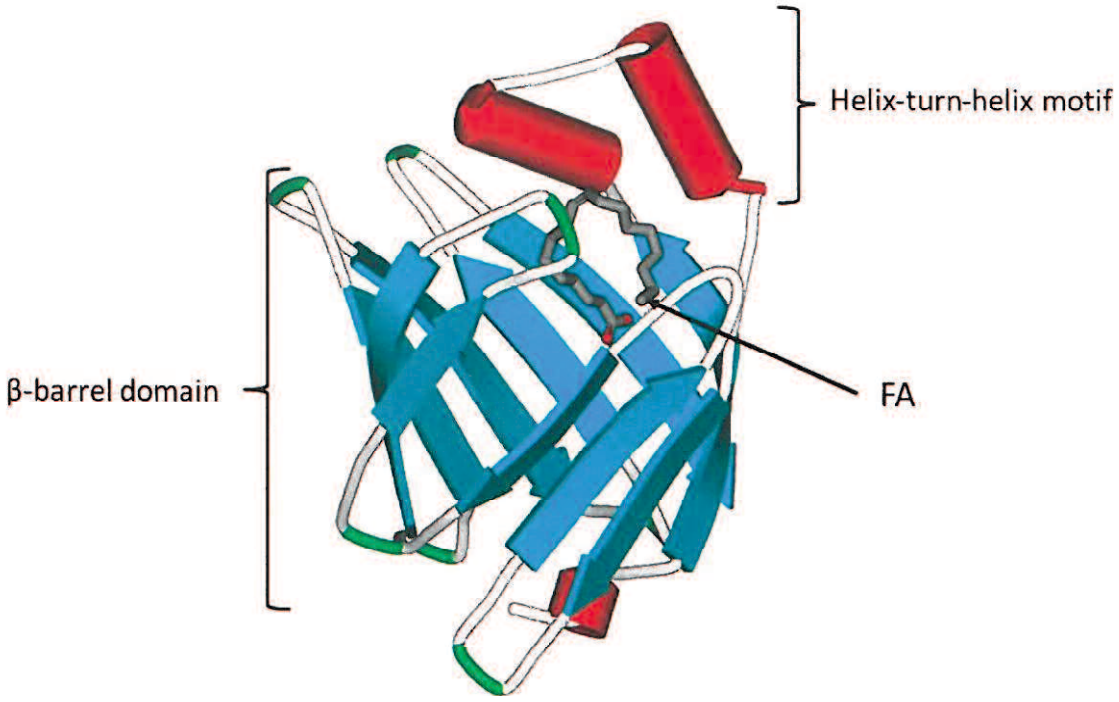

Fig 1.1 Similar 3D structure of FABPs including the β-barrel domain and the N-terminal helix-turnhelix motif. (Figure taken from [1])

FABPs regulate cellular functions through controlling lipid homeostasis

All aspects of FA homeostasis including uptake, storage in lipid droplets; transportation and membrane formation in ER; oxidation in peroxisomes or mitochondria; modulation of enzyme activity throughout the cytosol, conversion to eicosanoids, transcriptional regulation in the nucleus and even autocrine/paracrine signaling through FA efflux are supposed to be mediated by FABPs which selectively regulate intracellular FA trafficking [2-3] (Fig. 1.2).

FABPs are thought to regulate gene expression by accessing the nucleus and targeting their ligands (FAs) to nuclear receptors and transcription factors such as peroxisome proliferator-activated receptor family (PPARs) and retinoid X receptor family (RXRs) [9-12]. Interestingly, expression of some FABPs is reported to be regulated by these nuclear receptors

[2, 13]. Physical interaction of Some FABPs with PPARs has suggested them as PPAR co-activators [2, 11, 14-15].

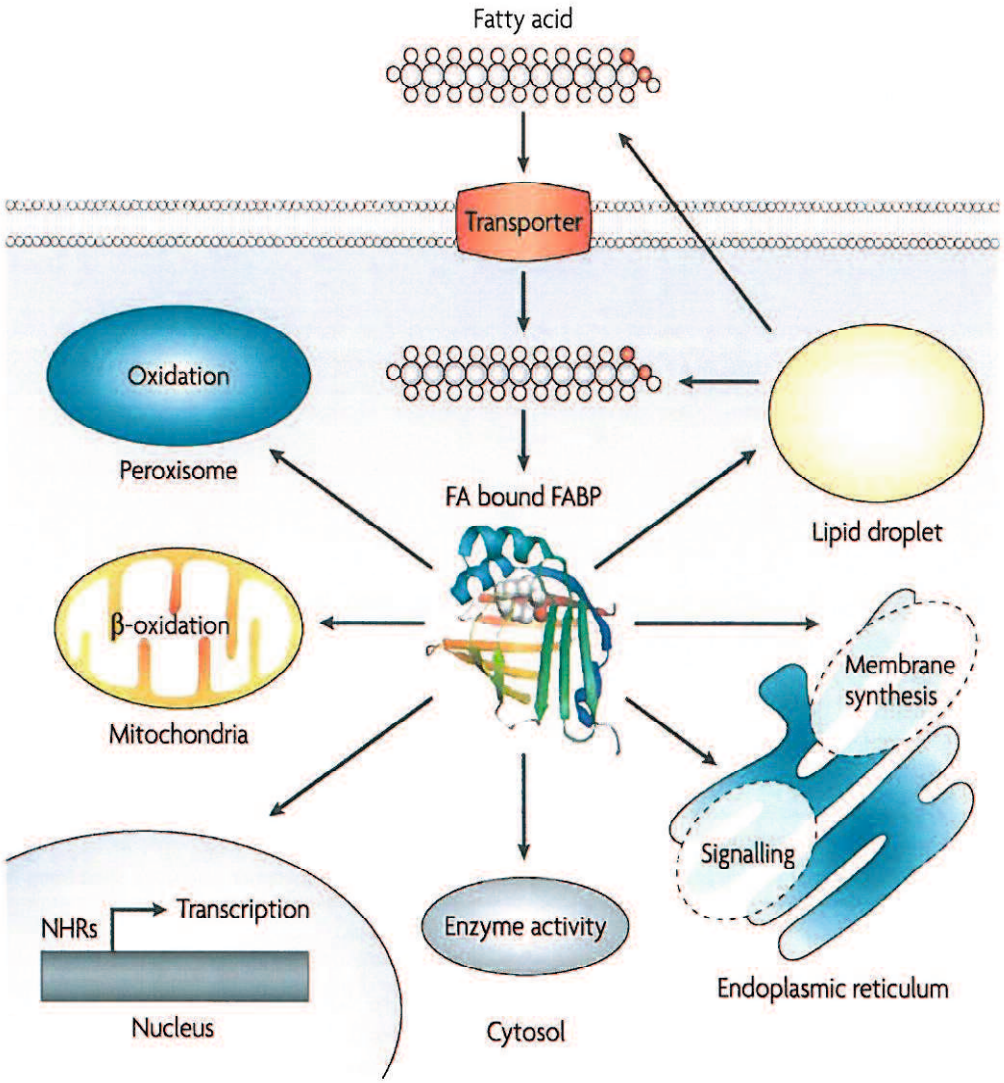

Fig. 1.2 Cellular functions of FABPs

Scheme showing putative cellular functions of FABPs through trafficking of FAs to specific compartments in the cell such as lipid droplets (storage); endoplasmic reticulum (signalling, transportation and membrane synthesis); mitochondria or peroxisome (oxidation); cytosolic or other enzymes (regulation of enzymatic activity); nucleus (transcriptional regulation via nuclear hormone receptors (NHRs) or other transcription factors); or even outside the cell for autocrine or paracrine signaling. (Figure taken from[2])

Details of FABP functions and their interacting protein partners are still poorly understood. However, development of FABP-KO transgenic mice has partly facilitated the studies on FABPs roles in different cells/tissues/organs as well as their systemic effects, leading to an increasing body of evidence which highlight the importance of FABPs in normal and pathological conditions and attract much attention to their diagnostic and therapeutic potentials [1-2].

FABPs have been used as tissue specific injury markers because of solubility in plasma, high tissue specificity, abundance in the tissue and low molecular weight as well as induced expression in pathological conditions [16]. Here, a brief review of the literature regarding major members of FABP family is provided which may highlight the increasing significance of FABPs in cellular and molecular medicine and the importance of further studies about these molecules.

FABP1: diagnostic and therapeutic potentials in liver, intestine and kidney diseases

FABP1 is abundantly expressed in the liver (up to 5% of total cytosolic proteins in hepatocytes). It has been also detected in several other organs such as intestine (absorptive columnar intestinal epithelium, proximal intestine, upper 2/3rd of the villus), kidney (renal proximal tubular epithelial cells of human kidney), pancreas, stomach and lung [2, 4, 17-18].

Fatty acids, dicarboxylic acids and retinoic acid cause increased transcription of FABP1 gene due to the presence of a peroxisome-proliferator response element in its promoter region [2, 19]. FABP1 can simultaneously bind two ligands with high and low affinities [2, 20]. FABP1 can bind to several bulky hydrophobic ligands other than fatty acids such as acyl-coenzyme A, eicosanoids, lysophospholipids, carcinogens, anticoagulantsand haem suggesting potential related functions [2, 19]. Recent studies suggest FABP1 as an indirect antioxidant with protective effects against hepatocellular oxidative stress, impairment of which could contribute to alcoholic liver disease [21-23]. Ethanol feeding in mice has been shown to decrease FABP1 protein and mRNA while increasing its poly-ubiquitinated form [24]. It has been reported that FABP1-KO mice are susceptible to oxidative stress in early-stage alcoholic liver [25].

FABP1 has been also reported to modulate hepatic stellate cell activation and diet-induced Nonalcoholic Fatty Liver Disease (NAFLD). FABP1 deletion in mice has been reported to decrease diet-induced hepatic steatosis and fibrogenesis [26]. Proteomic screens have revealed FABP1 to be overexpressed in obese subjects with simple steatosis, along with paradoxically decreased expression in the progressive versus mild forms of nonalcoholic steatohepatitis (NASH) [27].

FABP1 is used as a diagnostic marker for classification of hepatocellular adenoma (HCA), since a major subclass of HCA which contains HNF1α mutation (H-HCA) lacks FABP1 in immunostaining [28]. Several reports have shown the associations of FABP1 to various neoplasms in gastrointestinal tract (GI) and its diagnostic potentials. FABP1 is associated with intestinal adenoma formation [29]. It is intensly expressed in gastric metaplasia and subsets of gastric adenocarcinoma [30] and has been suggested as a prognostic indicator of peritoneal metastases in gastric cancer [31]. FABP1 is also associated with pancreatic adenocarcinoma[32], downregulated in colorectal cancers [33-34] and negatively correlated with lymph node matastasis in colorectal cancers [35].

Serum FABP1 has been suggested as a biomarker for liver injury [16, 36-37]. FABP1 expression is increased in renal injury and therefore urinary FABP1 has been considered as an effective marker for monitoring of chronic kidney diseases and an early predictive marker for acute kidney injury [18]. FABP1 has been recently shown to be protective against renal tubulointerstitial damage by reducing oxidative stress in renal injuries. [18, 38-39].

FABP2: diagnostic use for intestinal injury and link to metabolic syndrome

FABP2, is expressed throughout the intestinal epithelium with maximum mRNA expression in the medial segment of the small intestine [17, 40]

FABP2-KO mice show hyeperinsulineamia with normal glucose levels. Despite unimpaired fat absorption, male FABP2-KO mice show higher weight gain and higher triglyceride (TG) levels regardless of the diet and have larger livers on high fat diet, while female

FABP2-KO mice show less weight gain and smaller livers on high fat diet compared with WT counterparts [41].

Plasma and urinary FABP2 have been suggested by a large body of studies as diagnostic markers for injury to intestinal epithelium [16] used for early diagnosis, evaluation of prognosis, activity and severity of the disease or effectiveness of treatment in various conditions such as celiac disease [42-44], necrotizing enterocolitis [45-49], gut dysfunction in acute pancreatitis[50], identification of small bowel ischemia in patients with acute abdomen [51-52], Intestinal ischemic injury following cardiac operation [53]and multiple trauma [54-55].

A large and increasing body of reports in several different populations has shown that a variation (SNP) in exon2 (Ala 54 Thr) of FABP2 is associated with insulin resistance and type 2 diabetes as well as obesity and cardiovascular disease (with relative variations across populations) [56-59]. Increased FA absorption and increased risk for hearing impairment[60] and chronic kidney disease [61] in type 2 diabetes, higher levels of total cholesterol and LDL-cholestrol, and lower levels of HDL-cholestrol [62], higher levels of CRP and insulin levels in diabetes [63], increased risk for NAFLD [64], insulin resistance, leptin and adiponectin levels in morbid obese patients [65] and better response to high PUFA hypocaloric diet in obese patients[66] have been linked to Ala 54 Thr variant of FABP2, which has been also suggested as a potential genetic risk factor for myocardial infarction [57].

A variation in FABP2 Promoter (haplotype A,B) has been also found to be correlated with insulin resistance, type 2 diabetes, dyslipidemia and body mass index (BMI) [67-69]. Another variation of FABP2 (rs11724758 A>G in binding site of miR-132) has been shown to be correlated with decreased risk of metabolic syndrome and increased risk of T2DM in Chinese Han population, most likely through their effects on the specific miRNA-binding sites [70-71].

FABP3: diagnostic and therapeutic potentials for heart, CNS and kidney diseases

FABP3 is abundantly expressed in heart, skeletal muscles and brain. It has been also detected in several other organs such as mammary gland, placenta, testis, kidney (renal cortex/ distal

tubules), lung, aorta, adrenal gland, ovary, pancreas, retina (some populations of amacrine/ bipolar/horizontal interneurons) and brown adipose tissue [2, 4, 72-76].

FABP3 has an important role in the uptake and transportation of fatty acids towards the mitochondria. This causes a severe inhibition of FA uptake in the heart and skeletal muscle of FABP3-KO mice resulting in a subsequent increase in plasma free fatty acids [77] and a switch from fatty-acid oxidation towards glucose oxidation in cardiac and skeletal muscle metabolism [78-79]. FABP3 may also interact with PPAR to induce the expression of mitochondrial and peroxisomal-oxidation pathways [80]. FABP3-KO mice are rapidly exhausted by exercise and show cardiac hypertrophy in older animals [77].

FABP3 expression is up-regulated during cardiomyocyte and skeletal muscle differentiation [1, 81-82] and may inhibit cardiomyocyte proliferation [1, 81]. Dysregulation of FABP3 expression has been linked to embryonic cardiac malformations. FABP3 is highly upregulated in the myocardium of patients with ventricular septal defect. Either overexpression or silencing of FABP3 has been reported to decrease proliferation and increase apoptosis in embryonic carcinoma cell lines [83-84]. Consistently, Knock down of FABP3 in zebrafish results in impaired cardiac development through dysregulation of RA signaling, apoptosis induced mitochondrial dysfunction and stimulation of Wnt signaling [85-86]. Increased FABP3 mRNA has been found in the myocardium from left ventricle of failing heart [87].

Several studies have supported FABP3 as a marker of mayocardial cell damage with high diagnostic and prognostic value for myocardial infarction [16, 88-90]. It has been also suggested as a marker for risk stratification and estimation of prognosis in pulmonary embolism [91-94], severe sepsis and septic shock [95-97], endstage heart failure with left ventricular assist device[98], hypertrophic and dilated cardiomyopathy [99], obstructive sleep apnea syndrome [100]. Serum FABP3 has been also suggested as a biomarker in the evaluation of clinical severity and in the selection of patients for HBO therapy in acute CO poisoning [101]. Recent studies report an Inverse relationship between serum FABP3 and myocardial expression of miR-1, a microRNA with critical regulatory roles in myocardiac remodeling and progression to heart failure, suggesting FABP3 as biomarker for indirect evaluation of miR1 activity in the heart [102].

In brain, FABP3 is abundantly expressed in neurons and absent in other neural cells [3]. Brains of FABP3-KO mice show reduced arachidonic acid incorporation and imbalance in n-3/n-6 polyunsaturated fatty acid (PUFA) ratio, which may contribute to neuropsychiatric disorders [103]. FABP3 interacts with and regulates the functions of dopamine D2 receptor (long isoform), a target for antipsychotic drugs, in the dorsal striatum of the brain [104-105]. Decreased FABP3 expression has been reported in brains of patients with Down syndrome and Alzheimer disease [106].

FABP3 levels in serum or CSF has been suggested as a marker for several CNS diseases. Serum FABP3 has been suggested as a prognostic marker for survival and disability after traumatic brain injury, stroke and subarachnoid hemorrhage [16, 107-110]. FABP3 in CSF has been proposed as a marker of brain injury after subarachnoid hemorrhage which can identify the occurrence of vasospasm and predict the long-term outcome [111]. Increased CSF levels of FABP3 are found in several forms of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease with dementia, Lewy body dementia, vascular dementia, and Creutzfeldt-Jakob disease [112-114]. Recent studies show that CSF levels of FABP3 can predict conversion of mild congnitive impairment to AD suggesting FABP3 as a marker for early phases of AD [115]. Altered FABP3 expression has been also reported in CSF of Niemann-Pick disease, type C1 patients [116]. Increased FABP3 expression has been found in uveal melanoma which is associated with tumor invasion [117].

FABP3 is also considered as a marker for distal tubular damage in kidney. Urinary FABP3 is increased in experimental diabetic nephropathy [118] and human diabetic kidney disease [119] . Expression of FABP3 is increased in glomerular and tubular areas of patients with obesity-related glomerulopathy [120]. FABP3 is also suggested as a tool to evaluate the severity of initial kidney graft injury and predict graft dysfunction [121]. It is also proposed as a candidate predictive marker for mortality of hemodialysis patients [122].

Despite abundant expression of FABP3 in lactating mammary glands [123], no change in the development and function of the mammary gland was reported upon overexpression or ablation of FABP3 [124-125]. However, FABP3 expression in breast cancer cell lines inhibits

proliferation, migration, invasion and tumorigenicity. Consistently, expression of FABP3 results in a drastic improvement in prognosis of breast cancer [126-127]

FABP3 is essential for efficient fatty acid oxidation in brown adipose tissue and cold tolerance [128]. Expression of FABP3 in brown adipose tissue is 10 fold increased by exposure to cold [129]. FABP3-KO brown adipocytes fail to oxidize fatty acids, while overexpression of FABP3 promotes oxidation in brown adipocytes [128].

FABP4: the important therapeutic target for diabetes and atherosclerosis

FABP4 (Also called as ap2) have been so far the most attractive and the best studied FABP. FABP4 is abundant in adipose tissue, which is considered not only the major energy storage but also an endocrine organ, producing bioactive molecules involved in systemic energy metabolism and inflammatory processes [1-2]. FABP4 is also expressed in macrophages (10000 fold less than adipocytes in mRNA level), activated or differentiated monocytes [130-131] and dendritic cells [132].

Upon differentiation of adipocytes, FABP4 expression is drastically upregulated [133-134]. Transcriptional regulation of FABP4 by fatty acids, insulin and PPAR-γ agonists has been also reported [2]. FABP4-KO mice are protected against hyperinsulinaemia and insulin resistance following dietary and genetic obesity [2, 135-136]. FABP4-KO adipocytes show impaired lipolysis and overexpression of FABP5 which is minimally expressed in WT adipocytes [2, 137-139].

FABP4 is essential for inflammatory response and cholesterol ester accumulation in macrophages. ApoE-deficient mice (which show propensity to atheroschlerosis) are protected against atherosclerosis in the context of FABP4 deficiency, mainly due to altered functions of macrophages [130, 140]. FABP4 contributes to foam-cell formation in macrophages. Cholesterol efflux is increased in FABP4-KO macrophages by increased activity of PPAR-γ and its downstream targets including LXR-α which result in increased expression of the cholesterol transporter ABCA1 (ATP binding cassette A1) [141]. FABP4-KO macrophages also exhibit

suppressed inflammatory responses including production and function of pro-inflammatory enzymes such as iNOS (inducible nitric oxide synthase) and COX2 (cyclooxygenase 2) and production of cytokines such as TNF-α , IL6, IL1β, MCP1 (monocyte chemoattractant protein 1) [130, 141-142]. Immune response regulation by FABP4 has been suggested to be exerted through modulation of the IKK- NF-κB pathway (inhibitor of kappa kinase (IKK)-nuclear factor-κB (NF-κB) pathway) [141].

The expression of FABP4 in human bronchial epithelial cells and its upregulation in response to T helper 2 (TH2) cytokines; IL4 and IL13 have been also reported [131]. This finding together with protective phenotype of FABP4-KO mice against allergic airway inflammation has suggested the association of FABP4 to pathophysiology of asthma [131]. Recent studies have shown that FABP4 is induced by vascular endothelial growth factor (VEGF) in endothelial cells and promotes VEGF-induced airway angiogenesis, an underlying mechanism of asthma [143]. In addition, recent studies show that FABP4 (and FABP5) are expressed in endothelial cells and regulate FA transport to FA consuming tissues such as skeletal and cardiac muscles [144].

FABP4 expression in lipoblastoma and liposarcoma has been suggested for differential diagnosis of benign and malignant neoplasm of adipose tissue and distinguishing liposarcoma from malignant tumors of connective or epithelial tissue. [145]. In addition, loss of FABP4 expression has been linked to progression of human urothelial carcinomas, suggesting FABP4 as a prognostic biomarker of these tumors [146].

Discovery of the above mentioned regulatory roles on adipocyte and macrophages has turned FABP4 to an important therapeutic target for metabolic syndrome and thus the most outstanding member of FABP family. Accordingly, an inhibitor of FABP4 (BMS309403) have been invented and proved to have therapeutic effects against diabetes and atherosclesrosis [2, 147]. Plasma FABP4 concentration has been shown to be associated with obesity, diabetes type 2, cardiovascular diseases, atherosclerosis, metabolic syndrome and NASH with diagnostic and prognostic potentials [148]. Very recent studies suggest FABP4 as an adipokine actively secreted from adipocytes in response to fasting and lipolysis signals which stimulates

production of glucose by hepatocytes. This suggests neutralization of FABP4 as a novel therapeutic strategy for diabetes [149]. The concept of active release of FABP4 if applicable to other FABPs, questions the longstanding paradigm of intracellular lipid chaperoning of FABPs and extremely extends the scope of their signaling networks and enhances their therapeutic potentials throughout the body.

FABP5: immuonoregulatory roles and links to metabolic syndrome, psoriasis and cancer

FABP5 is highly expressed in epidermal cells . FABP5 is also expressed in macrophages, adipocytes and endothelial cells. It has been isolated from several other organs such as brain, mammary gland, lung, liver, kidney, and testis (sertoli cells) [4, 142, 150].

FABP5 shows close structural and functional similarities with FABP4. FABP4 deficiency in adipocytes (but not in macrophages) results in a compensatory overexpression of FABP5 [130]. FABP5-KO mice do not show gross abnormalities or compensatory FABP overexpressions, except enhanced FABP3 level in the developing liver [151]. FABP5-KO mice exhibit a minor reduction in transepidermal water loss [152] as well as mildly increased systemic insulin sensitivity in the context of genetic or diet induced obesity [151]. Consistently, adipose tissue specific overexpression of FABP5 causes decreased systemic insulin sensitivity [151].

Overlapping expression and function of FABP4 and FABP5 in adipocytes and macrophages has important association with insulin sensitivity, obesity, cardiovascular disease, type2 diabetes and metabolic syndrome. FABP4-FABP5 double KO mice in the context of genetic or diet induced obesity show drastic protection against insulin resistance, diabetes type 2, fatty liver and atherosclerosis (much more protective compared to single FABP-KO mice [2, 153-155]. Overall, FABP4 and FABP5 are candidate mediators and biomarkers for metabolic syndrome and cardiovascular disease in type 2 diabetes mellitus [156].

FABP5 has a suggested regulatory role in Retinoic Acid (RA) signaling since it competes with cellular RA binding protein II (CRABP-II) in shuttling RA. While CRABP-II carries RA to Retinoic Acid Receptors (RARs), alternatively FABP5 shuttles RA to PPARβ/δ which results in a distinct response [157]. This regulatory role of FABP5 has been described in several cell types in

normal and pathological conditions. For example, in preadipocytes RA- CRABPII-RAR pathway results in expression of genes which inhibit adipogenesis, while In mature adipocytes, due to upregulation of FABP5, RA can be alternatively shuttled to PPARβ/δ by FABP5 to induce the expression of genes related to energy expenditure and insulin responses [157]. Similar regulatory role for FABP5 has been suggested in skin development and homeostasis. Increased ratio of CRABP-II / FABP5 is thought to mediate allergen induced contact dermatitis through upregulation of PPARδ pathway and alteration of RAR pathway [158].

In the skin, FABP5 is expressed by differentiating cells in sebaceous gland, interfollicular epidermis and hair follicles. Accordingly, FABP5 is shown to be upregulated by RA treatment or Notch activation and downregulated by Wnt/beta-catenin signaling [159]. FABP5 is upregulated upon differentiation of keratinocyte stem cells towards postmitotic keratinocytes and induces keratinocyte differentiation [160]. In addition, FABP5-KO keratinocytes show reduced fatty acid uptake leading to decreased expression of differentiation markers through downregulation of NF-κB activity suggesting that FABP5 controls keratinocyte differentiation through FA mediated regulation of NF-κB activity [161]. FABP5 is overexpressed in psoriatic epidermis [162-163]. FABP5 (measured by skin stripping) has been also suggested as a predictive marker of clinical response to treatment in psoriatic skin lesions[164]. Increased expression of FABP5 has been linked with dysregulated differentiation of psoriatic keratinocytes. In vitro silencing of FABP5 resulted in decreased differentiation and downregulation of specific markers in psoriatic keratinocytes [160]. FABP5 is also strongly expressed in differentiated sebocytes. FABP5-KO mice exhibit smaller sebaceous glands with increased volume and altered lipid composition of sebum [165]. FABP5 has been found to be upregulated in skin lesions of atopic dermatitis and associated with severity of the disease. Therefore, FABP5 is considered as a candidate biomarker for evaluation of inflammation and skin barrier conditions in atopic dermatitis [166].

Proteomics and system biological studies have suggested FABP5 as a central regulator of metabolism in activated monocytes, which may control inflammation and lipid raft composition [167]. FABP5 is reported to have a proatherogenic effect by suppressing the activity of PPARγ. Bone marrow transplantation into low-density lipoprotein receptor-null

(LDLR-/-) mice has revealed that FABP5-KO monocytes show reduced recruitment in to atherosclerotic lesions, and suppression of inflammatory genes such as COX2 and IL6 [168]. Consistently, macrophages and dendritic cells from FABP5-KO mice show defects in production of inflammatory cytokines and promotion of proinflammatory T cell responses during antigen presentation [169]. FABP5 has been suggested as a positive regulator of Th17 differentiation and IL17 production, which are known to have modulatory roles in autoimmune responses, such as chronic asthma, rheumatoid arthritis, inflammatory bowel diseases, and multiple sclerosis [170]. It has been also shown that FABP5 (and FABP4) expression modulates cytokine production in the mouse thymic epithelial cells [171].

Immunoregulatory functions of FABP5 have been also shown in the respiratory system. Down regulation of FABP5 has been shown in airway epithelial cells of smokers with COPD. In vitro studies have revealed protective immunomodulatory functions of FABP5 against microbial infections in COPD patients [172]. In addition, a protective role of FABP5 has been reported against H1N1 influenza A by controlling oxidative damage and inflammation. Influenza A infection causes a decrease in FABP5 expression in lung tissue. Following Infection with influenza A virus, FABP5-KO mice show increased macrophage and neutrophil infiltration , increased adaptive immune response (T cell and B cell accumulation and production of H1N1-specific antibodies), increased oxidative damage and sustained inflammation [173].

Downregulation of FABP5 in the Retinal pigment epithelium (RPE)/choroid complex has been linked to aging and early age-related macular degeneration through altered lipid metabolism. It has been reported that FABP5 is downregulated in the RPE/choroidal complex in an experimental model of aging and early age-related macular degeneration (tian 2005). Besides, Knock down of FABP5 in human RPE cell line resulted in decreased levels of cholesterol and cholesterol esters, reduced apoB secretion and increased free fatty acids and triglycerides [174]. FABP5 (and FABP3) are expressed in pancreatic islets and upregulated in response to FA and glucose exposure [75].

FABP5 is detected in nervous system including astrocytes and glia of the prenatal and perinatal brain [73] and ganglion cells of retina [72]. FABP5 has been reported to promote

differentiation of neuronal progenitors to mature neurons through RA mediated regulation of PPARβ/δ target genes (namely PDK1) [175]. FABP5 (and FABP7) are induced in postischemic subgranular zone in monkeys suggesting their roles in neurogenesis [176]. Consistently, hippocampi of FABP5-KO mice show increased population of neuronal progenitor cells versus decreased population of mature neurons [175]. Furthermore, induction of FABP5 following peripheral nerve injury has been reported which suggests a role in neural regeneration [177-178].

FABP5 has been linked to several types of cancer, being studied as a diagnostic or therapeutic target. FABP5 is overexpressed in head and neck squamous cell carcinoma [179]. Upregulation of FABP5 has been shown to enhance proliferation and invasiveness in oral squamous cell carcinoma [180]. It has been reported that FABP5 is highly upregulated in human breast cancers and promotes EGFR induced tumorigenesis through activation of PPARδ [181]. Genetic FABP5 ablation suppresses mammary tumor development in MMTV-ErbB2/HER2 oncomouse (a mouse model of spontaneous breast tumors) suggesting FABP5 as a novel therapeutic target for cancers [182]. Similarly, FABP5 expression has been linked with high grade and poor prognosis of breast cancer tissues negative for estrogen receptor and progesterone receptor. Inhibition of tumor growth by in vitro silencing of FABP5, suggests FABP5 as a prognostic and therapeutic target for triple negative breast cancers [183]. FABP5 upregulation has been shown to enhance proliferation and invasion in human intrahepatic cholangiocarcinoma [184]. Proteomic studies have suggested FABP5 as a biomarker for colorectal cancer [185]. FABP5 has been identified to be upregulated in bladder cancer [186]. It is also associated with enhanced proliferation in prostate cancer cell lines through PPARβ/δ pathway [187]. Effective suppression of prostate cancer by experimental in vivo silencing has suggested FABP5 as a candidate therapeutic target for prostate cancer [188]. Proteomic studies have also suggested FABP5 overexpression as a diagnostic marker for lymph node metastasis in prostate cancer [189]. FABP5 is overexpressed in endometrial cancer and is associated with poor differentiation [190].

FABP6: regulatory roles in enterohepatic circulation of bile acids

FABP6 is mainly expressed in ileal enterocytes. It has been also detected in other cell types such as cholangiocytes and ovarian cells [191-195].

FABP6 can bind bile acids with high affinity. FABP6-KO mice show increased fecal bile acid excretion, increased activity of bile acid biosynthetic enzymes, and decreased mucosal to serosal bile acid transport. This suggests the essential roles of FABP6 for absorption and transport of bile acids in ileal enterocytes, and maintenance of bile acid homeostasis in the enterohepatic circulation [196].

FABP6 has been suggested as a predictive marker for colon cancer. Microarray gene expression analysis has revealed that FABP6 is overexpressed in sessile serrated adenomas/polyps, which are precursors of colon cancer, and can differentiate them from microvesicular hyperplastic polyp [197]. The Thr79Met polymorphism of the FABP6 is reported to protect obese individuals against type 2 diabetes [198].

FABP7: diagnostic and therapeutic potentials for major CNS diseases

FABP7 is abundantly expressed in embryonic brain (radial glia and NSCs) which gradually decreases and is weakly expressed in the adult brain (astroglia, bregman glia in cerebellum, muller cells and cone photoreceptor cells in retina...)[3, 72-73]. Although FABP7 is mainly expressed in CNS, it has been also detected in other places such as kupffer cells in the liver, fibroblastic reticular cells in the lymph nodes, melanoblasts and melanocytes in the skin and mammary gland [199-203].

FABP7 expression in neural stem cells and radial glia of the developing brain has important regulatory roles on neuronal migration and neurogenesis [3, 204]. Postnatal FABP7-KO mice show reduced neurogenesis in dentate gyrus of hippocampus [205]. Ex-vivo FABP7 knockdown has been reported to impair proliferation of neuroepithelial cells and induce neuronal differentiation [206]. Neutralization of FABP7 in a co-culture of immature cerebellar neurons and radial glia is reported to impair extension of glial fascicles and migration of

neurons along them [207]. Interestingly, high expression levels of FABP7 in neural stem cells has been recently used for efficient isolation of neural stem cells by developing a novel FABP7 binding dye CDr3 [208-209].

FABP7-KO mice have shown altered emotional behavior as well as impaired prepulse inhibition (PPI), an endophenotype of schizophrenia. FABP7 has been also found as a quantitative trait locus (QTL) for PPI. Upregulation of FABP7 mRNA has been reported in prefrontal cortex of schizophrenic brains and FABP7variations (SNPs) are linked to schizophrenia [205].

Increased serum levels of FABP7 has been suggested as a marker for CNS injuries [16]. Increased FABP7 have been found in the serum of dementia related diseases (Alzheimer's disease, Parkinson's disease) [210]. Consistently, FABP7 upregulation has been reported in brains of dnADAM10 mice, a mouse model for Alzheimer disease [211]. Cluster analysis on microarray data has shown association of FABP7 with Alzheimer disease [212]. proteomics studies have found altered expression of FABP7 protein (as well as FABP5 and FABP3) in cerebella of NPC1 mice, a mutant mouse model for Niemann-Pick disease [116].FABP7 upregulation has been found in fetal and adult brain in down syndrome (while FABP3 is downregulated) [106, 213].

Upregulation of FABP7 has been linked with invasiveness and poor prognosis of malignant glioma [214-215] which has suggested FABP7 as a therapeutic and diagnostic marker. FABP7 is also associated with tumor grade and invasiveness of meningioma [216] and differentiation status in Neuroblastic tumors [217].

In addition to CNS diseases, FABP7 has been associated with some diseases in other organs. FABP7 is associated with proliferation and invasion and tumor progression in malignant melanoma and a candidate diagnostic and therapeutic target [218-219]. FABP7 has been reported as a positive regulater of cell proliferation and migration in renal cell carcinoma [220]. In addition, FABP7 is upregulated in triple negative breast cancer and associated with poor prognosis, increased cell proliferation and higher tumor grade. In vitro silencing of FABP7 suppressed the growth of tumor cells, suggesting FABP7 as a therapeutic target for triple

negative breast cancer [221]. FABP7 has been also found in cervical squamous carcinoma [222]. Altered expression of FABP7 upon pyrazinamide hepatotoxicity has been shown in rat liver[223] and affected colons in Hirschsprung's disease [224]. miR-21 upregulation by lycopene has been reported to protect mice from high fat diet- induced hepatic steatosis through downregulation of FABP7 [225].

FABP8: structural role in peripheral myelin and link to autoimmune peripheral polyneuropathies

FABP8 (also called as peripheral myelin protein 2) constitutes up to 15% of total myelin proteins in peripheral nervous system [226-227]. Being localized at the major dense line of myelin sheaths, FABP8 is considered a structural protein which may regulate myelin assembly and turnover and stabilize the myelin membranes[227].

FABP8 is linked to autoimmune polyneuropathies. FABP8 fragments induce infammatory demyelination in experimental allergic neuritis [227-228]. Consistently, antibodies against FABP8 have been detected in Guillain- Barre` syndrome [227, 229-230]. FABP8 is also involved in the immune responses in chronic inflammatory demyelinating polyradiculoneuropathy [227, 231-232].

FABP9: roles in maturation and apoptosis of male germ cells

FABP9 (also called as PERF15) is the male germ cell specific FABP. Expression of FABP9 is increased from spermatocytes to spermatids with the peak in spermatid-derived residual bodies (RB), suggesting functional roles in the last stages of germ cell differentiation [150]. FABP9 has a suggested role in regulation of apoptosis in germ cells [233]. FABP9 is upregulated in the apoptotic germ cells. Consistently, FABP9 overexpressing mice, though fertile, show increased apoptosis in elongated spermatids, and reduced population of sperms carrying the transgene [234]. In addition, FABP9 shows increased phosphorylation during capacitation of

sperm, suggesting it's potential role in sperm maturation [235]. FABP9-KO male mice are fertile but show sperm head abnormalities [236].

Scientific rational and outline of the thesis

The increasing body of evidence about significance of FABPs, some of which are reviewed in the above, especially development of novel therapeutic strategy for treatment of diabetes and atherosclerosis originated from series of studies on biological roles of FABP4 and FABP5, highlight the necessity of exploring the biological significance of all members of FABP family in their cells/tissues of expression especially when evidences regarding the association with some diseases exist. Such studies should start from the very basic question about detailed expression pattern and biological regulatory roles in normal and pathological conditions.

Brain is the most important and most complex organ of the body which is partially isolated from the other organs by blood brain barrier. Although neuroscience has shaped a remarkable body of life science research in the last decades, cellular and molecular basis of CNS during normal development and pathological conditions is still poorly understood and preventive or therapeutic strategies against CNS diseases are very limited.

FAs, especially LCFAs have and in particular PUFAs are major structural components of the brain. 20% of brain dry weight is consisted of PUFAs such as docosahexanoic acid (DHA) and arachidonic acid (AA). Several studies have provided evidence regarding preventive effects of PUFAs balance against neuropsychiatric and neurodegenerative disorders. Lipid homeostasis is critical in the brain which highlights the potential roles of FABPs in the brain. However, still little is known about functional significance of FABPs in the brain.

FABP3, FABP5 and FABP7 are known to be expressed in the brain. FABP7 seems to be the most specific FABP for the brain, because brain is the main organ which expresses FABP7. Several studies in recent years have provided evidence regarding expression and regulatory roles of FABPs and their ligands during embryonic and postnatal brain development as well as

associations with CNS diseases. FABP7 is abundant in developing brain and their expression drastically decreases after birth toward weak levels in the adult[73].

Despite known associations between brain expressed FABPs, especially FABP7, with several CNS diseases such as neuropsychiatric and neurodegenerative diseases, CNS neoplasms and CNS injury and altered CSF levels of FABPs during various CNS pathologies, still identity of FABP expressing cells in the adult brain and biological significance of FABPs under normal and pathological conditions are poorly understood.

FABP7, the most specific FABP of the brain, is famous for its abundance in neural stem cells and radial glia of the developing brain and its roles in embryonic neurogenesis. As it will be described in the second chapter, in order to explore the association of FABP7 with adult CNS diseases, we evaluated the involvement of FABP7 in reactive astrogliosis, a common hallmark of most CNS pathologies which is considered a major component of the regenerative program in the adult brain including dedifferentiation and proliferation of astroglia and upregulation of genes abundant during brain development [237-239]. Using FABP7-KO mouse and a cortical stab injury model, we identified the FABP7 expressing cells in the normal and injured cortex and found the involvement of FABP7 in reactive gliosis and proliferation of reactive astrocytes. Besides, using in vitro cell biological investigations, we found the regulatory roles of FABP7 on proliferation and FA uptake in the astrocytes. In addition, our novel finding regarding the expression of FABP7 in oligodendrocyte progenitor cells, prompted us to further study the expression and functional significance of FABPs in the oligodendrocyte lineage, which will be described in the third chapter. In vivo and in vitro assessments showed the reciprocal expression of FABP7 and FABP5 in oligodendrocyte progenitors and mature oligodendrocytes respectively, suggesting their roles in oligodendrogenesis, another component of the regenerative program in the CNS. As a proof of concept, using embryonic neural stem cell derived culture of oligodendrocyte lineage cells, we showed that FABP7 regulates proliferation of oligodendrocyte progenitors and their early differentiation to oligedendrocytes while FABP5 regulates oligodendrocyte maturation.

In addition to neurogenesis which occurs during development or in adult neurogenic niches, reactive gliosis and oligodendrogenesis are important components of regenerative program in the brain which are topics of great importance in the field of regenerative medicine and attractive targets for molecular dissection by neuroscientists. While, involvement of FABPs in neurogenesis has been already described, we for the first time show the involvement of FABPs in reactive gliosis and oligodendrogenesis.

Our studies, described in the current thesis following publication of two articles, have resulted in several novel important findings which highlight the biological significance of FABPs in the biology of macroglia and their potential involvement in the pathophysiology of CNS diseases, provide new insights toward significance of lipid metabolism in regenerative programs of the brain, suggest several novel hypotheses which introduce FABPs as candidate diagnostic and therapeutic targets for major CNS diseases and open several fields of study.

References

1. Storch, J. and A.E. Thumser, *Tissue-specific functions in the fatty acid-binding protein family.* J Biol Chem, 2010. **285**(43): p. 32679-83.
2. Furuhashi, M. and G.S. Hotamisligil, *Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets.* Nat Rev Drug Discov, 2008. **7**(6): p. 489-503.
3. Owada, Y., *Fatty acid binding protein: localization and functional significance in the brain.* Tohoku J Exp Med, 2008. **214**(3): p. 213-20.
4. Chmurzynska, A., *The multigene family of fatty acid-binding proteins (FABPs): function, structure and polymorphism.* J Appl Genet, 2006. **47**(1): p. 39-48.
5. Haunerland, N.H. and F. Spener, *Fatty acid-binding proteins--insights from genetic manipulations.* Prog Lipid Res, 2004. **43**(4): p. 328-49.
6. Thompson, J., et al., *The crystal structure of the liver fatty acid-binding protein. A complex with two bound oleates.* J Biol Chem, 1997. **272**(11): p. 7140-50.
7. Storch, J. and L. McDermott, *Structural and functional analysis of fatty acid-binding proteins.* J Lipid Res, 2009. **50 Suppl**: p. S126-31.
8. Richieri, G.V., et al., *Fatty acid binding proteins from different tissues show distinct patterns of fatty acid interactions.* Biochemistry, 2000. **39**(24): p. 7197-204.
9. Schachtrup, C., et al., *Functional analysis of peroxisome-proliferator-responsive element motifs in genes of fatty acid-binding proteins.* Biochem J, 2004. **382**(Pt 1): p. 239-45.
10. Schroeder, F., et al., *Role of fatty acid binding proteins and long chain fatty acids in modulating nuclear receptors and gene transcription.* Lipids, 2008. **43**(1): p. 1-17.
11. Tan, N.S., et al., *Selective cooperation between fatty acid binding proteins and peroxisome proliferator-activated receptors in regulating transcription.* Mol Cell Biol, 2002. **22**(14): p. 5114-27.

12. Kitajka, K., et al., *Effects of dietary omega-3 polyunsaturated fatty acids on brain gene expression.* Proc Natl Acad Sci U S A, 2004. **101**(30): p. 10931-6.
13. Motojima, K., *Differential effects of PPARalpha activators on induction of ectopic expression of tissue-specific fatty acid binding protein genes in the mouse liver.* Int J Biochem Cell Biol, 2000. **32**(10): p. 1085-92.
14. Wolfrum, C., et al., *Fatty acids and hypolipidemic drugs regulate peroxisome proliferator-activated receptors alpha - and gamma-mediated gene expression via liver fatty acid binding protein: a signaling path to the nucleus.* Proc Natl Acad Sci U S A, 2001. **98**(5): p. 2323-8.
15. Ayers, S.D., et al., *Continuous nucleocytoplasmic shuttling underlies transcriptional activation of PPARgamma by FABP4.* Biochemistry, 2007. **46**(23): p. 6744-52.
16. Pelsers, M.M., W.T. Hermens, and J.F. Glatz, *Fatty acid-binding proteins as plasma markers of tissue injury.* Clin Chim Acta, 2005. **352**(1-2): p. 15-35.
17. Levy, E., et al., *Localization, function and regulation of the two intestinal fatty acid-binding protein types.* Histochem Cell Biol, 2009. **132**(3): p. 351-67.
18. Kamijo-Ikemori, A., et al., *Roles of human liver type fatty acid binding protein in kidney disease clarified using hL-FABP chromosomal transgenic mice.* Nephrology (Carlton), 2011. **16**(6): p. 539-44.
19. Coe, N.R. and D.A. Bernlohr, *Physiological properties and functions of intracellular fatty acid-binding proteins.* Biochim Biophys Acta, 1998. **1391**(3): p. 287-306.
20. Rolf, B., et al., *Analysis of the ligand binding properties of recombinant bovine liver-type fatty acid binding protein.* Biochim Biophys Acta, 1995. **1259**(3): p. 245-53.
21. Wang, G., et al., *Antioxidative function of L-FABP in L-FABP stably transfected Chang liver cells.* Hepatology, 2005. **42**(4): p. 871-9.
22. Yan, J., et al., *Regulation of liver fatty acid binding protein expression by clofibrate in hepatoma cells.* Biochem Cell Biol, 2010. **88**(6): p. 957-67.
23. Rajaraman, G., et al., *Role of cytosolic liver fatty acid binding protein in hepatocellular oxidative stress: effect of dexamethasone and clofibrate treatment.* Mol Cell Biochem, 2007. **295**(1-2): p. 27-34.
24. Smathers, R.L., et al., *Characterization of 4-HNE modified L-FABP reveals alterations in structural and functional dynamics.* PLoS One, 2012. **7**(6): p. e38459.
25. Smathers, R.L., et al., *Susceptibility of L-FABP-/- mice to oxidative stress in early-stage alcoholic liver.* J Lipid Res, 2013. **54**(5): p. 1335-45.
26. Chen, A., et al., *Liver fatty acid binding protein (L-Fabp) modulates murine stellate cell activation and diet-induced nonalcoholic fatty liver disease.* Hepatology, 2013. **57**(6): p. 2202-12.
27. Charlton, M., et al., *Differential expression of lumican and fatty acid binding protein-1: new insights into the histologic spectrum of nonalcoholic fatty liver disease.* Hepatology, 2009. **49**(4): p. 1375-84.
28. Bioulac-Sage, P., et al., *Revisiting the pathology of resected benign hepatocellular nodules using new immunohistochemical markers.* Semin Liver Dis, 2011. **31**(1): p. 91-103.
29. Dharmarajan, S., et al., *Liver fatty acid-binding protein (L-Fabp) modifies intestinal fatty acid composition and adenoma formation in ApcMin/+ mice.* Cancer Prev Res (Phila), 2013. **6**(10): p. 1026-37.
30. Hashimoto, T., et al., *Liver-type fatty acid-binding protein is highly expressed in intestinal metaplasia and in a subset of carcinomas of the stomach without association with the fatty acid synthase status in the carcinoma.* Pathobiology, 2004. **71**(3): p. 115-22.
31. Satoh, Y., et al., *Analysis for the combination expression of CK20, FABP1 and MUC2 is sensitive for the prediction of peritoneal recurrence in gastric cancer.* Jpn J Clin Oncol, 2012. **42**(2): p. 148-52.

32. Sharaf, R.N., et al., *Computational prediction and experimental validation associating FABP-1 and pancreatic adenocarcinoma with diabetes.* BMC Gastroenterol, 2011. **11**: p. 5.
33. Kheirelseid, E.A., et al., *Clinical applications of gene expression in colorectal cancer.* J Gastrointest Oncol, 2013. **4**(2): p. 144-57.
34. Lawrie, L.C., et al., *Liver fatty acid binding protein expression in colorectal neoplasia.* Br J Cancer, 2004. **90**(10): p. 1955-60.
35. Pei, H., et al., *Proteome analysis and tissue microarray for profiling protein markers associated with lymph node metastasis in colorectal cancer.* J Proteome Res, 2007. **6**(7): p. 2495-501.
36. Pelsers, M.M., et al., *Liver fatty acid-binding protein as a sensitive serum marker of acute hepatocellular damage in liver transplant recipients.* Clin Chem, 2002. **48**(11): p. 2055-7.
37. Akbal, E., et al., *Liver fatty acid-binding protein is a diagnostic marker to detect liver injury due to chronic hepatitis C infection.* Arch Med Res, 2013. **44**(1): p. 34-8.
38. Ichikawa, D., et al., *Renal liver-type fatty acid binding protein attenuates angiotensin II-induced renal injury.* Hypertension, 2012. **60**(4): p. 973-80.
39. Matsui, K., et al., *Renal liver-type fatty acid binding protein (L-FABP) attenuates acute kidney injury in aristolochic acid nephrotoxicity.* Am J Pathol, 2011. **178**(3): p. 1021-32.
40. Agellon, L.B., M.J. Toth, and A.B. Thomson, *Intracellular lipid binding proteins of the small intestine.* Mol Cell Biochem, 2002. **239**(1-2): p. 79-82.
41. Vassileva, G., et al., *The intestinal fatty acid binding protein is not essential for dietary fat absorption in mice.* FASEB J, 2000. **14**(13): p. 2040-6.
42. Adriaanse, M.P., et al., *Serum I-FABP as marker for enterocyte damage in coeliac disease and its relation to villous atrophy and circulating autoantibodies.* Aliment Pharmacol Ther, 2013. **37**(4): p. 482-90.
43. Derikx, J.P., et al., *A pilot study on the noninvasive evaluation of intestinal damage in celiac disease using I-FABP and L-FABP.* J Clin Gastroenterol, 2009. **43**(8): p. 727-33.
44. Vreugdenhil, A.C., et al., *Additional value of serum I-FABP levels for evaluating celiac disease activity in children.* Scand J Gastroenterol, 2011. **46**(12): p. 1435-41.
45. Ng, E.W., et al., *Gut-Associated Biomarkers L-FABP, I-FABP, and TFF3 and LIT Score for Diagnosis of Surgical Necrotizing Enterocolitis in Preterm Infants.* Ann Surg, 2013. **258**(6): p. 1111-8.
46. Reisinger, K.W., et al., *Noninvasive measurement of intestinal epithelial damage at time of refeeding can predict clinical outcome after necrotizing enterocolitis.* Pediatr Res, 2013. **73**(2): p. 209-13.
47. Aydemir, C., et al., *Serum intestinal fatty acid binding protein level for early diagnosis and prediction of severity of necrotizing enterocolitis.* Early Hum Dev, 2011. **87**(10): p. 659-61.
48. Thuijls, G., et al., *Non-invasive markers for early diagnosis and determination of the severity of necrotizing enterocolitis.* Ann Surg, 2010. **251**(6): p. 1174-80.
49. Evennett, N.J., et al., *Urinary intestinal fatty acid-binding protein concentration predicts extent of disease in necrotizing enterocolitis.* J Pediatr Surg, 2010. **45**(4): p. 735-40.
50. Pan, L., et al., *The intestinal fatty acid binding protein diagnosing gut dysfunction in acute pancreatitis: a pilot study.* Pancreas, 2010. **39**(5): p. 633-8.
51. Kanda, T., et al., *Diagnosis of ischemic small bowel disease by measurement of serum intestinal fatty acid-binding protein in patients with acute abdomen: a multicenter, observer-blinded validation study.* J Gastroenterol, 2011. **46**(4): p. 492-500.
52. Thuijls, G., et al., *Early diagnosis of intestinal ischemia using urinary and plasma fatty acid binding proteins.* Ann Surg, 2011. **253**(2): p. 303-8.
53. Camkiran, A., et al., *Clinical significance of intestinal type fatty acid binding protein in patients undergoing coronary artery bypass surgery.* Anadolu Kardiyol Derg, 2011. **11**(6): p. 536-41.

54. Relja, B., et al., *Intestinal-FABP and liver-FABP: Novel markers for severe abdominal injury.* Acad Emerg Med, 2010. **17**(7): p. 729-35.
55. de Haan, J.J., et al., *Rapid development of intestinal cell damage following severe trauma: a prospective observational cohort study.* Crit Care, 2009. **13**(3): p. R86.
56. Baier, L.J., et al., *An amino acid substitution in the human intestinal fatty acid binding protein is associated with increased fatty acid binding, increased fat oxidation, and insulin resistance.* J Clin Invest, 1995. **95**(3): p. 1281-7.
57. Kato, I., et al., *APOE and FABP2 Polymorphisms and History of Myocardial Infarction, Stroke, Diabetes, and Gallbladder Disease.* Cholesterol, 2011. **2011**: p. 896360.
58. Tavridou, A., et al., *Thr54 allele of fatty-acid binding protein 2 gene is associated with obesity but not type 2 diabetes mellitus in a Caucasian population.* Diabetes Res Clin Pract, 2009. **84**(2): p. 132-7.
59. Bu, L., et al., *Polymorphisms in fatty acid binding protein 5 show association with type 2 diabetes.* Diabetes Res Clin Pract, 2011. **92**(1): p. 82-91.
60. Uchida, Y., et al., *The Ala54Thr polymorphism in the fatty acid-binding protein 2 (FABP2) gene is associated with hearing impairment: a preliminary report.* Auris Nasus Larynx, 2010. **37**(4): p. 496-9.
61. Yoshida, T., et al., *Association of genetic variants with chronic kidney disease in Japanese individuals with type 2 diabetes mellitus.* Int J Mol Med, 2009. **23**(4): p. 529-37.
62. Zhao, T., M. Nzekebaloudou, and J. lv, *Ala54Thr polymorphism of fatty acid-binding protein 2 gene and fasting blood lipids: a meta-analysis.* Atherosclerosis, 2010. **210**(2): p. 461-7.
63. De Luis, D.A., et al., *Influence of Ala54Thr polymorphism of fatty acid-binding protein 2 on insulin resistance and adipocytokines in patients with diabetes mellitus type 2.* Eur Rev Med Pharmacol Sci, 2010. **14**(2): p. 89-95.
64. Peng, X., et al., *[Study on the relationship between FABP2 Ala54Thr polymorphism and the risk of non-alcoholic fatty liver diseases].* Wei Sheng Yan Jiu, 2009. **38**(4): p. 401-4.
65. de Luis, D.A., et al., *Ala54Thr polymorphism of fatty acid binding protein 2, role on insulin resistance and cardiovascular risk factors in presurgical morbid obesity patients.* Obes Surg, 2009. **19**(12): p. 1691-6.
66. de Luis, D., et al., *Effect of fatty acid-binding protein 2 Ala54Thr genotype on weight loss and cardiovascular risk factors after a high-polyunsaturated fat diet in obese patients.* J Investig Med, 2012. **60**(8): p. 1194-8.
67. Helwig, U., et al., *The association of fatty acid-binding protein 2 A54T polymorphism with postprandial lipemia depends on promoter variability.* Metabolism, 2007. **56**(6): p. 723-31.
68. Bohme, M., et al., *Association between functional FABP2 promoter haplotypes and body mass index: analyses of 8072 participants of the KORA cohort study.* Mol Nutr Food Res, 2009. **53**(6): p. 681-5.
69. Damcott, C.M., et al., *Variation in the FABP2 promoter alters transcriptional activity and is associated with body composition and plasma lipid levels.* Hum Genet, 2003. **112**(5-6): p. 610-6.
70. Zhao, X., et al., *Single-nucleotide polymorphisms inside microRNA target sites influence the susceptibility to type 2 diabetes.* J Hum Genet, 2013. **58**(3): p. 135-41.
71. Ye, Q., et al., *Polymorphisms in lipid metabolism related miRNA binding sites and risk of metabolic syndrome.* Gene, 2013. **528**(2): p. 132-8.
72. Saino-Saito, S., et al., *Discrete localization of various fatty-acid-binding proteins in various cell populations of mouse retina.* Cell Tissue Res, 2009. **338**(2): p. 191-201.
73. Owada, Y., T. Yoshimoto, and H. Kondo, *Spatio-temporally differential expression of genes for three members of fatty acid binding proteins in developing and mature rat brains.* J Chem Neuroanat, 1996. **12**(2): p. 113-22.

74. Watanabe, M., T. Ono, and H. Kondo, *Immunohistochemical studies on the localisation and ontogeny of heart fatty acid binding protein in the rat.* J Anat, 1991. **174**: p. 81-95.
75. Hyder, A., et al., *Expression of fatty acid binding proteins 3 and 5 genes in rat pancreatic islets and INS-1E cells: regulation by fatty acids and glucose.* Islets, 2010. **2**(3): p. 174-84.
76. Zschiesche, W., et al., *Histochemical localization of heart-type fatty-acid binding protein in human and murine tissues.* Histochem Cell Biol, 1995. **103**(2): p. 147-56.
77. Binas, B., et al., *Requirement for the heart-type fatty acid binding protein in cardiac fatty acid utilization.* FASEB J, 1999. **13**(8): p. 805-12.
78. Schaap, F.G., et al., *Impaired long-chain fatty acid utilization by cardiac myocytes isolated from mice lacking the heart-type fatty acid binding protein gene.* Circ Res, 1999. **85**(4): p. 329-37.
79. Binas, B., et al., *A null mutation in H-FABP only partially inhibits skeletal muscle fatty acid metabolism.* Am J Physiol Endocrinol Metab, 2003. **285**(3): p. E481-9.
80. Zanotti, G., *Muscle fatty acid-binding protein.* Biochim Biophys Acta, 1999. **1441**(2-3): p. 94-105.
81. Tang, M.K., et al., *Heart-type fatty acid binding proteins are upregulated during terminal differentiation of mouse cardiomyocytes, as revealed by proteomic analysis.* Cell Tissue Res, 2004. **316**(3): p. 339-47.
82. Rump, R., et al., *Differentiation-dependent expression of heart type fatty acid-binding protein in C2C12 muscle cells.* Eur J Cell Biol, 1996. **69**(2): p. 135-42.
83. Zhu, C., et al., *Fabp3 inhibits proliferation and promotes apoptosis of embryonic myocardial cells.* Cell Biochem Biophys, 2011. **60**(3): p. 259-66.
84. Shen, Y., et al., *Silencing of FABP3 inhibits proliferation and promotes apoptosis in embryonic carcinoma cells.* Cell Biochem Biophys, 2013. **66**(1): p. 139-46.
85. Wang, X., et al., *Knockdown of FABP3 Impairs Cardiac Development in Zebra fi sh through the Retinoic Acid Signaling Pathway.* Int J Mol Sci, 2013. **14**(7): p. 13826-41.
86. Liu, Y.Q., et al., *Silencing of FABP3 leads to apoptosis-induced mitochondrial dysfunction and stimulates Wnt signaling in zebrafish.* Mol Med Rep, 2013. **8**(3): p. 806-12.
87. Garcia-Rua, V., et al., *Increased expression of fatty-acid and calcium metabolism genes in failing human heart.* PLoS One, 2012. **7**(6): p. e37505.
88. Bruins Slot, M.H., et al., *Heart-type fatty acid-binding protein in the early diagnosis of acute myocardial infarction: a systematic review and meta-analysis.* Heart, 2010. **96**(24): p. 1957-63.
89. Lippi, G., C. Mattiuzzi, and G. Cervellin, *Critical review and meta-analysis on the combination of heart-type fatty acid binding protein (H-FABP) and troponin for early diagnosis of acute myocardial infarction.* Clin Biochem, 2013. **46**(1-2): p. 26-30.
90. Carroll, C., et al., *Heart-type fatty acid binding protein as an early marker for myocardial infarction: systematic review and meta-analysis.* Emerg Med J, 2013. **30**(4): p. 280-6.
91. Lankeit, M., et al., *The predictive value of heart-type fatty acid-binding protein is independent from symptom duration in normotensive patients with pulmonary embolism.* Thromb Res, 2013. **132**(5): p. 543-7.
92. Arnalich, F., et al., *Plasma levels of mitochondrial and nuclear DNA in patients with massive pulmonary embolism in the emergency department: a prospective cohort study.* Crit Care, 2013. **17**(3): p. R90.
93. Lankeit, M., et al., *A simple score for rapid risk assessment of non-high-risk pulmonary embolism.* Clin Res Cardiol, 2013. **102**(1): p. 73-80.
94. Boscheri, A., et al., *Correlation of heart-type fatty acid-binding protein with mortality and echocardiographic data in patients with pulmonary embolism at intermediate risk.* Am Heart J, 2010. **160**(2): p. 294-300.
95. Jo, Y.H., et al., *Heart-type fatty acid-binding protein as a prognostic factor in patients with severe sepsis and septic shock.* Am J Emerg Med, 2012. **30**(9): p. 1749-55.

96. Zhang, Z.C., et al., *Usefulness of heart-type fatty acid-binding protein in patients with severe sepsis.* J Crit Care, 2012. **27**(4): p. 415 e13-8.
97. Yan, G.T., et al., *Heart-type fatty acid-binding protein is a useful marker for organ dysfunction and leptin alleviates sepsis-induced organ injuries by restraining its tissue levels.* Eur J Pharmacol, 2009. **616**(1-3): p. 244-50.
98. Cabiati, M., et al., *High peripheral levels of h-FABP are associated with poor prognosis in end-stage heart failure patients with mechanical circulatory support.* Biomark Med, 2013. **7**(3): p. 481-92.
99. Komamura, K., et al., *Heart-type fatty acid binding protein is a novel prognostic marker in patients with non-ischaemic dilated cardiomyopathy.* Heart, 2006. **92**(5): p. 615-8.
100. Oktay, B., et al., *Evaluation of the relationship between heart type fatty acid binding protein levels and the risk of cardiac damage in patients with obstructive sleep apnea syndrome.* Sleep Breath, 2008. **12**(3): p. 223-8.
101. Erenler, A.K., et al., *Heart-type fatty acid-binding protein as a potential biomarker of acute carbon monoxide poisoning.* Am J Emerg Med, 2013. **31**(8): p. 1165-9.
102. Varrone, F., et al., *The circulating level of FABP3 is an indirect biomarker of microRNA-1.* J Am Coll Cardiol, 2013. **61**(1): p. 88-95.
103. Murphy, E.J., et al., *Brain arachidonic acid incorporation is decreased in heart fatty acid binding protein gene-ablated mice.* Biochemistry, 2005. **44**(16): p. 6350-60.
104. Shioda, N., Y. Takeuchi, and K. Fukunaga, *Advanced research on dopamine signaling to develop drugs for the treatment of mental disorders: proteins interacting with the third cytoplasmic loop of dopamine D2 and D3 receptors.* J Pharmacol Sci, 2010. **114**(1): p. 25-31.
105. Shioda, N., et al., *Heart-type fatty acid binding protein regulates dopamine D2 receptor function in mouse brain.* J Neurosci, 2010. **30**(8): p. 3146-55.
106. Cheon, M.S., et al., *Heart type fatty acid binding protein (H-FABP) is decreased in brains of patients with Down syndrome and Alzheimer's disease.* J Neural Transm Suppl, 2003(67): p. 225-34.
107. Walder, B., et al., *The prognostic significance of the serum biomarker heart-fatty acidic binding protein in comparison with s100b in severe traumatic brain injury.* J Neurotrauma, 2013. **30**(19): p. 1631-7.
108. Park, S.Y., et al., *Predictive value of circulating interleukin-6 and heart-type fatty acid binding protein for three months clinical outcome in acute cerebral infarction: multiple blood markers profiling study.* Crit Care, 2013. **17**(2): p. R45.
109. Yilman, M., et al., *Is there a relationship between serum heart- type fatty acid binding protein level and clinical severity in patients with subarachnoid hemorrhage?* Turk Neurosurg, 2012. **22**(6): p. 695-700.
110. Turck, N., et al., *A multiparameter panel method for outcome prediction following aneurysmal subarachnoid hemorrhage.* Intensive Care Med, 2010. **36**(1): p. 107-15.
111. Zanier, E.R., et al., *Heart-fatty acid-binding and tau proteins relate to brain injury severity and long-term outcome in subarachnoid haemorrhage patients.* Br J Anaesth, 2013. **111**(3): p. 424-32.
112. Guo, L.H., P. Alexopoulos, and R. Perneczky, *Heart-type fatty acid binding protein and vascular endothelial growth factor: cerebrospinal fluid biomarker candidates for Alzheimer's disease.* Eur Arch Psychiatry Clin Neurosci, 2013. **263**(7): p. 553-60.
113. Olsson, B., et al., *Cerebrospinal fluid levels of heart fatty acid binding protein are elevated prodromally in Alzheimer's disease and vascular dementia.* J Alzheimers Dis, 2013. **34**(3): p. 673-9.

114. Ohrfelt, A., et al., *Screening for new biomarkers for subcortical vascular dementia and Alzheimer's disease.* Dement Geriatr Cogn Dis Extra, 2011. **1**(1): p. 31-42.
115. Chiasserini, D., et al., *CSF levels of heart fatty acid binding protein are altered during early phases of Alzheimer's disease.* J Alzheimers Dis, 2010. **22**(4): p. 1281-8.
116. Cologna, S.M., et al., *Quantitative proteomic analysis of Niemann-Pick disease, type C1 cerebellum identifies protein biomarkers and provides pathological insight.* PLoS One, 2012. **7**(10): p. e47845.
117. Linge, A., et al., *Differential expression of fourteen proteins between uveal melanoma from patients who subsequently developed distant metastases versus those who did Not.* Invest Ophthalmol Vis Sci, 2012. **53**(8): p. 4634-43.
118. Alter, M.L., et al., *Early urinary and plasma biomarkers for experimental diabetic nephropathy.* Clin Lab, 2012. **58**(7-8): p. 659-71.
119. Nauta, F.L., et al., *Glomerular and tubular damage markers are elevated in patients with diabetes.* Diabetes Care, 2011. **34**(4): p. 975-81.
120. Chen, H.M., et al., *Heart-type fatty acid binding protein is associated with proteinuria in obesity.* PLoS One, 2012. **7**(9): p. e45691.
121. Jochmans, I., et al., *Circulating AST, H-FABP, and NGAL are early and accurate biomarkers of graft injury and dysfunction in a preclinical model of kidney transplantation.* Ann Surg, 2011. **254**(5): p. 784-91; discussion 791-2.
122. Arsov, S., et al., *Increase in skin autofluorescence and release of heart-type fatty acid binding protein in plasma predicts mortality of hemodialysis patients.* Artif Organs, 2013. **37**(7): p. E114-22.
123. Binas, B., et al., *Hormonal induction of functional differentiation and mammary-derived growth inhibitor expression in cultured mouse mammary gland explants.* In Vitro Cell Dev Biol, 1992. **28A**(9-10): p. 625-34.
124. Clark, A.J., et al., *Deletion of the gene encoding H-FABP/MDGI has no overt effects in the mammary gland.* Transgenic Res, 2000. **9**(6): p. 439-44.
125. Binas, B., et al., *Epithelial proliferation and differentiation in the mammary gland do not correlate with cFABP gene expression during early pregnancy.* Dev Genet, 1995. **17**(2): p. 167-75.
126. Nevo, J., et al., *Mammary-derived growth inhibitor (MDGI) interacts with integrin alpha-subunits and suppresses integrin activity and invasion.* Oncogene, 2010. **29**(49): p. 6452-63.
127. Huynh, H.T., et al., *Tumor suppressor activity of the gene encoding mammary-derived growth inhibitor.* Cancer Res, 1995. **55**(11): p. 2225-31.
128. Vergnes, L., et al., *Heart-type fatty acid-binding protein is essential for efficient brown adipose tissue fatty acid oxidation and cold tolerance.* J Biol Chem, 2011. **286**(1): p. 380-90.
129. Yamamoto, T., et al., *Quantitative evaluation of the effects of cold exposure of rats on the expression levels of ten FABP isoforms in brown adipose tissue.* Biotechnol Lett, 2011. **33**(2): p. 237-42.
130. Makowski, L., et al., *Lack of macrophage fatty-acid-binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis.* Nat Med, 2001. **7**(6): p. 699-705.
131. Shum, B.O., et al., *The adipocyte fatty acid-binding protein aP2 is required in allergic airway inflammation.* J Clin Invest, 2006. **116**(8): p. 2183-2192.
132. Rolph, M.S., et al., *Regulation of dendritic cell function and T cell priming by the fatty acid-binding protein AP2.* J Immunol, 2006. **177**(11): p. 7794-801.
133. Spiegelman, B.M., M. Frank, and H. Green, *Molecular cloning of mRNA from 3T3 adipocytes. Regulation of mRNA content for glycerophosphate dehydrogenase and other differentiation-dependent proteins during adipocyte development.* J Biol Chem, 1983. **258**(16): p. 10083-9.

134. Hunt, C.R., et al., *Adipocyte P2 gene: developmental expression and homology of 5'-flanking sequences among fat cell-specific genes.* Proc Natl Acad Sci U S A, 1986. **83**(11): p. 3786-90.
135. Hotamisligil, G.S., et al., *Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein.* Science, 1996. **274**(5291): p. 1377-9.
136. Uysal, K.T., et al., *Improved glucose and lipid metabolism in genetically obese mice lacking aP2.* Endocrinology, 2000. **141**(9): p. 3388-96.
137. Shen, W.J., et al., *Interaction of rat hormone-sensitive lipase with adipocyte lipid-binding protein.* Proc Natl Acad Sci U S A, 1999. **96**(10): p. 5528-32.
138. Scheja, L., et al., *Altered insulin secretion associated with reduced lipolytic efficiency in aP2-/- mice.* Diabetes, 1999. **48**(10): p. 1987-94.
139. Coe, N.R., M.A. Simpson, and D.A. Bernlohr, *Targeted disruption of the adipocyte lipid-binding protein (aP2 protein) gene impairs fat cell lipolysis and increases cellular fatty acid levels.* J Lipid Res, 1999. **40**(5): p. 967-72.
140. Boord, J.B., et al., *Adipocyte fatty acid-binding protein, aP2, alters late atherosclerotic lesion formation in severe hypercholesterolemia.* Arterioscler Thromb Vasc Biol, 2002. **22**(10): p. 1686-91.
141. Makowski, L., et al., *The fatty acid-binding protein, aP2, coordinates macrophage cholesterol trafficking and inflammatory activity. Macrophage expression of aP2 impacts peroxisome proliferator-activated receptor gamma and IkappaB kinase activities.* J Biol Chem, 2005. **280**(13): p. 12888-95.
142. Makowski, L. and G.S. Hotamisligil, *The role of fatty acid binding proteins in metabolic syndrome and atherosclerosis.* Curr Opin Lipidol, 2005. **16**(5): p. 543-8.
143. Ghelfi, E., et al., *Fatty acid binding protein 4 regulates VEGF-induced airway angiogenesis and inflammation in a transgenic mouse model: implications for asthma.* Am J Pathol, 2013. **182**(4): p. 1425-33.
144. Iso, T., et al., *Capillary endothelial fatty acid binding proteins 4 and 5 play a critical role in fatty acid uptake in heart and skeletal muscle.* Arterioscler Thromb Vasc Biol, 2013. **33**(11): p. 2549-57.
145. Bennett, J.H., et al., *Immunohistochemical identification of tumours of adipocytic differentiation using an antibody to aP2 protein.* J Clin Pathol, 1995. **48**(10): p. 950-4.
146. Ohlsson, G., et al., *Loss of expression of the adipocyte-type fatty acid-binding protein (A-FABP) is associated with progression of human urothelial carcinomas.* Mol Cell Proteomics, 2005. **4**(4): p. 570-81.
147. Furuhashi, M., et al., *Treatment of diabetes and atherosclerosis by inhibiting fatty-acid-binding protein aP2.* Nature, 2007. **447**(7147): p. 959-65.
148. Kralisch, S. and M. Fasshauer, *Adipocyte fatty acid binding protein: a novel adipokine involved in the pathogenesis of metabolic and vascular disease?* Diabetologia, 2013. **56**(1): p. 10-21.
149. Cao, H., et al., *Adipocyte lipid chaperone AP2 is a secreted adipokine regulating hepatic glucose production.* Cell Metab, 2013. **17**(5): p. 768-78.
150. Oresti, G.M., et al., *Cell-type-specific regulation of genes involved in testicular lipid metabolism: fatty acid-binding proteins, diacylglycerol acyltransferases, and perilipin 2.* Reproduction, 2013. **146**(5): p. 471-80.
151. Maeda, K., et al., *Role of the fatty acid binding protein mal1 in obesity and insulin resistance.* Diabetes, 2003. **52**(2): p. 300-7.
152. Owada, Y., et al., *Analysis on the phenotype of E-FABP-gene knockout mice.* Mol Cell Biochem, 2002. **239**(1-2): p. 83-6.
153. Maeda, K., et al., *Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes.* Cell Metab, 2005. **1**(2): p. 107-19.

154. Cao, H., et al., *Regulation of metabolic responses by adipocyte/macrophage Fatty Acid-binding proteins in leptin-deficient mice.* Diabetes, 2006. **55**(7): p. 1915-22.
155. Boord, J.B., et al., *Combined adipocyte-macrophage fatty acid-binding protein deficiency improves metabolism, atherosclerosis, and survival in apolipoprotein E-deficient mice.* Circulation, 2004. **110**(11): p. 1492-8.
156. Bagheri, R., et al., *Relation of plasma fatty acid binding proteins 4 and 5 with the metabolic syndrome, inflammation and coronary calcium in patients with type-2 diabetes mellitus.* Am J Cardiol, 2010. **106**(8): p. 1118-23.
157. Wolf, G., *Retinoic acid activation of peroxisome proliferation-activated receptor delta represses obesity and insulin resistance.* Nutr Rev, 2010. **68**(1): p. 67-70.
158. Gericke, J., et al., *Allergen-induced dermatitis causes alterations in cutaneous retinoid-mediated signaling in mice.* PLoS One, 2013. **8**(8): p. e71244.
159. Collins, C.A. and F.M. Watt, *Dynamic regulation of retinoic acid-binding proteins in developing, adult and neoplastic skin reveals roles for beta-catenin and Notch signalling.* Dev Biol, 2008. **324**(1): p. 55-67.
160. Dallaglio, K., et al., *E-FABP induces differentiation in normal human keratinocytes and modulates the differentiation process in psoriatic keratinocytes in vitro.* Exp Dermatol, 2013. **22**(4): p. 255-61.
161. Ogawa, E., et al., *Epidermal FABP (FABP5) regulates keratinocyte differentiation by 13(S)-HODE-mediated activation of the NF-kappaB signaling pathway.* J Invest Dermatol, 2011. **131**(3): p. 604-12.
162. Siegenthaler, G., et al., *Characterization and expression of a novel human fatty acid-binding protein: the epidermal type (E-FABP).* Biochem Biophys Res Commun, 1993. **190**(2): p. 482-7.
163. Siegenthaler, G., et al., *Purification and characterization of the human epidermal fatty acid-binding protein: localization during epidermal cell differentiation in vivo and in vitro.* Biochem J, 1994. **302 (Pt 2)**: p. 363-71.
164. Miyake, T., et al., *Epidermal-type FABP is a predictive marker of clinical response to systemic treatment and ultraviolet therapy in psoriatic skin lesions.* J Dermatol Sci, 2012. **68**(3): p. 199-202.
165. Sugawara, T., et al., *Reduced size of sebaceous gland and altered sebum lipid composition in mice lacking fatty acid binding protein 5 gene.* Exp Dermatol, 2012. **21**(7): p. 543-6.
166. Yamane, Y., et al., *New horny layer marker proteins for evaluating skin condition in atopic dermatitis.* Int Arch Allergy Immunol, 2009. **150**(1): p. 89-101.
167. Shipman, M., et al., *Proteomic and systems biology analysis of monocytes exposed to securinine, a GABA(A) receptor antagonist and immune adjuvant.* PLoS One, 2012. **7**(9): p. e41278.
168. Babaev, V.R., et al., *Macrophage Mal1 deficiency suppresses atherosclerosis in low-density lipoprotein receptor-null mice by activating peroxisome proliferator-activated receptor-gamma-regulated genes.* Arterioscler Thromb Vasc Biol, 2011. **31**(6): p. 1283-90.
169. Reynolds, J.M., et al., *Deficiency of fatty acid-binding proteins in mice confers protection from development of experimental autoimmune encephalomyelitis.* J Immunol, 2007. **179**(1): p. 313-21.
170. Li, B., et al., *Regulation of Th17 differentiation by epidermal fatty acid-binding protein.* J Immunol, 2009. **182**(12): p. 7625-33.
171. Adachi, Y., et al., *Fatty acid-binding protein 4 (FABP4) and FABP5 modulate cytokine production in the mouse thymic epithelial cells.* Histochem Cell Biol, 2012. **138**(3): p. 397-406.
172. Gally, F., H.W. Chu, and R.P. Bowler, *Cigarette smoke decreases airway epithelial FABP5 expression and promotes Pseudomonas aeruginosa infection.* PLoS One, 2013. **8**(1): p. e51784.

173. Gally, F., et al., *FABP5 deficiency enhances susceptibility to H1N1 influenza A virus-induced lung inflammation.* Am J Physiol Lung Cell Mol Physiol, 2013. **305**(1): p. L64-72.
174. Wu, T., et al., *Knockdown of FABP5 mRNA decreases cellular cholesterol levels and results in decreased apoB100 secretion and triglyceride accumulation in ARPE-19 cells.* Lab Invest, 2010. **90**(6): p. 906-14.
175. Yu, S., et al., *Retinoic acid induces neurogenesis by activating both retinoic acid receptors (RARs) and peroxisome proliferator-activated receptor beta/delta (PPARbeta/delta).* J Biol Chem, 2012. **287**(50): p. 42195-205.
176. Boneva, N.B., et al., *Expression of fatty acid-binding proteins in adult hippocampal neurogenic niche of postischemic monkeys.* Hippocampus, 2011. **21**(2): p. 162-71.
177. De Leon, M., et al., *Fatty acid binding protein is induced in neurons of the dorsal root ganglia after peripheral nerve injury.* J Neurosci Res, 1996. **44**(3): p. 283-92.
178. Owada, Y., et al., *Changes in gene expression for skin-type fatty acid binding protein in hypoglossal motor neurons following nerve crush.* Neurosci Lett, 1997. **223**(1): p. 25-8.
179. Han, J., et al., *Identification of potential therapeutic targets in human head & neck squamous cell carcinoma.* Head Neck Oncol, 2009. **1**: p. 27.
180. Fang, L.Y., et al., *Fatty-acid-binding protein 5 promotes cell proliferation and invasion in oral squamous cell carcinoma.* J Oral Pathol Med, 2010. **39**(4): p. 342-8.
181. Kannan-Thulasiraman, P., et al., *Fatty acid-binding protein 5 and PPARbeta/delta are critical mediators of epidermal growth factor receptor-induced carcinoma cell growth.* J Biol Chem, 2010. **285**(25): p. 19106-15.
182. Levi, L., et al., *Genetic ablation of the fatty acid-binding protein FABP5 suppresses HER2-induced mammary tumorigenesis.* Cancer Res, 2013. **73**(15): p. 4770-80.
183. Liu, R.Z., et al., *Association of FABP5 expression with poor survival in triple-negative breast cancer: implication for retinoic acid therapy.* Am J Pathol, 2011. **178**(3): p. 997-1008.
184. Jeong, C.Y., et al., *Fatty acid-binding protein 5 promotes cell proliferation and invasion in human intrahepatic cholangiocarcinoma.* Oncol Rep, 2012. **28**(4): p. 1283-92.
185. Koshiyama, A., T. Ichibangase, and K. Imai, *Comprehensive fluorogenic derivatization-liquid chromatography/tandem mass spectrometry proteomic analysis of colorectal cancer cell to identify biomarker candidate.* Biomed Chromatogr, 2013. **27**(4): p. 440-50.
186. Jin, B.Y., et al., *CRABP2 and FABP5 identified by 2D DIGE profiling are upregulated in human bladder cancer.* Chin Med J (Engl), 2013. **126**(19): p. 3787-9.
187. Morgan, E., P. Kannan-Thulasiraman, and N. Noy, *Involvement of Fatty Acid Binding Protein 5 and PPARbeta/delta in Prostate Cancer Cell Growth.* PPAR Res, 2010. **2010**.
188. Forootan, S.S., et al., *Atelocollagen-delivered siRNA targeting the FABP5 gene as an experimental therapy for prostate cancer in mouse xenografts.* Int J Oncol, 2010. **36**(1): p. 69-76.
189. Pang, J., et al., *Profiling protein markers associated with lymph node metastasis in prostate cancer by DIGE-based proteomics analysis.* J Proteome Res, 2010. **9**(1): p. 216-26.
190. Li, Z., et al., *Prognostic evaluation of epidermal fatty acid-binding protein and calcyphosine, two proteins implicated in endometrial cancer using a proteomic approach.* Int J Cancer, 2008. **123**(10): p. 2377-83.
191. Gong, Y.Z., et al., *Molecular cloning, tissue distribution, and expression of a 14-kDa bile acid-binding protein from rat ileal cytosol.* Proc Natl Acad Sci U S A, 1994. **91**(11): p. 4741-5.
192. Fujita, M., et al., *Molecular cloning, expression, and characterization of a human intestinal 15-kDa protein.* Eur J Biochem, 1995. **233**(2): p. 406-13.
193. Watanabe, K., et al., *Immunohistochemical distribution of intestinal 15 kDa protein in human tissues.* Arch Histol Cytol, 1995. **58**(3): p. 303-6.

194. Alpini, G., et al., *gamma-Interferon inhibits secretin-induced choleresis and cholangiocyte proliferation in a murine model of cirrhosis.* J Hepatol, 1997. **27**(2): p. 371-80.
195. Zwicker, B.L. and L.B. Agellon, *Transport and biological activities of bile acids.* Int J Biochem Cell Biol, 2013. **45**(7): p. 1389-98.
196. Praslickova, D., et al., *The ileal lipid binding protein is required for efficient absorption and transport of bile acids in the distal portion of the murine small intestine.* PLoS One, 2012. **7**(12): p. e50810.
197. Gonzalo, D.H., et al., *Gene expression profiling of serrated polyps identifies annexin A10 as a marker of a sessile serrated adenoma/polyp.* J Pathol, 2013. **230**(4): p. 420-9.
198. Fisher, E., et al., *Evidence for the Thr79Met polymorphism of the ileal fatty acid binding protein (FABP6) to be associated with type 2 diabetes in obese individuals.* Mol Genet Metab, 2009. **98**(4): p. 400-5.
199. Abdelwahab, S.A., et al., *Localization of brain-type fatty acid-binding protein in Kupffer cells of mice and its transient decrease in response to lipopolysaccharide.* Histochem Cell Biol, 2003. **119**(6): p. 469-75.
200. Tokuda, N., et al., *Identification of FABP7 in fibroblastic reticular cells of mouse lymph nodes.* Histochem Cell Biol, 2010. **134**(5): p. 445-52.
201. Colombo, S., et al., *Transcriptomic analysis of mouse embryonic skin cells reveals previously unreported genes expressed in melanoblasts.* J Invest Dermatol, 2012. **132**(1): p. 170-8.
202. Shi, Y.E., et al., *Antitumor activity of the novel human breast cancer growth inhibitor, mammary-derived growth inhibitor-related gene, MRG.* Cancer Res, 1997. **57**(15): p. 3084-91.
203. Hohoff, C. and F. Spener, *Correspondence re: Y.E. Shi et al., Antitumor activity of the novel human breast cancer growth inhibitor, mammary-derived growth inhibitor-related gene, MRG. Cancer Res., 57: 3084-3091, 1997.* Cancer Res, 1998. **58**(17): p. 4015-7.
204. Liu, R.Z., et al., *Fatty acid binding proteins in brain development and disease.* Int J Dev Biol, 2010. **54**(8-9): p. 1229-39.
205. Watanabe, A., et al., *Fabp7 maps to a quantitative trait locus for a schizophrenia endophenotype.* PLoS Biol, 2007. **5**(11): p. e297.
206. Arai, Y., et al., *Role of Fabp7, a downstream gene of Pax6, in the maintenance of neuroepithelial cells during early embryonic development of the rat cortex.* J Neurosci, 2005. **25**(42): p. 9752-61.
207. Feng, L., M.E. Hatten, and N. Heintz, *Brain lipid-binding protein (BLBP): a novel signaling system in the developing mammalian CNS.* Neuron, 1994. **12**(4): p. 895-908.
208. Yun, S.W., et al., *Neural stem cell specific fluorescent chemical probe binding to FABP7.* Proc Natl Acad Sci U S A, 2012. **109**(26): p. 10214-7.
209. Leong, C., et al., *Neural stem cell isolation from the whole mouse brain using the novel FABP7-binding fluorescent dye, CDr3.* Stem Cell Res, 2013. **11**(3): p. 1314-22.
210. Teunissen, C.E., et al., *Brain-specific fatty acid-binding protein is elevated in serum of patients with dementia-related diseases.* Eur J Neurol, 2011. **18**(6): p. 865-71.
211. Prinzen, C., et al., *Differential gene expression in ADAM10 and mutant ADAM10 transgenic mice.* BMC Genomics, 2009. **10**: p. 66.
212. Guttula, S.V., A. Allam, and R.S. Gumpeny, *Analyzing microarray data of Alzheimer's using cluster analysis to identify the biomarker genes.* Int J Alzheimers Dis, 2012. **2012**: p. 649456.
213. Sanchez-Font, M.F., et al., *Overexpression of FABP7 in Down syndrome fetal brains is associated with PKNOX1 gene-dosage imbalance.* Nucleic Acids Res, 2003. **31**(11): p. 2769-77.
214. Kaloshi, G., et al., *FABP7 expression in glioblastomas: relation to prognosis, invasion and EGFR status.* J Neurooncol, 2007. **84**(3): p. 245-8.
215. Liang, Y., et al., *Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme.* Proc Natl Acad Sci U S A, 2005. **102**(16): p. 5814-9.

216. Jiang, J., et al., *The expression of fatty acid metabolism-associated proteins is correlated with the prognosis of meningiomas.* APMIS, 2013. **121**(10): p. 997-1003.
217. Retrosi, G., et al., *Brain lipid-binding protein: a marker of differentiation in neuroblastic tumors.* J Pediatr Surg, 2011. **46**(6): p. 1197-200.
218. Slipicevic, A., et al., *The fatty acid binding protein 7 (FABP7) is involved in proliferation and invasion of melanoma cells.* BMC Cancer, 2008. **8**: p. 276.
219. Goto, Y., et al., *Aberrant fatty acid-binding protein-7 gene expression in cutaneous malignant melanoma.* J Invest Dermatol, 2010. **130**(1): p. 221-9.
220. Tolle, A., et al., *Importance of braintype fatty acid binding protein for cell-biological processes in human renal carcinoma cells.* Oncol Rep, 2011. **25**(5): p. 1307-12.
221. Liu, R.Z., et al., *A fatty acid-binding protein 7/RXRbeta pathway enhances survival and proliferation in triple-negative breast cancer.* J Pathol, 2012. **228**(3): p. 310-21.
222. Song, J.Y., et al., *Candidates for tumor markers of cervical cancer discovered by proteomic analysis.* J Korean Med Sci, 2012. **27**(12): p. 1479-85.
223. Zhang, Y., et al., *Gene expression profiling reveals potential key pathways involved in pyrazinamide-mediated hepatotoxicity in Wistar rats.* J Appl Toxicol, 2013. **33**(8): p. 807-19.
224. Gao, H., et al., *Proteomic analysis of differentially expressed proteins between stenotic and normal colon segment tissues derived from patients with Hirschsprung's disease.* Protein J, 2011. **30**(2): p. 138-42.
225. Ahn, J., et al., *Lycopene inhibits hepatic steatosis via microRNA-21-induced downregulation of fatty acid-binding protein 7 in mice fed a high-fat diet.* Mol Nutr Food Res, 2012. **56**(11): p. 1665-74.
226. Greenfield, S., et al., *Protein composition of myelin of the peripheral nervous system.* J Neurochem, 1973. **20**(4): p. 1207-16.
227. Majava, V., et al., *Structural and functional characterization of human peripheral nervous system myelin protein P2.* PLoS One, 2010. **5**(4): p. e10300.
228. Brostoff, S.W., J.M. Powers, and M.J. Weise, *Allergic encephalomyelitis induced in guinea pigs by a peptide from the NH2-terminus of bovine P2 protein.* Nature, 1980. **285**(5760): p. 103-4.
229. Inglis, H.R., P.A. Csurhes, and P.A. McCombe, *Antibody responses to peptides of peripheral nerve myelin proteins P0 and P2 in patients with inflammatory demyelinating neuropathy.* J Neurol Neurosurg Psychiatry, 2007. **78**(4): p. 419-22.
230. Khalili-Shirazi, A., et al., *Antibody responses to P0 and P2 myelin proteins in Guillain-Barre syndrome and chronic idiopathic demyelinating polyradiculoneuropathy.* J Neuroimmunol, 1993. **46**(1-2): p. 245-51.
231. Makowska, A., et al., *Immune responses to myelin proteins in Guillain-Barre syndrome.* J Neurol Neurosurg Psychiatry, 2008. **79**(6): p. 664-71.
232. Sanvito, L., et al., *Humoral and cellular immune responses to myelin protein peptides in chronic inflammatory demyelinating polyradiculoneuropathy.* J Neurol Neurosurg Psychiatry, 2009. **80**(3): p. 333-8.
233. Kido, T. and H. Namiki, *Expression of testicular fatty acid-binding protein PERF 15 during germ cell apoptosis.* Dev Growth Differ, 2000. **42**(4): p. 359-66.
234. Kido, T., et al., *The testicular fatty acid binding protein PERF15 regulates the fate of germ cells in PERF15 transgenic mice.* Dev Growth Differ, 2005. **47**(1): p. 15-24.
235. Platt, M.D., et al., *Use of differential isotopic labeling and mass spectrometry to analyze capacitation-associated changes in the phosphorylation status of mouse sperm proteins.* J Proteome Res, 2009. **8**(3): p. 1431-40.
236. Selvaraj, V., et al., *Mice lacking FABP9/PERF15 develop sperm head abnormalities but are fertile.* Dev Biol, 2010. **348**(2): p. 177-89.

237. Buffo, A., et al., *Origin and progeny of reactive gliosis: A source of multipotent cells in the injured brain.* Proc Natl Acad Sci U S A, 2008. **105**(9): p. 3581-6.
238. Shimada, I.S., et al., *Self-renewal and differentiation of reactive astrocyte-derived neural stem/progenitor cells isolated from the cortical peri-infarct area after stroke.* J Neurosci, 2012. **32**(23): p. 7926-40.
239. Sirko, S., et al., *Reactive glia in the injured brain acquire stem cell properties in response to sonic hedgehog. [corrected].* Cell Stem Cell, 2013. **12**(4): p. 426-39.

CHAPTER 2

FABP7 expression in normal and stab injured cortex and it's role in astrocyte proliferation

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SUMMARY

Reactive gliosis, in which astrocytes as well as other types of glial cells undergo massive proliferation, is a common hallmark of all brain pathologies and a part of brain regenerative program. Brain-type fatty acid binding protein (FABP7) is abundantly expressed in neural stem cells and astrocytes of developing brain, suggesting its role in differentiation and/or proliferation of glial cells through regulation of lipid metabolism and/or signaling. However, the role of FABP7 in proliferation of glial cells during reactive gliosis is unknown. In this study, we examined the expression of FABP7 in mouse cortical stab injury model and also the phenotype of FABP7-KO mice in glial cell proliferation. Western blotting showed that FABP7 expression was increased significantly in the injured cortex compared with the contralateral side. By immunohistochemistry, FABP7 was localized to $GFAP^+$ astrocytes (21% of $FABP7^+$ cells) and $NG2^+$ oligodendrocyte progenitor cells (62%) in the normal cortex. In the injured cortex there was no change in the population of $FABP7^+/NG2^+$ cells, while there was a significant increase in $FABP7^+/GFAP^+$ cells. In the stab injured cortex of FABP7-KO mice there was decrease in the total number of reactive astrocytes and in the number of $BrdU^+$ astrocytes compared with wild-type mice. Primary cultured astrocytes from FABP7-KO mice also showed a significant decrease in proliferation and omega-3 fatty acid incorporation compared with wild-type astrocytes. Overall, these data suggest that FABP7 is involved in reactive astrogliosis and regulates the proliferation of astrocytes by controlling cellular fatty acid homeostasis.

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) have important metabolic, structural and signal transducing roles. PUFAs are abundant in the developing brain and are essential for normal development of the brain. Several studies have suggested a negative correlation between dietary consumption of PUFAs and risk of several neurodegenerative and neuropsychiatric diseases such as multiple sclerosis [1-3], Parkinson disease [4], Alzheimer disease [5-6], schizophrenia [7-8], depression, other psychotic and mood disorders and developmental disorders like ADHD and autism [9-10]. Therapeutic effects of PUFAs have also been suggested even in these diseases, and PUFA supplements are currently recommended to these patients as alternative or complementary medicine [11-13]. Nevertheless, there is limited evidence to support preventive and therapeutic effects of PUFAs [14-15].

Fatty acid binding proteins (FABPs) are intracellular low molecular weight (14-15 kDa) polypeptides, and are key molecules in the uptake, transportation and storage of long chain fatty acids and in mediating their signal transduction and gene regulation activities. Twelve members (9 in mammals) of the FABP family have been recognized so far, which are differentially expressed in different organs, tissues and cell types. FABP7 (Brain-type FABP), a strong binder of omega-3 PUFAs, is abundantly expressed in neural stem cells and astrocytes of the developing brain, suggesting a role in glial cell differentiation and proliferation of glial cells [16-17]. However, the functional significance of FABP7 expression in cortical astrocytes under normal and pathological conditions remains unknown.

Reactive gliosis, whereby astrocytes and other types of glial cells undergo massive proliferation, is a common hallmark of numerous brain pathologies. However, the underlying mechanisms of gliosis are largely unknown. Reactive gliosis consists of a range of molecular and morphological changes, especially in astrocytes, including cellular hypertrophy, alteration in molecular expression, proliferation and scar formation. Several studies have shown that upon injury, post mitotic mature astrocytes undergo dedifferentiation, upregulate molecules enriched in neural stem /progenitor cells and become capable of self renewal and multipotency both in vitro and in vivo[18-20]. Although several studies have shown that due to presence or absence of some factors, reactive astrocytes fail to regenerate neurons in mammal brains, reactive astrogliosis is

considered as a major component of regenerative program in the brain conserved across species [21] and molecular dissection of reactive astrocytes has been an attractive target in regenerative medicine. FABP7 is a well known marker of neural stem /progenitor cells in the developing brain and a regulator of their proliferation [22]. However, involvement of FABP7 in reactive astrogliosis is not known.

Recently, it was reported that docosahexaenoic acid (DHA), a ligand of FABP7, prevents degradation of neurons and astrocytes following brain ischemia and spinal cord injury by protection of neurons and astrocytes from cell degeneration [23], suggesting a role for FABP7 in regulating CNS injury. In addition, there is an association of FABP7 with glioma [24-27]. This suggests a role of FABP7 in reactive astrogliosis and astrocyte proliferation, considering the similarities in molecular and cellular phenotypes between reactive astrocytes and glioma, especially induced proliferation and upregulation of neural stem/progenitor markers. Furthermore, while FABP7 is abundantly expressed in neural stem/progenitor cells during early developmental stages [22], it might be also important in adult cortical progenitors, which share some characteristics with neural stem cells.

In the present study, we examined the detailed localization of FABP7 in the normal and stab injured brain cortex of adult mice and determined its role in astrocyte proliferation *in vivo and in vitro* using FABP7 knockout (KO) mice [28]. We found that FABP7 regulated the proliferative response of astrocytes to injury. Furthermore, we observed novel expression of FABP7 in oligodendrocyte progenitor cells (OPCs).

MATERIALS AND METHODS

Animals

Three month-old male C57BL/6 mice (14 wild-type and 14 FABP7-KO) [28] were used for brain cortex stab injury experiments. All experimental protocols were reviewed by the Ethics Committee for Animal Experimentation of Yamaguchi University School of Medicine, and were

performed according to the Guidelines for Animal Experimentation of the Yamaguchi University School of Medicine and Under Law and Notification requirements of the Japanese Government.

Stab injury model

Animals were anesthetized (Isoflurane; Abbott, IL, USA) using a small animal anesthetizer (Muromachi. Co. Tokyo, Japan) and a 1 mm deep stab wound was made on the right cerebral neocortex (Bregma, -1 mm to -2.5 mm. Latero-lateral, 1.5 to 2.5 mm) [29] (Fig. 2.1 a, b). Mice were sacrificed at 3 (n=7 for wild-type mice, n=7 for FABP7-KO mice) and 7 (n=7) days post lesion (DPL).

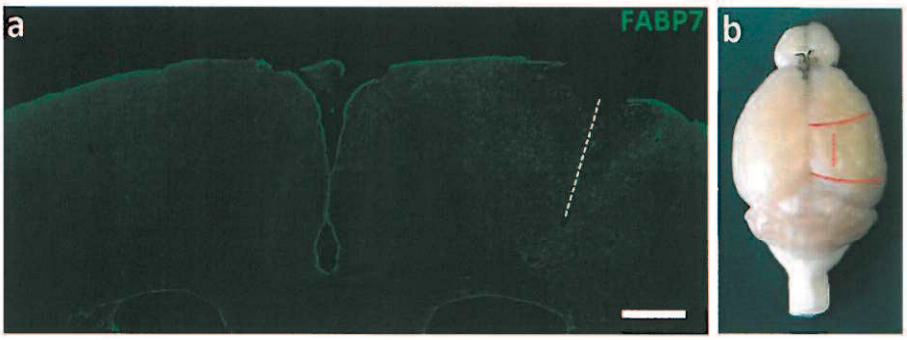

Fig. 2.1 Experimental design of stab injury.
a) Low magnification immunofluorescent micrograph showing intact and stab-injured cortex immunostained for FABP7. **b)** Image showing the location of stab injury in the cerebral cortex. Scale bars = 500 μm

Cell culture

For primary cultured astrocytes, the cerebral cortices of newborn mice (wild-type and FABP7-KO) were dissected, and the meninges were carefully removed. Tissues were cut into small pieces in HBSS (Hanks Buffer Salt Solution) supplemented with glucose, penicillin and streptomycin, and further dissociated by trypsin treatment (0.25%) at 37 °C for 20 minutes. Debris was removed by filtering through a 100 μm mesh, and the cell suspension was centrifuged at 1000 rpm and 20 °C for 5 min. The supernatant was removed and the pellet was resuspended in DMEM supplemented with 10% FBS, penicillin, streptomycin and amphotericin B, and then seeded at a density of 2 x 10^6 cells per T25 flask. Cells were incubated in a 5 % CO_2 incubator at 37 °C until cells became confluent. The culture flasks were shaken on an orbital

shaker (BR-40LF; TAITEC, Koshigaya, Japan) at 200 rpm and 37 °C for more than 14 h to detach loosely attached microglia and OPCs from the astrocytic bed. Immediately after shaking, the cells were washed three times with PBS and culture medium was refreshed. After 2-day incubation in 5% CO_2 at 37 °C, flasks were washed with PBS for three times and treated with trypsin /EDTA/PBS for 5 min at 37 °C. Trypsination was stopped by adding equal amount of culture medium and cell suspension was centrifuged at 1000 rpm for 5 minutes at room temperature. Supernatant was removed and pellet was resuspended in fresh medium. Cells were seeded at a density of $1.5X10^5$ cells/ml in 35mm or 10 cm dishes. The purity of astrocytes was confirmed to be > 95 % by immunostaining.

For establishment of FABP7 stably over-expressing cells, the coding region of mouse FABP7 gene was amplified by PCR and sub-cloned into the pcDNA3 mammalian expression vector. The constructed expression vector (pcDNA3 for mock, pcDNA3/FABP7) was transfected into NG108 cells (rodent neuroblastoma/glioma hybrid cells; a kind gift from Dr. K Fukunaga, Tohoku University) using Lipofectamine 2000 (Invitrogen Co., Carlsbad, CA, USA) following the manufacturer's instructions, and the cells were selected for two weeks in culture media supplemented with 0.5 mg/mL G418 (Sigma-Aldrich, St. Louis, MO, USA). The resistant clones were harvested, and the over-expression of the gene and protein was confirmed by RT-PCR and western blot analysis, respectively.

Immunohistochemistry

Animals were transcardially perfused with 4 % paraformaldehyde (PFA) in 0.1 M sodium phosphate buffer (pH = 7.4). Brains were removed, post fixed in 4 % PFA (at least for 4 h) and cryoprotected with graded sucrose (10 %, 20 %, and 30 %; each for approximately 10 h) at 4 °C, and then stored at -80 °C. Brain samples were cut into serial coronal 12 - 14 μm thick sections with 50-60 μm intervals using a cryostat (Leica CM1850; Leica, Nussloch, Germany), and then mounted onto glass slides. Sections were rehydrated in PBS containing 0.01% Tween20 (TPBS) for 10 minutes, permeabilized with 0.3 % triton X-100 in PBS for 30 min, washed three times in TPBS over 15 min and then blocked in 5 % skim milk in TPBS as blocking solution for 1 h at room temperature. When using the antibody of mouse origin, we used M.O.M Kit (Vector

Laboratories, Burlingame, CA, USA) for masking the endogenous Fc receptors. Sections were incubated in combinations of the following primary antibodies for 16 h at 4 °C: rabbit anti-FABP7 (0.5 μg/ml) [30], rabbit anti-NG2, guinea pig anti-NG2 and rabbit anti-PDGFR-α (1:1000, 1:50 and 1:1000 respectively, kindly provided by W. Stallcup, Burnham Institute, www.burnham.org), rat anti-GFAP (1:200, Invitrogen), mouse anti-NeuN (1:100, Millipore, Billerica, MA, USA), mouse anti-MAP2 (1:500, Sigma-Aldrich), rat anti-CD11b (1:50, Chemicon, Temecula, CA, USA), mouse anti-BrdU (1:100, BD pharmingens, San Diego, CA, USA) (See Table 2.1).

Table 2.1 List of primary antibodies used in this study

Antibody	Species	Dilution	Reference or vendor	Target cells
anti-FABP7	rabbit	0.5μg/ml	Owada,et al. 2006	
anti-GFAP	rat	1:200	Invitrogen	astrocytes
anti-NG2	guinea pig	1:50	W. Stallcup, Burnham institute*	oligodendrocyte progenitor cells(OPCs)
anti-NG2	rabbit	1:1000	W. Stallcup, Burnham institute*	oligodendrocyte progenitor cells(OPCs)
anti-PDGFRα	rabbit	1:1000	W. Stallcup, Burnham institute *	oligodendrocyte progenitor cells(OPCs)
anti-NeuN	mouse	1:100	Millipore	Neurons
anti-MAP2	mouse	1:500	Sigma Aldrich	Neurons
anti-CD11b	rat	1:100	Chemicon	Microglia
anti-BrdU	mouse	1:100	BD Pharmingens	Proliferating cells (S phase)

*http://www.burnham.org

After primary antibody incubation, sections were washed three times in TPBS for 30 min, and incubated with combinations of the following secondary antibodies for 30 min at room temperature: anti-rabbit IgG-Alexa488, anti-rabbit IgG-Alexa 633, anti-mouse IgG-Alexa568, anti-rat IgG-Alexa 568, and anti-guinea pig IgG-Alexa 555 (1:1000 for all; Invitrogen Co.). 4', 6-diamidino-2-phenylindole (DAPI) (0.5 μg/ml, Invitrogen Co.) was added as a nuclear marker. When required, Zenon rabbit IgG labeling reagents (Molecular probes, Invitrogen Co.) labeled with Alexa Fluor 594 and Alexa Fluor 488 were used for double-staining of rabbit primary antibodies. Slides were cover-slipped using Fluoromount (DBS, CA, USA). For negative controls of the antibodies used in this study, we omitted the primary antibody reaction in the immunohistochemical procedure, and confirmed the absence of the reaction under such condition. Furthermore, for FABP7 antibody, the authenticity was confirmed by the complete absence of the reaction in FABP7-KO mouse tissues/cells.

BrdU uptake assay

For proliferation assay after stab injury, single intraperitoneal injection (100 mg/kg) of Brdu (Sigma-Aldrich) was performed at 3 days after lesion (DPL3), and animals were sacrificed after 2 h (n=4 for wild-type, n=4 for FABP7-KO mice) or 4 days after injection (DPL7: n=4 for wild-type, n=4 for FABP7-KO mice) [31]. For cultured astrocytes, BrdU (10 μM) was added to culture medium at 3 days after seeding when the astrocytes reached a subconfluent state, and was incubated for 5 h. $BrdU^+$ cells were visualized immunocytochemically using an anti-BrdU antibody as described previously with slight modifications [31-32]. Briefly, cells were incubated with 2 M HCl at room temperature for 30 min followed by washing twice with 0.1 M sodium borate (pH = 8.5) over 10 min. After blocking with 5 % goat serum at room temperature for 30 min, they were incubated with mouse anti-BrdU antibody (1:100) at 4°C overnight, and incubated with anti-mouse IgG-Alexa568 (1:1000) at room temperature for 1 h and then counterstained with DAPI. After several washes with PBS, they were mounted and cover slipped. Brain sections, were pretreated by microwave heating in citrate buffer (PH=6) at 96 °C for 10 minutes followed by incubation in 2 N HCl at 37 °C for 11 min and stained as described above.

Morphometric analysis

Sections were observed by a confocal laser scanning microscope (LSM510 META; Carl Zeiss, Oberkochen, Germany) and images were obtained in a 0.1 mm^2 area (x 40 lens with 0.7 zoom, 317 x 317 μm), approximately 0.1 mm either lateral or medial to the edge of the lesion in the gray matter of the cortex (mostly layers III and IV, where astrocyte population includes only protoplasmic astrocytes) [33]. Corresponding areas in the contralateral cortex were also imaged. For BrdU assessment, counts were performed in 0.4 mm^2 areas (x 20 lens with 0.7 zoom, 635 x 635 μm) from the edge of the lesion in both the lateral and medial sides (Fig. 2.2).

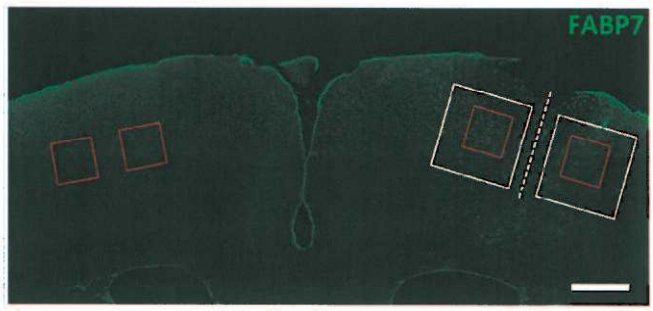

Fig 2.2 Description of imaging area
Identification of FABP7^{+} cells was performed in 0.1 mm^2 areas (red rectangles), 100μm medial or lateral to the edge of the stab injury and corresponding areas in the intact cortex. Proliferation assay was performed in 0.4mm^2 areas (white rectangle) very close to the medial or lateral edges of the stab injury.

Western Blot analysis

Mice were sacrificed 3 days after stab injury. The ipsilateral cortex containing the stab injury and the corresponding contralateral cortex were dissected (2 x 2 x 2 mm blocks). Whole tissue lysate was prepared in 2 x SDS-PAGE sample buffer containing protease inhibitors (Roche Diagnostics, Mannheim, Germany). The lysates were electrophoresed on a 15 % SDS-polyacrylamide gel and immunoblotted onto Immobilon-P^{SQ} PVDF membrane (Millipore). After blocking with skim milk, membranes were incubated with rabbit anti-FABP7 antibody (0.125 μg/ml) [30] and mouse anti-β-actin antibody (1:5000, Santa Cruz Biotech, Santa Cruz, CA, USA) overnight at 4 °C followed by incubation with HRP conjugated goat anti-rabbit antibody (1:1000, Chemicon) and goat anti-mouse antibody (1:1000, Chemicon). Detection of reactive bands was performed using the ECL kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

[^{3}H] thymidine incorporation assay

Primary cultured astrocytes were incubated in 24-well plates at the subconfluent status. The cells were then incubated in fresh medium containing 10 % fetal calf serum and [^{3}H]thymidine (1 μCi per well; Amersham, Piscataway, NJ, USA) for 24 h at 37 °C. After incubation, the cells were washed twice with 500 μl of ice-cold PBS (0.1 M; pH 7.4), detached with trypsin-EDTA solution, and collected on GF/C filters (Whatman, Clifton, NJ, USA). The filters were washed three times with 1 ml of 10 % trichloroacetic acid and rinsed twice with 1 ml of absolute

ethanol. The radioactivity contained on each filter was determined using a scintillation counter (LSC-5100; Aloca, Tokyo, Japan).

Fatty acid incorporation assay

Primary cultured astrocytes were incubated in 12-well plates. A 0.1 μCi/ml of [^{14}C] linoleic acid or [^{14}C]α-linolenic acid (Amersham Pharmacia Biotech) was added to confluent cultured astrocytes. After incubation for 30-120 min, the cells were washed thoroughly with cold PBS and lysed with 0.1 M NaOH. Radioactivity was measured using a β-scintillation counter. Radioactivity was normalized to the DNA content of the sample.

Cell titer assay

The cell titer was quantified using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI, USA) according to the manufacturer's manual. Briefly, after seeding the cells onto 96-well plates at the density of 1 x 10^4 cells/well, the coloring solution was added to the culture media at each time point (0, 1, 2, 3, 4 and 5 days after seeding). Following incubation for 2 h, chromogenic development was measured at 490 nm by spectrophotometer (Beckman Coulter, Fullerton, CA,USA). The experiment was done in quadruplicate and the mean value of optical density in each time point was calculated.

Statistical analysis

All data are shown as mean ± SD. Two-tailed unpaired Student's t-test was performed using Microsoft Excel. P values less than 0.05 were considered statistically significant.

RESULTS

Localization of FABP7 in normal cortex

In the normal (intact) cortex, FABP7$^+$ cells exhibiting several cellular processes were evenly scattered throughout the cortex. In these cells, FABP7 immunopositive staining was observed in the nuclei and cytoplasm (Fig. 2.3). The majority (62.7 ± 6.3%, n=3) of the FABP7$^+$ cells were

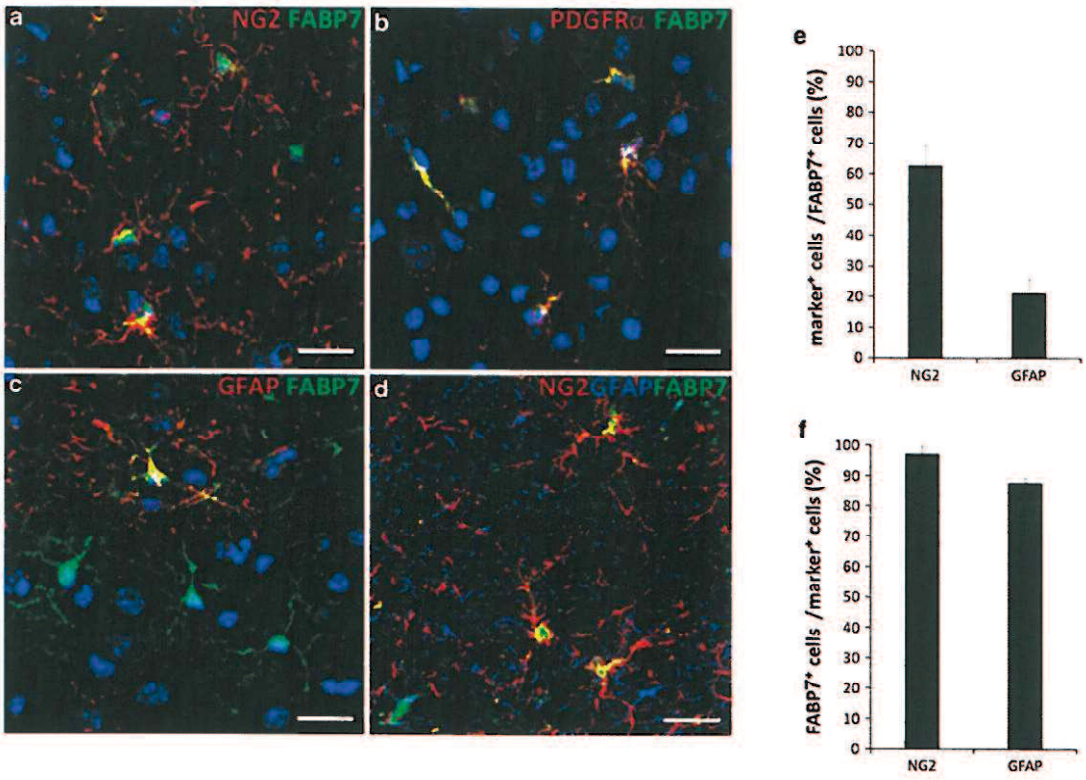

Fig. 2.3 Identification of FABP7 expressing cells in the normal adult cerebral cortex.
a-d) Immunofluorescence micrographs showing expression of FABP7 in OPCs and astrocytes. **a)** Expression of FABP7 (green) in NG2$^+$ OPCs(red). **b)** Expression of FABP7 in OPCs confirmed by colocalization of FABP7 (green) and PDGFRα (red). **c)** Expression of FABP7 (green) in GFAP$^+$ protoplasmic astrocyte. **d)** Localization of FABP7 (green) in NG2$^+$ OPCs (red) and GFAP$^+$ astrocytes (blue). Note that the majority of FABP7$^+$ cells are OPC. **e)** Bar graph showing the percentage of NG2$^+$ OPCs and GFAP$^+$ astrocytes among total FABP7$^+$ cells. **f)** Bar graph showing the percentage of FABP7$^+$ cells in NG2$^+$OPCs and GFAP$^+$ astrocytes. Data in **e, f** are obtained from 0.1 mm^2 area. Scale bars= 20 μm.

positive for NG2 proteoglycan, a marker for oligodendrocyte progenitor cells (termed polydendrocytes) [34] (Fig. 2.3 a, f).

Confirmation of FABP7$^+$ cells as OPCs was further performed by staining with PDGFRα, a specific marker of OPCs [34-37] (Fig. 2.3 b). Furthermore, 97.1 ± 2.5% of NG2$^+$ OPCs in the intact cortex were FABP7$^+$ (Fig. 2.3 f). By contrast, 21.6 ± 3.7% of FABP7$^+$ cells in the intact cortex were GFAP$^+$ astrocytes (Fig. 2.3 c) and 82.4±10.6% of GFAP$^+$ astrocytes were FABP7$^+$. In the upper layers near the dorsal surface of the cortex where GFAP$^+$ cells exhibit a higher population density, thicker and less branched processes and more intensive expression of GFAP (also

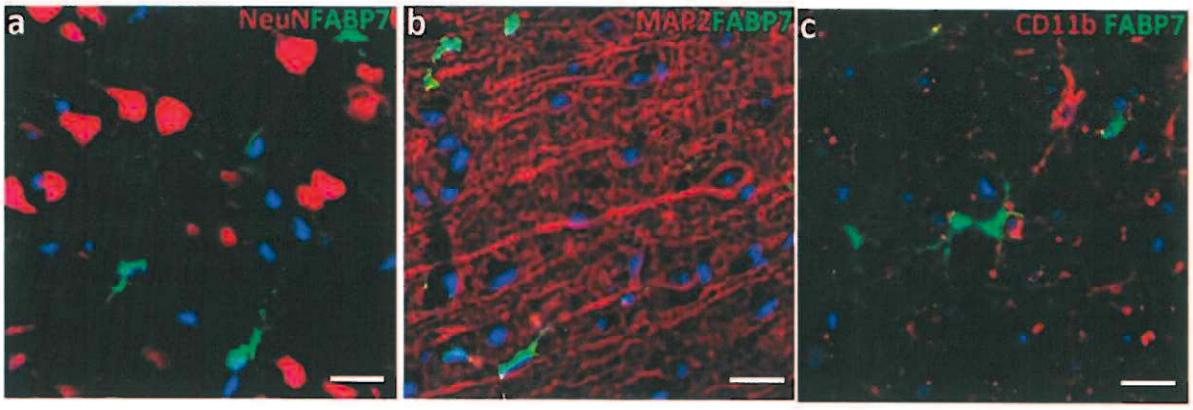

Fig. 2.4 Identification of FABP7$^-$ cells in the intact adult cortex.
a-d) Immunofluorescence micrograph showing that FABP7 is not expressed on neurons, microglia, or a subpopulation of oligodendrocyte lineage cells. **a)** FABP7 (green) is not expressed on NeuN$^+$ neurons (red). **b)** FABP7 (green) is not expressed on MAP2$^+$ neurons (red). **c)** FABP7 (green) is not expressed on CD11b$^+$ microglia (red). Scale bars = 20 μm.

known as interlaminar astrocytes, [33]), GFAP$^+$ astrocytes comprised near half of all FABP7$^+$ cells, with the remainder being NG2$^+$ OPCs (data not shown).

FABP7 was not colocalized with markers for neurons including NeuN (Fig. 2.4 a) and MAP2 (Fig. 2.4 b), or with makers for microglia including CD11b (Fig. 2.4 c).

Taken together these data suggest that mainly OPCs and astrocytes comprise almost the entire population of FABP7 expressing cells in the intact adult cortex. To our knowledge, this is the first time FABP7 has been shown on OPCs.

Localization of FABP7 in the stab injured cortex

To evaluate changes in expression of FABP7 after cortical stab injury, the stab injured cortices at DPL3 and the contralateral intact cortices were dissected for western blotting. FABP7 protein expression was significantly increased in the injured cortex compared with the contralateral side (Fig. 2.5 a). When assessed by immunohistochemistry, the population density of FABP7$^+$ cells significantly increased (approximately 35%) compared with the intact cortex (Fig. 2.5 b-d; 28.8 ± 1.9 cells at DPL3, 29.1 ± 1.3 cells at DPL7 *vs.* 19.1 ± 1.4 cells in the intact cortex: $p < 0.05$ and $p < 0.01$, respectively) in a 0.1 mm^2 area (0.1 mm lateral or medial to the edge of injury).

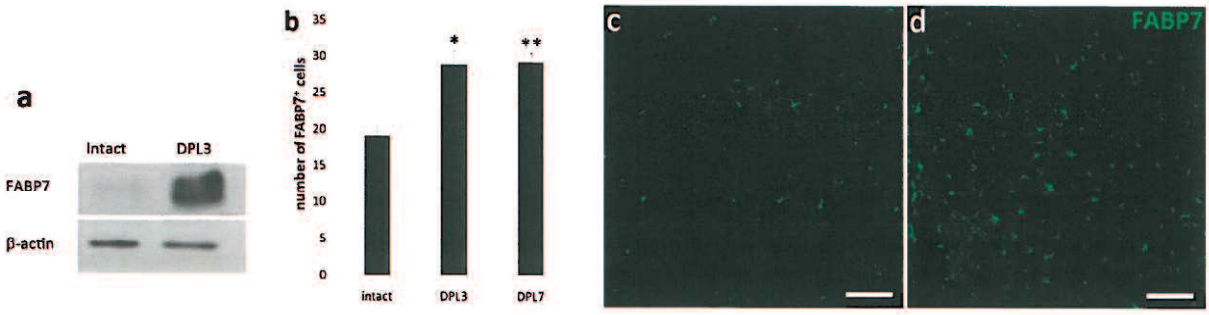

Fig. 2.5 Increased expression of FABP7 after stab injury

a) Western blotting showing increased expression of FABP7 in stab-injured cortex compared with the intact cortex. **b)** Bar graph showing increased population density of FABP7$^+$ cells in injured cortex compared to intact cortex. **c, d)** FABP7$^+$ cells in intact (**c**) and injured cortex (**d**). Note the hypertrophy and increased population of FABP7$^+$ cells in the stab-injured cortex compared with intact cortex. **e)** Image showing the location of stab injury in the cerebral cortex. Scale bars = 100 μm (**c,d**). Data in **b** are obtained from 0.1 mm^2 area; DPL: days post lesion; *p<0.05, **p<0.01, ***p<0.001.

In contrast to the intact cortex, the majority of FABP7$^+$ cells (80.1 ± 0.2 % and 82.8 ± 5.3 % at DPL3 and DPL7, respectively) in the stab-injured cortex were GFAP$^+$ (Fig. 2.6 a, d, e), while almost all GFAP$^+$ astrocytes (94.5 ± 0.8 % and 97.0 ± 0.01 % at DPL3 and DPL7, respectively) were positive for FABP7 (Fig. 2.6 f). The number of GFAP$^+$/FABP7$^+$ cells significantly increased in number in the injured cortex (Fig. 2.6 g; 23.3 ± 1.7 cells and 24.4 ± 0.1 cells at DPL3 and DPL7 respectively vs. 4.1 ± 0.5 cells in intact cortex in a 0.1 mm^2 area), indicating a role for FABP7 in astrocyte proliferation.

A minor proportion of FABP7$^+$ cells (35.2 ± 5.6 % and 29.4 ± 2.5 % at DPL3 and DPL7, respectively) in the stab-injured cortex co-expressed NG2 (Fig. 2.6 b, d). The expression of FABP7$^+$/NG2$^+$ cells in the injured cortex was similar to that in the intact cortex, and could be distinguished from NG2$^+$ pericytes in the stab-injured cortex as characterized by their specific elongated morphology and their location around the vessels and close to the injury core. Furthermore, these cells co-expressed PDGFRα (Fig. 2.6 c), indicative of OPCs rather than vascular pericytes.

While the total number of NG2$^+$ cells significantly increased (approximately 30 %) in the stab injured cortex compared with the intact cortex (21 ± 1.1 and 19.1 ± 2.1 cells/0.1 mm^2

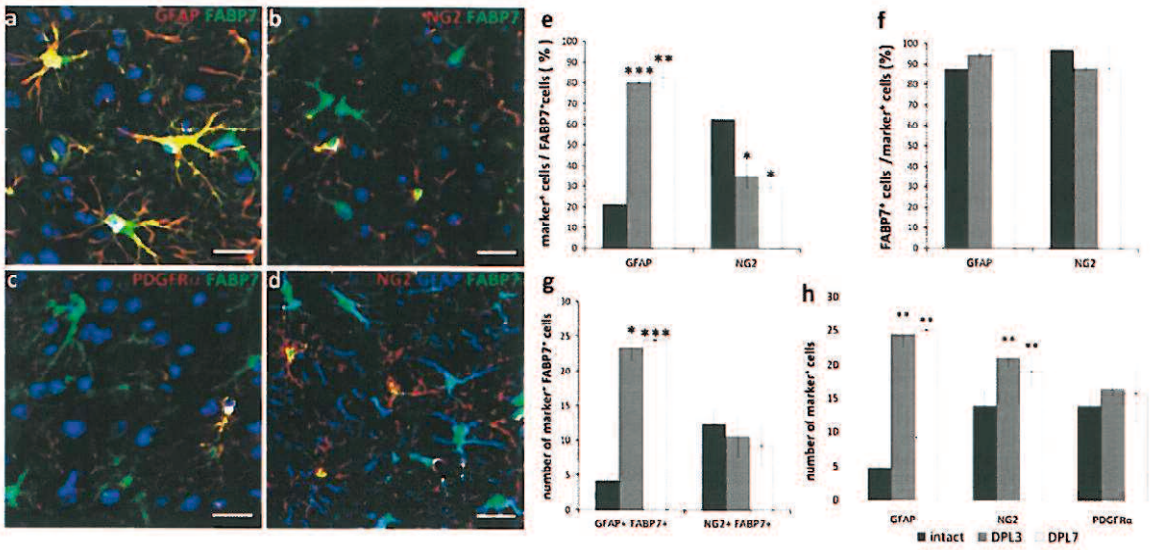

Fig. 2.6 FABP7 expression in stab injured cortex.

a-d) Immunofluorescence micrographs showing expression of FABP7 in OPCs and astrocytes after cortical stab injury. **a)** Intensive expression of FABP7 (green) in hypertrophied GFAP$^+$ reactive astrocytes (red). **b)** Exprerssion of FABP7(green) in NG2$^+$ OPCs (red). **c)** Expression of FABP7 in OPCs after stab injury confirmed by colocalization of FABP7 (green) and PDGFRα (red). **d)** Localization of FABP7 (green) in GFAP$^+$ astrocytes (blue) and NG2$^+$ OPCs (red) after stab injury. Note that the majority of FABP7$^+$ cells in stab-injured cortex are astrocytes. **e)** Quantification of astrocytes and OPCs among FABP7$^+$ cells in intact and injured cortex. **f)** Quantification of FABP7$^+$ cells among astrocytes and OPCs in intact and injured cortex. **g)** Population density of FABP7$^+$/GFAP$^+$ astrocytes and FABP7$^+$/ NG2$^+$ OPCs in intact and injured cortex. **h)** Bar graph showing that the population density of GFAP$^+$ astrocytes is significantly increased after stab injury, while the population density of PDGFRα$^+$ OPCs does not change. The increased population of NG2$^+$ cells may be due to expression of NG2 in pericytes and activated microglia in stab-injured cortex which could not be excluded just by their different morphology. Data in **e-h** are obtained from 0.1 mm^2 area; DPL: days post lesion; *p<0.05, **p<0.01, ***p<0.001. Scale bars= 20 μm.

at DPL3 and DPL7, respectively *vs.* 14 ± 1.9 cells/0.1 mm^2 in the intact cortex; Fig. 2.6. h), the total number of NG2$^+$/FABP7$^+$ cells did not change (10.5 ± 2.8 and 9.2 ± 2.7 cells/0.1 mm^2 at DPL3 and DPL7, respectively vs. 12.3 ± 2.2 cells/0.1 mm^2 in the intact cortex; Fig. 2.6 g). Furthermore, the total number of PDGFRα$^+$ cells did not significantly differ between the intact and injured cortex (16.4 ± 0.8 and 15.9 ± 4.4 cells/0.1 mm^2 at DPL3 and DPL7, respectively *vs.* 14 ± 1.1 cells/0.1 mm^2 in the intact cortex; Fig. 2.6 h).

Similar to the intact cortex, in the stab-injured cortex, FABP7 was not seen in neurons positive for MAP2 or NeuN, or in microglia and/or monocyte derived cells positive for F4/80 or CD11b

(data not shown).Based on these results, localization of FABP7$^+$ in mainly NG2$^+$ OPCs in the intact cortex is changed into mainly GFAP$^+$ astrocytes in the stab-injured cortex, due to a profound increase in population density of GFAP$^+$ astrocytes. Taken together, these results suggest an association between FABP7 and proliferation of reactive astrocytes after stab injury.

Role of FABP7 in proliferation of astrocytes after stab injury

For further evaluation of the role of FABP7 in proliferation of astrocytes, we examined whether FABP7-deficient astrocytes showed an altered proliferation in response to stab injury. We quantified the number of BrdU$^+$ astrocytes and the total population of astrocytes in the cortices of wild-type and FABP7-KO mice after stab injury. BrdU was administered intraperitoneally to wild-type and FABP7-KO mice at 3 days after cortical stab injury, and the number of BrdU$^+$ astrocytes was examined immunohistochemically at 2 h (DPL3; n = 4 for each genotype) and 4 d (DPL7; n = 4 for each genotype) after administration (Fig. 2.7 a). In the contralateral side of DPL3 and DPL7 brain cortices, a few cells (1-2 cells per section) were labeled with BrdU, and these were PDGFRα$^+$/GFAP$^-$ cells, presumably proliferating OPCs (Fig. 2.7 b).

There was no differences in the total number of BrdU$^+$ cells (including blood derived cells and activated microglia, reactive astrocytes, OPCs, pericytes and/or endothelial cells) between the stab-injured cortex of wild-type and FABP7-KO mice at either time points (Fig. 2.8 f). The total number of BrdU$^+$ cells increased approximately 2-fold at DPL7, indicating that some of the cells that were in S-phase at DPL3 proceeded to mitosis or reentered the cell cycle untill DPL7. Moreover, it is possible that some cells may have proliferated several times resulting in BrdU levels below the detectable limit.

At DPL3, there was no difference in the total number of BrdU$^+$/GFAP$^+$ cells between wild-type and FABP7-KO mice (8.6 ± 2 cells/0.4 mm^2 in wild-type *vs.* 6.3 ± 3 cells/0.4 mm^2 in FABP7-KO) (Fig. 2.7 c, d, i), while at DPL7 there was a significant difference between two groups (29.3 ± 5.7 cells/0.4 mm^2 in wild-type vs. 18.3 ± 0.5 cells/0.4 mm^2 in FABP7-KO, $p < 0.05$) (Fig. 2.7 e, f, i). Consistent with these data, the total number of GFAP$^+$ astrocytes was significantly decreased in FABP7-KO mice at DPL7 (125.7 ± 7.6 cells/0.4 mm^2 in wild-type *vs.* 106.7 ± 5.5 cells/0.4 mm^2 in FABP7-KO, $p < 0.05$,) (Fig. 2.7 g, Fig. 2.8 a-d). The proportion of BrdU$^+$ astrocytes among the total astrocyte population also significantly decreased at DPL7 (25.67 ±

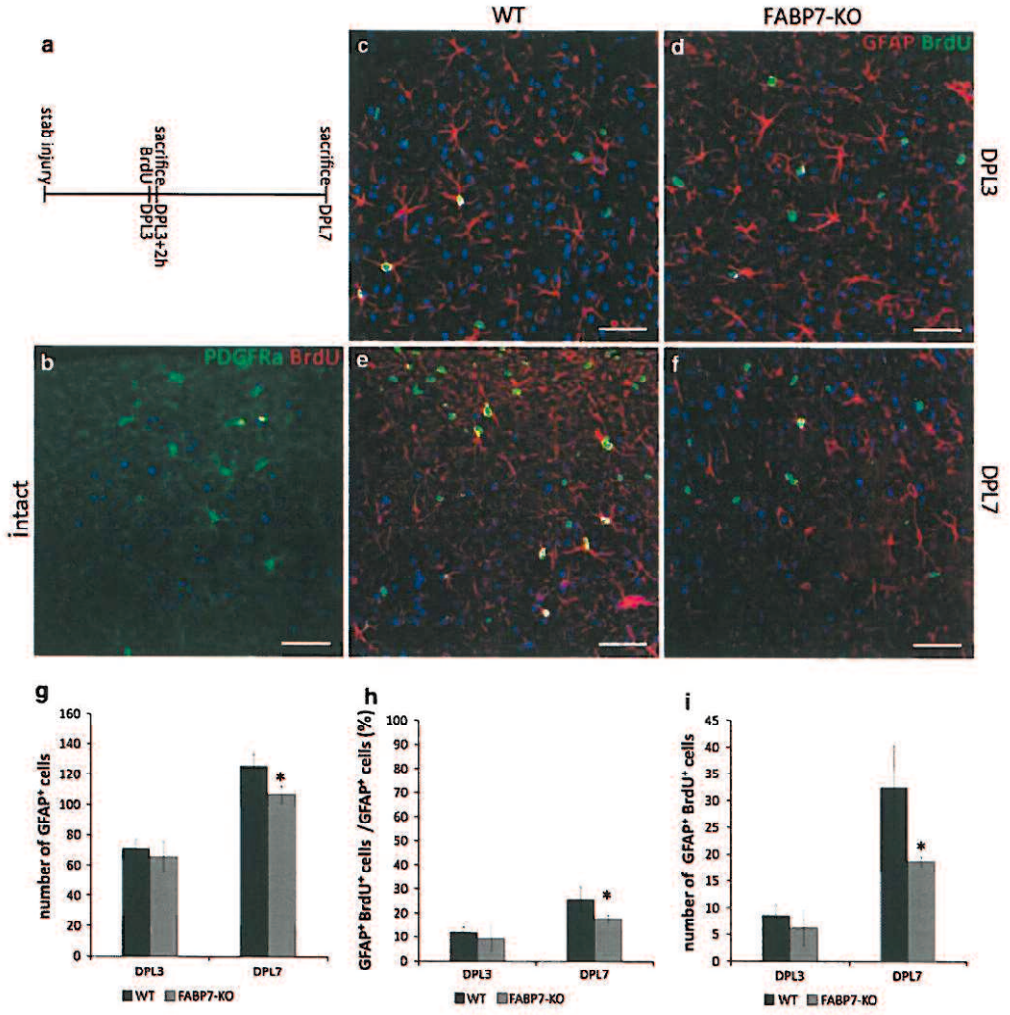

Fig. 2.7 Decreased proliferation of reactive astrocytes after cortical stab injury in FABP7-KO mice. **a)** BrdU administration paradigm: single injection of BrdU was performed at 3 days after stab injury, and mice were killed 2 hrs or 4 days later. **b)** Immunofluorescence micrograph showing that few $BrdU^+$ (red) cells were detected in the intact cortex which were $PDGFR\alpha^+$ (green) OPCs. **c-f)** Representative immunofluorescence micrographs showing $BrdU^+$ (green) cells and $GFAP^+$ (red) astrocytes in stab-injured cortex in wild-type and FABP7-KO mice at 3 and 7 days after stab injury. Note the lower number of $BrdU^+/GFAP^+$ astrocytes in FABP7-KO mice compared with wild-type mice, especially in DPL7. **g)** Bar graph showing the population density of $GFAP^+$ astrocytes in the stab-injured cortex of wild type and FABP7-KO mice. **h)** Bar graph showing the proportion of $BrdU^+/GFAP^+$ astrocytes among total $GFAP^+$ astrocytes in the stab-injured cortex of wild-type and FABP7-KO mice. **i)** Bar graph quantifying the population density of $BrdU^+/GFAP^+$ astrocytes in stab-injured cortex of wild-type and FABP7-KO mice. Data in **g, h, i** are obtained from 0.4 mm^2 area. DPL: days post lesion. *$p<0.05$. Scale bars = 50 μm.

5.1% in wild-type *vs.* 17.55 ± 1.5% in FABP7-KO, $p < 0.05$) (Fig. 2.7 h). These data show that astrocyte proliferation is decreased in FABP7-KO after stab injury compared with wild-type, suggesting a role of FABP7 in proliferation of astrocytes.

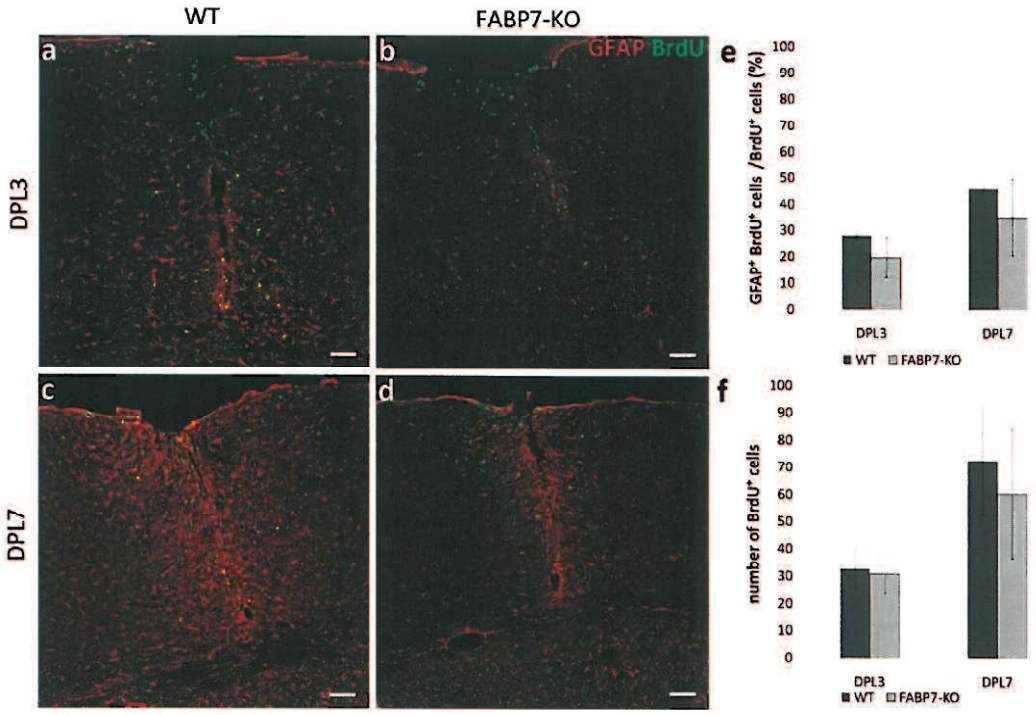

Fig. 2.8 Decreased proliferation and population of astrocytes in stab-injured cortex of FABP7-KO mice compared with wild-type.

a-d) Representative low magnification immunofluorescence micrographs showing population density of $GFAP^+$ (red) and $BrdU^+$ (green) cells in the cerebral cortex of wild-type and FABP7-KO mice at 3 and 7 days after stab injury. Note the lower population of $GFAP^+$ and $GFAP^+/BrdU^+$ cells in the FABP7-KO compared with wild-type mice, especially in DPL7. **e)** Bar graph showing the proportion of $BrdU^+/GFAP^+$ astrocytes among total $BrdU^+$ cells in the stab-injured cortex of wild-type and FABP7-KO mice. **f)** Bar graph showing the population density of $BrdU^+$ astrocytes in the stab-injured cortex of wild-type and FABP7-KO mice. Data in **e, f** are obtained from 0.4 mm^2 area. DPL: days post lesion. Scale bars = 100 μm.

Role of FABP7 in astrocyte proliferation *in vitro*

FABP7 was abundantly expressed in primary cultured astrocytes at gene and protein levels (Fig. 2.9 a, b), and in immunocytochemistry FABP7 was predominantly localized to the cell nuclei of cultured astrocytes (Fig. 2.9 c).

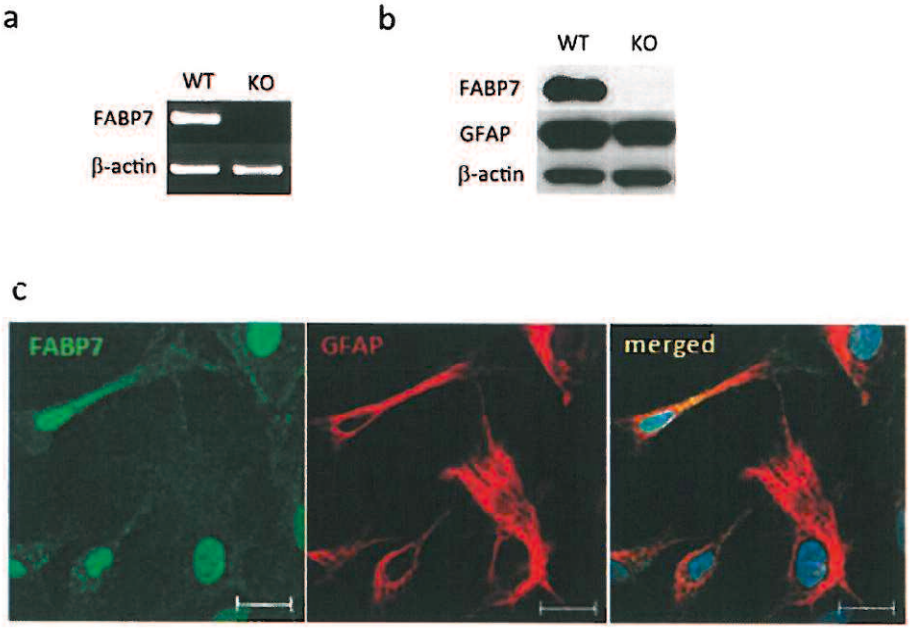

Fig. 2.9 Expression of FABP7 in the mouse primary cultured astrocytes.
a) RT-PCR showing the gene expression of FABP7 in primary cultured astrocytes. **b)** Western blotting showing the protein expression of FABP7 in primary cultured astrocytes. In a) and b), expression of β-actin was shown as an internal control. In b), expression of GFAP was shown as a reference. **c)** Immunofluorescence micrographs showing the localization of FABP7 in primary cultured astrocytes. FABP7 (green) was predominantly localized to the nuclei of $GFAP^+$ astrocytes (red). Cell nuclei were stained with DAPI (blue). Scale bars = 20 μm.

To study the role of FABP7 in astrocyte proliferation, we examined BrdU and ^{3}H-thymidine incorporation in subconfluent primary cultured astrocytes from wild-type and FABP7-KO mice. The BrdU labeling index (proportion of $BrdU^+$ astrocytes among all astrocytes) was significantly decreased (by approximately 30%, $p<0.05$) in FABP7-KO astrocytes compared with wild-type (Fig. 2.10 c, d, e). Similarly, the uptake of ^{3}H-thymidine in FABP7-KO astrocytes was significantly decreased (by approximately 20%, $p<0.05$) compared to wild-type (Fig. 2.10 f). Delayed confluency was consistently observed in FABP7-KO astrocytes compared with wild-type (Fig. 2.10 a, b). These data further confirm the role of FABP7 in astrocyte proliferation.

Next, we transfected FABP7 into NG108 cells, neuroblastoma and glioma hybrid cells, which show no endogenous FABP7 expression (Fig. 2.11 a). There was a significant increase in the proliferation of FABP7 transfected cells compared with mock transfected cells (Fig. 2.11 b).

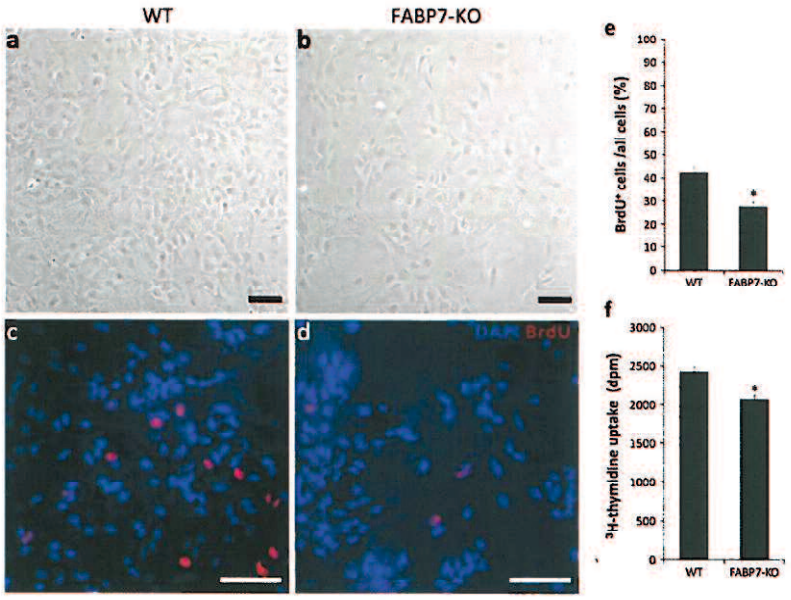

Fig. 2.10 Decreased proliferation in primary cultured FABP7-KO astrocytes.
a, b) Representative phase contrast images of primary astrocyte cultures from wild-type and FABP7-KO mice at 6 days after passage. Note that while wild-type astrocytes reached confluent status, FABP7-KO astrocytes were not confluent. **c, d)** Representative immunofluorescence micrographs showing BrdU uptake in primary astrocyte culture from wild-type and FABP7-KO mice. Note to lower $BrdU^+$ (red) nuclei among total $DAPI^+$ (blue) nuclei. **e)** Bar graph showing decreased $BrdU^+$ cells at 3 days after passage in FABP7-KO cultured astrocytes compared with wild-type. **f)** Bar graph showing decreased H-thymidine uptake in FABP7-KO cultured astrocytes compared to wild-type astrocytes. *$p<0.05$; Scale bars = 100 μm.

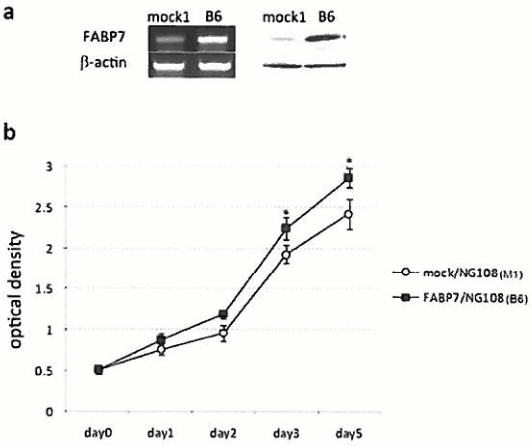

Fig. 2.11 Role of FABP7 in controlling cell proliferation.
a,b) Increased proliferation of NG108 cells after overexpression of FABP7. **e)** Western blotting and PCR showing the increased expression of FABP7 in NG108 cells transfected with FABP7 gene (clone B6) compared with control (mock transfected cells). **f)** Numbers of FABP7-overexpressing cells (B6) increased significantly faster than mock-transfected cells (mock/NG108) in cell titer assay. $p<0.01$.

We also evaluated the uptake of α-linolenic acid ($_{18}C_3$, n-3) and linoleic acid ($_{18}C_2$, n-6) in FABP7-KO astrocytes. There was a decrease in incorporation of α-linolenic acid ($_{18}C_3$, n-3) in FABP7-KO astrocytes ($p<0.05$; Fig. 2.12 a), while no difference was observed in that of linoleic acid ($_{18}C_2$, n-6) (Fig. 2.12 b). Taken together these data suggest that FABP7 is involved in the proliferation of astrocytes by controlling fatty acid homeostasis (metabolism/signal transduction).

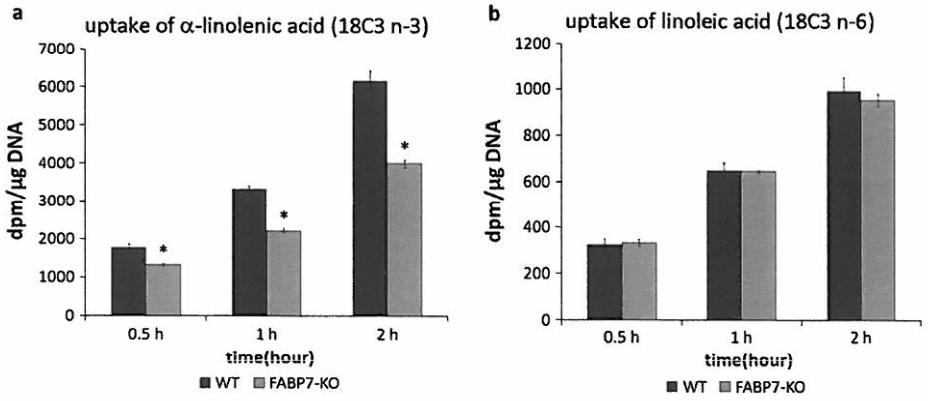

Fig. 2.12 Decreased n-3 fatty acid incorporation in primary cultured FABP7-KO astrocytes.
a, b) Bar graphs showing incorporation of n-3 (**a**) and n-6 (**b**) fatty acids in wild-type and FABP7-KO cultured astrocytes. *$p<0.05$.

DISCUSSION

FABP7 is abundantly expressed in neural stem cells and radial glia of the developing brain, and has been associated with a number of brain diseases including psychotic disorders [16-17] or brain tumors [24-27]. However, the underlying mechanisms of the actions of FABP7 are poorly understood, particularly in the adult brain under normal and pathologic conditions. Herein, we identified FABP7 expressing cells in the adult mouse cortex as both oligodendrocyte progenitor cells and astrocytes, and found that FABP7 expression was markedly increased in the astrocytes after cortical injury. Furthermore, we examined the phenotype of FABP7-KO mice after cortical stab injury to study the role of FABP7 in gliosis, and found that FABP7 played a critical role in regulating astrocytes proliferation *in vivo*.

Decreased proliferation of reactive astrocytes in FABP7-KO mice is one of the main findings in this study. We previously reported that FABP7 was substantially expressed by $GFAP^+$ astrocytes in the cortex of early postnatal developing brain [16-17], and that the number of $GFAP^+$ cells (neural stem cells and early progenitors) and total BrdU uptake were decreased in the hippocampal dentate gyrus of the FABP7-KO mice at postnatal 4 weeks [38]. Our present finding suggests that in addition to regulating the proliferation of neural stem / progenitor cells in early developmental stages, FABP7 is also involved in reactive astrogliosis as a major component of regenerative program in the adult brain. Although the proliferation of astrocytes in reactive gliosis and normal developing brain may partly differ in mechanism, they may have some common features, as reactive astrocytes undergo partial dedifferentiation and upregulation of neural stem cell markers such as nestin and vimentin [39] and restore the ability of self renewal and multipotency[18-20]. However, no significant difference in the astrocyte population was detected in the normal adult cortex between wild type and FABP7-KO mice in this study (not shown). One possible explanation is that the cellular demand of fatty acids was developmentally compensated in the FABP7-KO brain by the redundant molecule such as FABP5, another FABP expressed weakly in the astrocytes, while the dramatic increase in the fatty acid requirement for astrocyte proliferation during reactive gliosis, possibly for rapid remodeling of membrane lipid, was not fully covered. Another possibility is that the mechanism by which FABP7 controls astrocyte biology under pathological condition (gliosis process) is

partly distinct from that in the normal brain development. However, this study demonstrated that the primary cultured FABP7-KO astrocytes showed the decrease in proliferation and the late confluency, suggesting that FABP7-KO mice may reach to the normal population of astrocytes in the *in vivo* cortex later than wild type mice. This hypothesis should be further examined through careful quantification of cortical astrocytes in different developmental stages in FABP7-KO mice.

Primary cultured astrocytes from FABP7-KO mice showed a decrease in proliferation and a decrease in the cellular uptake of α-linolenic acid ($_{18}C_3$, n-3), while there was no decrease in that of linoleic acid ($_{18}C_2$, n-6). These data are consistent with the previous finding that FABP7 binds to n-3 PUFAs with high affinity [40], suggesting that FABP7 is involved in proliferation of astrocytes by controlling fatty acid homeostasis (metabolism/signal transduction). Although the role of n-3 PUFA in the astrocyte proliferation remains unknown, Tian et al. (2011) recently reported that dietary supplementation of the female mice during pregnancy and lactation period with n-3 PUFA results in increased numbers of $GFAP^+$ astrocytes and increased expression of PPARγ in the developing brain [41] . Champeil et al. (2004) reported an increased proliferation of primary cultured astrocytes supplemented with DHA by assessing DNA concentration. They also found an increased n-3 PUFAs and DHA composition of membrane phospholipids in astrocytes after supplementation with DHA [42]. Although the molecular mechanism by which FABP7 regulates astrocyte proliferation remains to be studied, it has been recently shown that transfection of FABP7 in $FABP7^-$ U87 cells (malignant glioma cell line) causes increased expression of growth factors such as FGF2 [43], which is known to promote astrocyte proliferation [44-46]. The same group also reported the morphological difference between $FABP7^+$ and $FABP7^-$ U87 cells [47], in contrast that we did not notice any difference in the morphology of cortical astrocytes in the adult brain [28] and in the primary cultured astrocytes between wild type and FABP7-KO mice. Although the immortalized glioma cells and astrocytes are distinct in their biological characteristics, it is crucial and interesting to measure the expression levels of growth factors in the FABP7-KO astrocytes, and further to examine whether decreased proliferation of reactive astrocytes in FABP7-KO mice has any impact on neuronal outcome including neuronal survival and/or regeneration after the brain damage.

We found that the major population of FABP7$^+$ cells in the adult cortex was OPCs. OPCs are distributed throughout the grey and white matter and are the most prevalent cycling progenitors in the adult CNS [48-50]. OPCs are functionally and antigenically distinct from neurons, mature oligodendrocytes, astrocytes and microglia, and as such, are regarded as the fourth major glial population [34]. Several studies have reported that OPCs generate oligodendrocytes in the developing and mature CNS [34, 50] and that OPCs respond by proliferating, migrating, and differentiating into myelinating oligodendrocytes following demyelination [51-56]. This novel finding opens a new field of study regarding expression and biological roles of FABPs in oligodendrocyte lineage which will be described in the next chapter.

In contrast to several previous studies that reported increased number of OPCs after stab injury [29, 50, 57-61], we did not detect any significant changes in OPC numbers between normal and stab-injured cortex. This discrepancy can be partly explained by different sizes of the observation area and its distance from the edge of the injury as well as the different markers used for OPC labeling. Previous reports used mainly NG2 for identification of OPCs, which is also expressed by pericytes and activated microglia, in the injured cortex. In this study, quantification of OPC in the stab-injured cortex was confirmed by using PDGFRα positivities as the most specific marker for OPCs, combined with co-expression of NG2 and FABP7. Interestingly, Hompton et al. (2004) showed that the total number of PDGFRα$^+$ cells remained constant after injury, while the number of NG2$^+$ cells increased [58]. These results were, however, explained by proposing a NG2$^+$/PDGFRα$^-$ subpopulation of OPCs, which is not consistent with the current understanding of the antigenic characteristics of OPCs.

REFERENCES

1. Swank, R.L., et al., *Multiple sclerosis in rural Norway its geographic and occupational incidence in relation to nutrition.* N Engl J Med, 1952. **246**(19): p. 722-8.
2. Esparza, M.L., S. Sasaki, and H. Kesteloot, *Nutrition, latitude, and multiple sclerosis mortality: an ecologic study.* Am J Epidemiol, 1995. **142**(7): p. 733-7.
3. Lauer, K., *The risk of multiple sclerosis in the U.S.A. in relation to sociogeographic features: a factor-analytic study.* J Clin Epidemiol, 1994. **47**(1): p. 43-8.
4. de Lau, L.M., et al., *Dietary fatty acids and the risk of Parkinson disease: the Rotterdam study.* Neurology, 2005. **64**(12): p. 2040-5.

5. Barberger-Gateau, P., et al., *Fish, meat, and risk of dementia: cohort study.* BMJ, 2002. **325**(7370): p. 932-3.
6. Morris, M.C., et al., *Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease.* Arch Neurol, 2003. **60**(7): p. 940-6.
7. Fedorova, I., et al., *Deficit in prepulse inhibition in mice caused by dietary n-3 fatty acid deficiency.* Behav Neurosci, 2009. **123**(6): p. 1218-25.
8. Amminger, G.P., et al., *Long-chain omega-3 fatty acids for indicated prevention of psychotic disorders: a randomized, placebo-controlled trial.* Arch Gen Psychiatry, 2010. **67**(2): p. 146-54.
9. Muskiet, F.A. and R.F. Kemperman, *Folate and long-chain polyunsaturated fatty acids in psychiatric disease.* J Nutr Biochem, 2006. **17**(11): p. 717-27.
10. Hedelin, M., et al., *Dietary intake of fish, omega-3, omega-6 polyunsaturated fatty acids and vitamin D and the prevalence of psychotic-like symptoms in a cohort of 33,000 women from the general population.* BMC Psychiatry, 2010. **10**: p. 38.
11. Peet, M., et al., *Two double-blind placebo-controlled pilot studies of eicosapentaenoic acid in the treatment of schizophrenia.* Schizophr Res, 2001. **49**(3): p. 243-51.
12. Nordvik, I., et al., *Effect of dietary advice and n-3 supplementation in newly diagnosed MS patients.* Acta Neurol Scand, 2000. **102**(3): p. 143-9.
13. Weinstock-Guttman, B., et al., *Low fat dietary intervention with omega-3 fatty acid supplementation in multiple sclerosis patients.* Prostaglandins Leukot Essent Fatty Acids, 2005. **73**(5): p. 397-404.
14. Ross, B.M., J. Seguin, and L.E. Sieswerda, *Omega-3 fatty acids as treatments for mental illness: which disorder and which fatty acid?* Lipids Health Dis, 2007. **6**: p. 21.
15. Mehta, L.R., R.H. Dworkin, and S.R. Schwid, *Polyunsaturated fatty acids and their potential therapeutic role in multiple sclerosis.* Nat Clin Pract Neurol, 2009. **5**(2): p. 82-92.
16. Owada, Y., T. Yoshimoto, and H. Kondo, *Spatio-temporally differential expression of genes for three members of fatty acid binding proteins in developing and mature rat brains.* J Chem Neuroanat, 1996. **12**(2): p. 113-22.
17. Maekawa, M., Y. Owada, and T. Yoshikawa, *Role of polyunsaturated fatty acids and fatty acid binding protein in the pathogenesis of schizophrenia.* Curr Pharm Des, 2011. **17**(2): p. 168-75.
18. Buffo, A., et al., *Origin and progeny of reactive gliosis: A source of multipotent cells in the injured brain.* Proc Natl Acad Sci U S A, 2008. **105**(9): p. 3581-6.
19. Shimada, I.S., et al., *Self-renewal and differentiation of reactive astrocyte-derived neural stem/progenitor cells isolated from the cortical peri-infarct area after stroke.* J Neurosci, 2012. **32**(23): p. 7926-40.
20. Sirko, S., et al., *Reactive glia in the injured brain acquire stem cell properties in response to sonic hedgehog. [corrected].* Cell Stem Cell, 2013. **12**(4): p. 426-39.
21. Baumgart, E.V., et al., *Stab wound injury of the zebrafish telencephalon: a model for comparative analysis of reactive gliosis.* Glia, 2012. **60**(3): p. 343-57.
22. Arai, Y., et al., *Role of Fabp7, a downstream gene of Pax6, in the maintenance of neuroepithelial cells during early embryonic development of the rat cortex.* J Neurosci, 2005. **25**(42): p. 9752-61.
23. Belayev, L., et al., *Docosahexaenoic Acid Therapy of Experimental Ischemic Stroke.* Transl Stroke Res, 2011. **2**(1): p. 33-41.
24. Godbout, R., et al., *Correlation of B-FABP and GFAP expression in malignant glioma.* Oncogene, 1998. **16**(15): p. 1955-62.
25. Tso, C.L., et al., *Primary glioblastomas express mesenchymal stem-like properties.* Mol Cancer Res, 2006. **4**(9): p. 607-19.
26. Liang, Y., et al., *Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme.* Proc Natl Acad Sci U S A, 2005. **102**(16): p. 5814-9.

27. Kaloshi, G., et al., *FABP7 expression in glioblastomas: relation to prognosis, invasion and EGFR status.* J Neurooncol, 2007. **84**(3): p. 245-8.
28. Owada, Y., et al., *Altered emotional behavioral responses in mice lacking brain-type fatty acid-binding protein gene.* Eur J Neurosci, 2006. **24**(1): p. 175-87.
29. Buffo, A., et al., *Expression pattern of the transcription factor Olig2 in response to brain injuries: implications for neuronal repair.* Proc Natl Acad Sci U S A, 2005. **102**(50): p. 18183-8.
30. Abdelwahab, S.A., et al., *Localization of brain-type fatty acid-binding protein in Kupffer cells of mice and its transient decrease in response to lipopolysaccharide.* Histochem Cell Biol, 2003. **119**(6): p. 469-75.
31. White, B.D., et al., *Beta-catenin signaling increases in proliferating NG2+ progenitors and astrocytes during post-traumatic gliogenesis in the adult brain.* Stem Cells, 2010. **28**(2): p. 297-307.
32. Yang, H., et al., *De-differentiation response of cultured astrocytes to injury induced by scratch or conditioned culture medium of scratch-insulted astrocytes.* Cell Mol Neurobiol, 2009. **29**(4): p. 455-73.
33. Nag, S., *Morphology and properties of astrocytes.* Methods Mol Biol, 2011. **686**: p. 69-100.
34. Nishiyama, A., et al., *Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity.* Nat Rev Neurosci, 2009. **10**(1): p. 9-22.
35. Zhu, L., et al., *Induced NG2 expressing microglia in the facial motor nucleus after facial nerve axotomy.* Neuroscience, 2010. **166**(3): p. 842-51.
36. Gao, Q., et al., *NG2, a member of chondroitin sulfate proteoglycans family mediates the inflammatory response of activated microglia.* Neuroscience, 2010. **165**(2): p. 386-94.
37. Ozerdem, U., et al., *NG2 proteoglycan is expressed exclusively by mural cells during vascular morphogenesis.* Dev Dyn, 2001. **222**(2): p. 218-27.
38. Watanabe, A., et al., *Fabp7 maps to a quantitative trait locus for a schizophrenia endophenotype.* PLoS Biol, 2007. **5**(11): p. e297.
39. Sofroniew, M.V., *Molecular dissection of reactive astrogliosis and glial scar formation.* Trends Neurosci, 2009. **32**(12): p. 638-47.
40. Balendiran, G.K., et al., *Crystal structure and thermodynamic analysis of human brain fatty acid-binding protein.* J Biol Chem, 2000. **275**(35): p. 27045-54.
41. Tian, C., et al., *Brain histological changes in young mice submitted to diets with different ratios of n-6/n-3 polyunsaturated fatty acids during maternal pregnancy and lactation.* Clin Nutr, 2011.
42. Champeil-Potokar, G., et al., *Astrocytes in culture require docosahexaenoic acid to restore the n-3/n-6 polyunsaturated fatty acid balance in their membrane phospholipids.* J Neurosci Res, 2004. **75**(1): p. 96-106.
43. Kipp, M., et al., *BLBP-expression in astrocytes during experimental demyelination and in human multiple sclerosis lesions.* Brain Behav Immun, 2011.
44. Stachowiak, M.K., et al., *Growth factor regulation of cell growth and proliferation in the nervous system. A new intracrine nuclear mechanism.* Mol Neurobiol, 1997. **15**(3): p. 257-83.
45. Joy, A., et al., *Nuclear accumulation of FGF-2 is associated with proliferation of human astrocytes and glioma cells.* Oncogene, 1997. **14**(2): p. 171-83.
46. Gomez-Pinilla, F., L. Vu, and C.W. Cotman, *Regulation of astrocyte proliferation by FGF-2 and heparan sulfate in vivo.* J Neurosci, 1995. **15**(3 Pt 1): p. 2021-9.
47. Mita, R., et al., *B-FABP-expressing radial glial cells: the malignant glioma cell of origin?* Neoplasia, 2007. **9**(9): p. 734-44.
48. Dawson, M.R., et al., *NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS.* Mol Cell Neurosci, 2003. **24**(2): p. 476-88.

49. Geha, S., et al., *NG2+/Olig2+ cells are the major cycle-related cell population of the adult human normal brain.* Brain Pathol, 2010. **20**(2): p. 399-411.
50. Simon, C., M. Gotz, and L. Dimou, *Progenitors in the adult cerebral cortex: Cell cycle properties and regulation by physiological stimuli and injury.* Glia, 2011. **59**(6): p. 869-81.
51. Levine, J.M., R. Reynolds, and J.W. Fawcett, *The oligodendrocyte precursor cell in health and disease.* Trends Neurosci, 2001. **24**(1): p. 39-47.
52. Levine, J.M. and R. Reynolds, *Activation and proliferation of endogenous oligodendrocyte precursor cells during ethidium bromide-induced demyelination.* Exp Neurol, 1999. **160**(2): p. 333-47.
53. Islam, M.S., et al., *Olig2-expressing progenitor cells preferentially differentiate into oligodendrocytes in cuprizone-induced demyelinated lesions.* Neurochem Int, 2009. **54**(3-4): p. 192-8.
54. Franklin, R.J., *Why does remyelination fail in multiple sclerosis?* Nat Rev Neurosci, 2002. **3**(9): p. 705-14.
55. Franklin, R.J., C. Zhao, and F.J. Sim, *Ageing and CNS remyelination.* Neuroreport, 2002. **13**(7): p. 923-8.
56. Watanabe, M., Y. Toyama, and A. Nishiyama, *Differentiation of proliferated NG2-positive glial progenitor cells in a remyelinating lesion.* J Neurosci Res, 2002. **69**(6): p. 826-36.
57. Rhodes, K.E., G. Raivich, and J.W. Fawcett, *The injury response of oligodendrocyte precursor cells is induced by platelets, macrophages and inflammation-associated cytokines.* Neuroscience, 2006. **140**(1): p. 87-100.
58. Hampton, D.W., et al., *The responses of oligodendrocyte precursor cells, astrocytes and microglia to a cortical stab injury, in the brain.* Neuroscience, 2004. **127**(4): p. 813-20.
59. Levine, J.M., *Increased expression of the NG2 chondroitin-sulfate proteoglycan after brain injury.* J Neurosci, 1994. **14**(8): p. 4716-30.
60. McTigue, D.M., P. Wei, and B.T. Stokes, *Proliferation of NG2-positive cells and altered oligodendrocyte numbers in the contused rat spinal cord.* J Neurosci, 2001. **21**(10): p. 3392-400.
61. Alonso, G., *NG2 proteoglycan-expressing cells of the adult rat brain: possible involvement in the formation of glial scar astrocytes following stab wound.* Glia, 2005. **49**(3): p. 318-38.

Chapter 3

Differential expression and regulatory roles of FABP5 and FABP7 in oligodendrocyte lineage cells

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SUMMARY

Fatty acid binding proteins (FABPs) are key intracellular molecules involved in the uptake, transportation, and storage of fatty acids and in the mediation of signal transduction and gene transcription. However, little is known regarding their expression and function in the oligodendrocyte lineage. In this study, we evaluated the *in vivo* and *in vitro* expression of FABP5 and FABP7 in the oligodendrocyte lineage cells in the cortex and corpus callosum of adult mice, mixed cortical culture, and oligosphere culture by immunofluorescent counter-staining with major oligodendrocyte lineage markers. In all settings, FABP7 expression was detected in $NG2^+/PDGFR\alpha^+$ oligodendrocyte progenitor cells (OPCs) that did not express FABP5. FABP5 was detected in mature $CC1^+/MBP^+$ oligodendrocytes that did not express FABP7. Analysis of cultured OPCs showed a significant decrease in the population of FABP7-knockout (KO) OPCs and their BrdU uptake compared with wild-type (WT) OPCs. Upon incubation of OPCs in oligodendrocyte differentiation medium, a significantly lower percentage of FABP7-KO OPCs differentiated into $O4^+$ oligodendrocytes. The percentage of mature MBP^+ oligodendrocytes relative to whole $O4^+/MBP^+$ oligodendrocytes was significantly lower in FABP7-KO and FABP5-KO than WT cell populations. The percentage of terminally mature oligodendrocytes with membrane sheet morphology was significantly lower in FABP5-KO compared with WT cell populations. Our data show that FABP7 and FABP5 are differentially expressed in oligodendrocyte lineage cells and regulate their proliferation and/or differentiation. These findings suggest the involvement of FABP7 and FABP5 in the pathophysiology of demyelinating disorders, neuropsychiatric disorders, and glioma, conditions in which OPCs/oligodendrocytes play central roles.

INTRODUCTION

Oligodendrocyte lineage cells have critical roles in normal development and function of the brain and are major players in the pathophysiology of various central nervous system (CNS) diseases, including demyelinating disorders, neuropsychiatric disorders, and glioma. Differentiation of oligodendrocyte progenitor cells (OPCs) into mature myelinating oligodendrocytes is a complex and still poorly understood process which may be regulated by several intrinsic factors as well as interactions with other neural cell types [1]. Furthermore, following demyelination, OPCs respond by proliferating, migrating, and differentiating into myelinating oligodendrocytes. Failure of this response, also known as "spontaneous remyelination", is critical in the pathophysiology of demyelinating disorders such as multiple sclerosis [2-3]. Identifying the factors which regulate myelination and spontaneous remyelination together with the OPC transplantation are major strategies toward developing treatments for demyelinating disorders such as leukodystrophies and multiple sclerosis. In addition to demyelinating disorders, the significance of oligodendrocyte biology is increasing in the pathophysiology of schizophrenia [4-5] and glioma [6-7].

Fatty acid binding proteins (FABPs) are intracellular chaperones that mediate the uptake, transportation, storage, and signal transduction of long-chain fatty acids. Among the 12 identified members of the FABP family which show high tissue/cell type specificity and different ligand affinity, FABP3, FABP5, and FABP7 are expressed in the brain [8-9]. While FABP3 expression is restricted to neurons, FABP7 and FABP5 are known to be abundantly expressed in neural stem cells and radial glia of the developing brain, suggesting their roles in the proliferation and differentiation of glial cells [8]. FABP7 is well known to be involved in the pathophysiology of schizophrenia [10-11] and glioma [12]. Studies in rats have shown that *in vitro* and *in vivo* supplementation with n-3 poly unsaturated fatty acids (PUFAs) enhances oligodendrocyte differentiation and expression of myelin proteins [13-14]. Despite the increasing body of evidence regarding the significance of lipid homeostasis in oligodendrocyte lineage, little is known about expression and importance of FABPs in this lineage.

During our studies on role of FABPs in pathophysiology of CNS diseases, we for the first time detected the expression of FABP7 in OPCs [15], which prompted us for a comprehensive

study on FABPs expression and significance in oligodendrocyte lineage. In this study, we examined the expression of FABPs in the oligodendrocyte lineage in the cerebral cortex and corpus callosum of adult mice and in primary cultured oligodendrocyte lineage cells in mixed cortical culture as well as oligosphere culture by immunofluorescent counter-staining with different markers of oligodendrocyte lineage. Furthermore, we explored the roles of FABPs in the oligodendrocyte lineage by evaluating the phenotype of cultured oligodendrocyte lineage cells from FABP5-KO and FABP7-KO mice.

MATERIALS AND METHODS

Animals

Twelve-week-old male C57BL/6 mice (FABP7-KO, FABP5-KO, and their wild-type [WT] littermates [10, 16]) were used for immunohistochemical analysis. Embryonic day 14.5 (E14.5) and postnatal day 2-4 (P2-P4) mice from each genotype were used for cell culture. All experimental protocols were reviewed by the Ethics Committee for Animal Experimentation of the Yamaguchi University School of Medicine and were performed according to the Guidelines for Animal Experimentation of the Yamaguchi University School of Medicine and under the law and notification requirements of the Japanese Government.

Immunostaining

For immunohistochemical analysis, the mice were transcardially perfused with 4% paraformaldehyde (PFA) in 0.1 M sodium phosphate buffer (pH=7.4). Brains were removed, postfixed in 4 % PFA (for at least 4 h) and cryoprotected using graded sucrose (10 %, 20 %, and 30 %; each for approximately 10 h) at 4°C, and then stored at –80°C. Brain samples were cut into serial coronal 12-14 μm thick sections (Bregma -1.5~-2.0 mm) at 50-60 μm intervals by using a cryostat (Leica CM1850; Leica, Nussloch, Germany), and then mounted onto glass slides. The sections were rehydrated in Tween containing phosphate-buffered saline (TPBS) for 10 min, permeabilized with 0.3% Triton X-100 in PBS for 30 min, washed 3 times in TPBS over 15 min, and blocked in 5% skim milk in TPBS for 1 h at room temperature. The sections were incubated

with combinations of the following primary antibodies for 16 h at 4°C: rabbit anti-FABP7 (0.5 μg/mL) [10], rabbit anti-FABP5 (0.5 μg/mL) [10], rabbit anti-NG2, guinea pig anti-NG2, and rabbit anti-PDGFR-α (1:1000, 1:50, and 1:1000, respectively; kindly provided by W. Stallcup, Burnham Institute, www.burnham.org), rat anti-PDGFR-α (1:500, eBioscience), mouse anti-olig2 (1:500, Millipore) and mouse anti-CC1 (1:50, Calbiochem) (Table 3.1). For identification of oligodendrocytes, CC1 immunoreaction localized in the cell body with round or oval morphology was considered [17].

Table 3.1 List of primary antibodies used in this study

Antibody	Species	Dilution	Reference or vendor	Target cells
anti-FABP7	rabbit	0.5μg/ml	Owada et al., 2006	
anti-FABP5	rabbit	0.5μg/ml	Owada et al., 2006	
anti-NG2	guinea pig	1:50	W. Stallcup, Burnham institute*	oligodendrocyte progenitor cells(OPCs)
anti-NG2	rabbit	1:1000	W. Stallcup, Burnham institute*	oligodendrocyte progenitor cells(OPCs)
anti-PDGFRα	rabbit	1:1000	W. Stallcup, Burnham institute *	oligodendrocyte progenitor cells(OPCs)
anti-PDGFRα	rat	1:500	eBioscience	oligodendrocyte progenitor cells(OPCs)
anti-Olig2	mouse	1:500	Millipore	whole oligodendrocyte lineage cells
anti-O4	mouse	1:200	Millipore	immature and mature oligodendrocytes
anti-CC1	mouse	1:50	Calbiochem	mature oligodenrocytes
anti-MBP	rat	1:200	abcam	mature oligodenrocytes
anti-BrdU	mouse	1:100	BD Pharmingens	proliferating cells (S phase)
anti-Sox2	Rabbit	1:100	Millipore	Neural stem/progenitor cells
anti-caspase3 (ASP175)	Rabbit	1:400	Cell signalling	Apoptotic cells

*http://www.burnham.org

For immunocytochemical analysis, cultured cells were fixed with 4 % PFA for 20 min at room temperature and washed 3 times with cold D-PBS. For BrdU staining, cells were incubated with 2 M HCl for 30 min at room temperature followed by twice washing with 0.1 M sodium borate (pH = 8.5) over 10 min. After blocking with 5 % goat serum in PBS for 20 min, the cells were incubated with combinations of the primary antibodies (Table 3.1) overnight at 4 °C.

Following incubation with primary antibodies, sections or cells were washed three times in TPBS or D-PBS, respectively, for 30 min and incubated with suitable combinations of the following secondary antibodies for 30 min at room temperature: anti-rabbit IgG-Alexa 488, anti-rabbit IgG-Alexa 568, anti-rabbit IgG-Alexa 633, anti-mouse IgG-Alexa 568, anti-mouse IgG-Alexa 488, anti-mouse IgM-Alexa 568, anti-rat IgG-Alexa 568, anti-rat IgG-Alexa 488, and anti-guinea pig IgG-Alexa 555 (1:1000 for all; Invitrogen). 4', 6-diamidino-2-phenylindole (DAPI) (0.5 μg/ml, Invitrogen) was added as a nuclear marker. Slides were cover-slipped using

Fluoromount (DBS). Brains and/or cultured cells from FABP5-KO and FABP7-KO mice were used as negative controls for the FABP5 and FABP7 immunoreaction, respectively. Images were obtained by using a confocal laser scanning microscope (LSM510 META; Carl Zeiss, Oberkochen, Germany). For *in vivo* quantification of FABPs expression among oligodendrocyte lineage cells, images from brain sections (n=3; 3-4 sections per sample; 1 image per section) were taken from the cortex (20x, 0.4 mm^2; 200µm from the midline) and medial corpus callosum (20x, 0.2 mm^2).

Primary culture of oligodendrocyte lineage cells (mixed cortical culture)

Primary OPC culture was performed as described previously [18]. The cerebral cortices of P2–P4 mice (WT, FABP5-KO, and FABP7-KO) were dissected, and the meninges were carefully removed. Tissues were cut into small pieces in SMEM medium (Gibco, 11380), and further dissociated by trypsin treatment (0.2 %) at 37°C for 15 min. Trypsin treatment was stopped by adding DMEM medium (Gibco, 12685) supplemented with 10% fetal bovine serum (FBS). Digested tissue was homogenized by pipetting (10 times). Debris was removed by filtering through a 100 µm mesh, and the cell suspension was centrifuged at 1100 rpm and 20°C for 3 min. The supernatant was removed, and the pellet was resuspended in DMEM supplemented with 10% FBS, penicillin, streptomycin, and L-glutamine, and then seeded at a density of $1 \times 10^5/cm^2$ onto coverslips coated with poly-L-lysine (Sigma, MW > 300,000) in 24-well plates and incubated in a 5 % CO_2

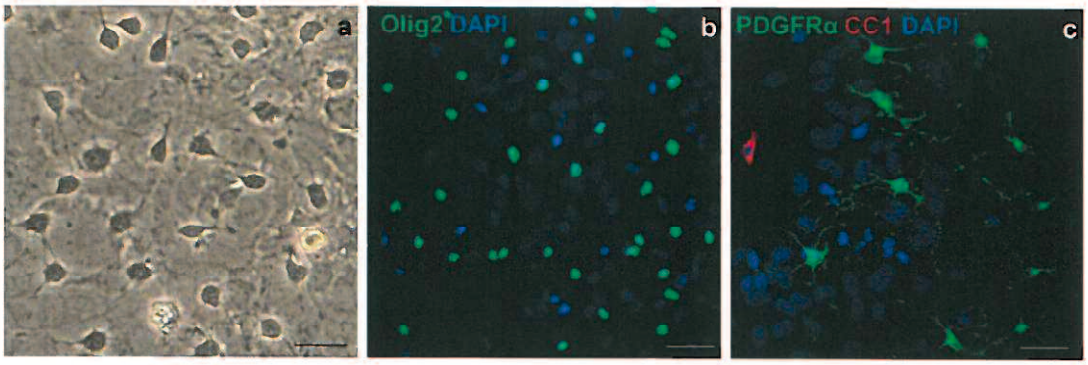

Fig. 3. 1 Oligodendrocyte lineage cells in mixed cortical culture.
Phase contrast (**a**) and immunofluorescent (**b, c**) micrographs showing oligodendrocyte lineage cells in mixed cortical culture. **a**) Oligodendrocyte lineage cells appear as dark process-bearing cells over an underlying monolayer of astrocytes. **b**) Oligodendrocyte lineage cells labeled with olig2. **c**) Most oligodendrocyte lineage cells in this culture are $PDGFR\alpha^+$ OPCs and a minor subpopulation are $CC1^+$ oligodendrocytes. Scale bars= 20 µm.

incubator at 37°C for 7 days. The medium was refreshed every 3–4 days. Mouse OPCs are known to begin differentiating in mixed culture; this enabled us to evaluate the expression of FABPs in oligodendrocyte lineage. The oligodendrocyte lineage cells, characterized by olig2 expression (Fig. 3.1 a, b), in this culture system included mostly PDGFRα$^+$/NG2$^+$ OPCs and a minor subpopulation of CC1$^+$ oligodendrocytes (Fig. 3.1 c).

Primary culture of oligodendrocyte lineage cells (oligosphere culture)

Oligosphere culture was performed as described previously [19]. Neurospheres were developed from embryos at 14.5 days of gestation from timed pregnant females (WT, FABP7-KO, and FABP5-KO). Embryonic brains were microdissected, and the meninges and cerebellum were removed. Cerebral tissue was mechanically triturated using a 1 mL Gilson pipette and passed through a 70-μm cell strainer (BD falcon). Harvested cells from every two brains were plated onto a T25 flask in DMEM-F12 medium (Gibco, 11320) in the presence of epidermal growth factor (EGF; 10 ng/mL, Peprotech AF-100-15) and B27 supplement (1:50, Gibco, 17504). After 2-3 days, neurospheres (Fig. 3. 2 a, b, f) were passaged at a ratio of 1:3 every 3-4 days.

Neurospheres from the 2nd passage were mechanically dissociated into single cells and resuspended in DMEM-F12 supplemented with B27 (1:50, Gibco, 17504), platelet derived growth factor (PDGF AA; 10 ng/mL, Peprotech 100-13 A) and basic fibroblast growth factor (FGF; 10 ng/mL, Peprotech 100-18B). After 3 days, oligospheres were formed, which were passaged at a ratio of 1:2 every 4-7 days. Upon plating of oligospheres, PDGFRα$^+$ NG2$^+$ OPCs began a typical radial migration (Fig. 3.2 c, g). Passage 2 oligospheres were dissociated into single cells using NeuroCult chemical dissociation kit (STEMCELL Technologies), passed through 40 μm cell strainers, counted using a hemocytometer and trypan blue staining and seeded at a density of 3-5X10^4 cells/ml/well on poly-D-lysine-coated coverslips in multi-well plates

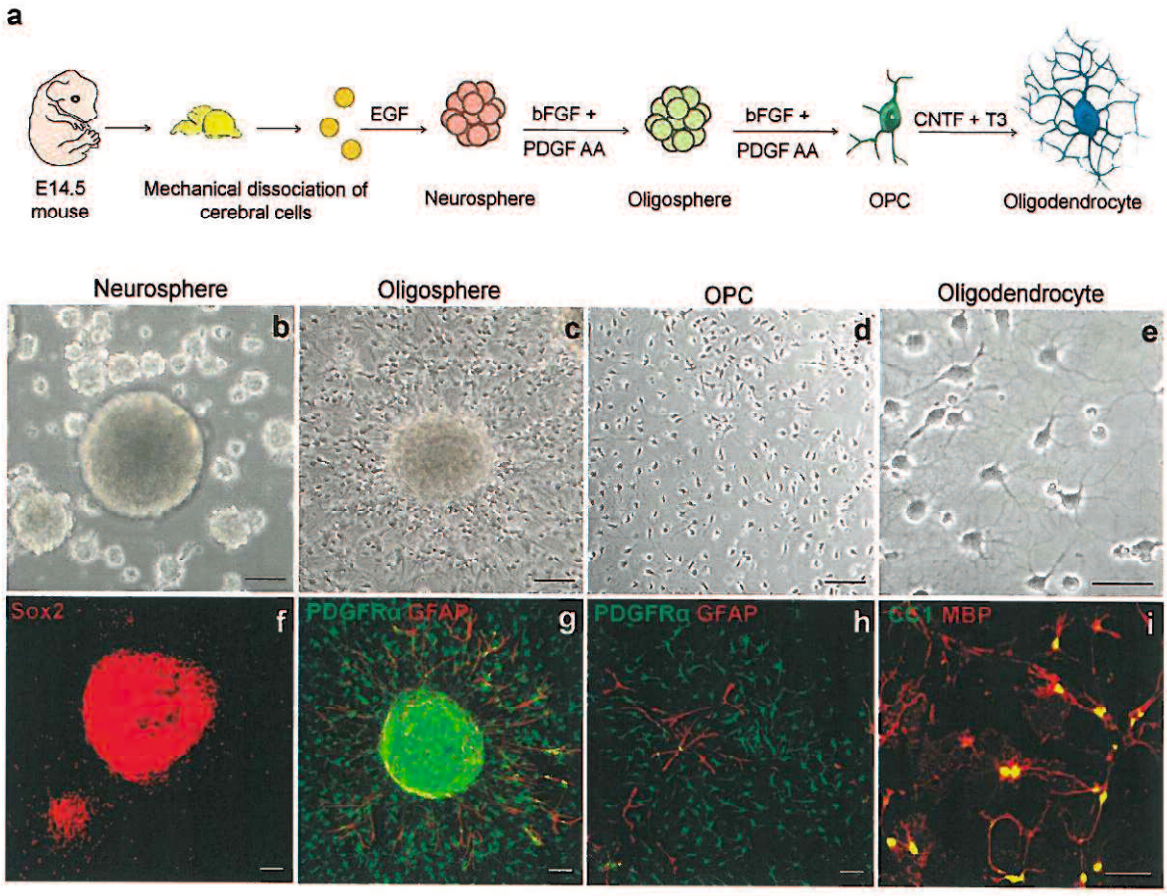

Fig. 3.2 Primary culture of oligodendrocyte lineage cells (oligosphere method).
a) Schematic outline of oligodendrocyte lineage culture from embryonic mouse neural stem cells. **b-e)** Phase contrast images showing floating neurospheres (b), seeded oligosphere, and radially migrating OPCs (c), seeded OPCs upon dissociation of oligospheres (d), and oligodednrocytes (e). **f-i)** Immunofluorescent micrographs showing sox2$^+$ neural stem cells in seeded neurospheres (f), PDGFRα$^+$ OPCs radially migrating from seeded oligospheres and extended processes of GFAP$^+$ astrocytes (g), seeded PDGFRα$^+$ OPCs from dissociated oligospheres and contaminating GFAP$^+$ astrocytes (h), and CC1$^+$ MBP$^+$ mature oligodendrocytes.

containing oligosphere medium and incubated for 3-5 days (Fig. 3. 2 d, h). Alternatively, for dissociation of oligospheres, they were plated in poly-D-lysine-coated flasks and after 3-4 days incubation in prolipheration medium, radialy migrated OPCs were detached by mild trypsination (0.05% trypsin, 1-2min, RT) [20]. About 15% of the cells in the OPC culture were GFAP+ astrocytes (Fig. 3.2 g, h). To produce oligodendrocytes, OPCs were incubated in DMEM-F12 supplemented with B27 (1:50, Gibco, 17504), ciliary neurotrophic factor (CNTF; 10 ng/mL,

Peprotech 450-50) and Triiodothyronine (T3; 40 ng/mL, Sigma T2752) for 5 days. Mature oligodendrocytes were characterized by expression of CC1 and MBP (Fig. 3.2 e, i). The population of contaminating astrocytes further increased to about 25% after incubation in oligodendrocyte differentiation medium (not shown). To examine immature oligodendrocytes, OPCs were incubated in differentiation medium for 2 days when most oligodendrocytes were MBP^{-} $O4^{+}$.

Proliferation assay

For the proliferation assay, WT, FABP5-KO, and FABP7-KO OPCs from passage 2 oligospheres were seeded on coverslips in 24-well plates (5×10^4 cells/well; three coverslips per genotype). Five days after incubation in OPC culture medium, BrdU (10 μM) was added to the OPC culture medium 2 h prior to fixation. OPCs and $BrdU^{+}$ cells were immunocytochemically visualized using antibodies against PDGFRα and BrdU as described above. Cells were observed by using a confocal laser scanning microscope; five random images (0.4 mm^2 ×20) were obtained from each coverslip. The population density of OPCs and the percentage of $BrdU^{+}$ OPCs were quantified.

Differentiation assay

Differentiation assay was performed as described previously [21-22] with some modifications. Briefly, WT, FABP5-KO, and FABP7-KO OPCs from passage 2 oligospheres were seeded onto coverslips in 24-well plates (5×10^4 cells/well; three coverslips per genotype). After incubation of OPCs in the proliferation medium for 5 days, the cells were incubated for a further 5 days in the differentiation medium. Cells were visualized by using antibodies against NG2, O4, and MBP as described above. Cells were observed by using a confocal laser scanning microscope; 16 random images (0.4 mm^2 ×20) were obtained from each coverslip. Early differentiation was evaluated by quantifying the percentage of $O4^{+}$ oligodendrocytes among whole lineage cells (total population of cells positive for either NG2 or O4). To evaluate late differentiation of oligodendrocytes, the ratio of mature MBP^{+} oligodendrocytes to whole oligodendrocytes ($O4^{+}$ cells) was determined. In addition, differentiation and maturation were morphologically

quantified by classifying the cells as having "simple" (short, non-interdigitating processes), "complex" (longer, interdigitating processes), or "membrane" (processes containing membrane sheets) morphology.

Statistical analysis

Data are presented as the mean ± SD. Two-tailed unpaired Student's *t*-test was performed using Microsoft Excel. P values less than 0.05 were considered statistically significant.

RESULTS

Expression of FABP5 and FABP7 in oligodendrocyte lineage in mouse brain

To study the *in vivo* expression of FABPs in the oligodendrocyte lineage, we performed immunofluorescent counterstaining of FABPs with specific markers of this lineage in adult WT mouse brain (N=3, 3-4 sections per sample, 1 image per section). In cortex 26.6±2.5% of oligodendrocyte lineage cells (olig2$^+$ cells) were FABP7$^+$ and 73.6±1.9% of them were FABP5$^+$ (Fig. 3.3 a, b, m). In the corpus callusom which contains a higher density of mature myelinating oligodendrocytes, 9.2±0.9% of olig2$^+$ cells were FABP7$^+$ and 86.8±2.4% of them were FABP5$^+$ (Fig. 3.3 c, d, n). Almost all PDGFRα$^+$ OPCs expressed FABP7 (99.4±0.9% and 100% in cortex and corpus callosum respectively; Fig. 3.3 e, g, m, n) while almost none of them expressed FABP5(0.3% ± 0.6% and 0.3± 0.6% in cortex and corpus callosum respectively; Fig. 3.3 f, h, m, n). In contrast to OPCs, almost all CC1$^+$ oligodendrocytes expressed FABP5 (99±0.9% and 99.27± 0.4% in cortex and corpus callosum respectively; Fig 3.3 j, l, m, n) while few of them expressed FABP7 (2.6±0.9% and 4.13±1.6; Fig. 3.3 i, k, m, n). Expression pattern of FABPs in OPCs was further confirmed by using NG2, another marker for OPCs (Fig. 3.4). These data suggest a reciprocal expression of FABP5 and FABP7 in the oligodendrocyte lineage.

Similar expression patterns were detected in other parts of the brain and in the spinal cord (Fig. 3.5). In addition, we found the expression of FABP5 in GFAP$^+$ astrocytes, while we could not detect its expression in NeuN$^+$ neurons and CD11b$^+$ microglia (Fig. 3.6). Similarly, we have previously reported that FABP7 is expressed in astrocytes and is not expressed in neurons and microglia [15].

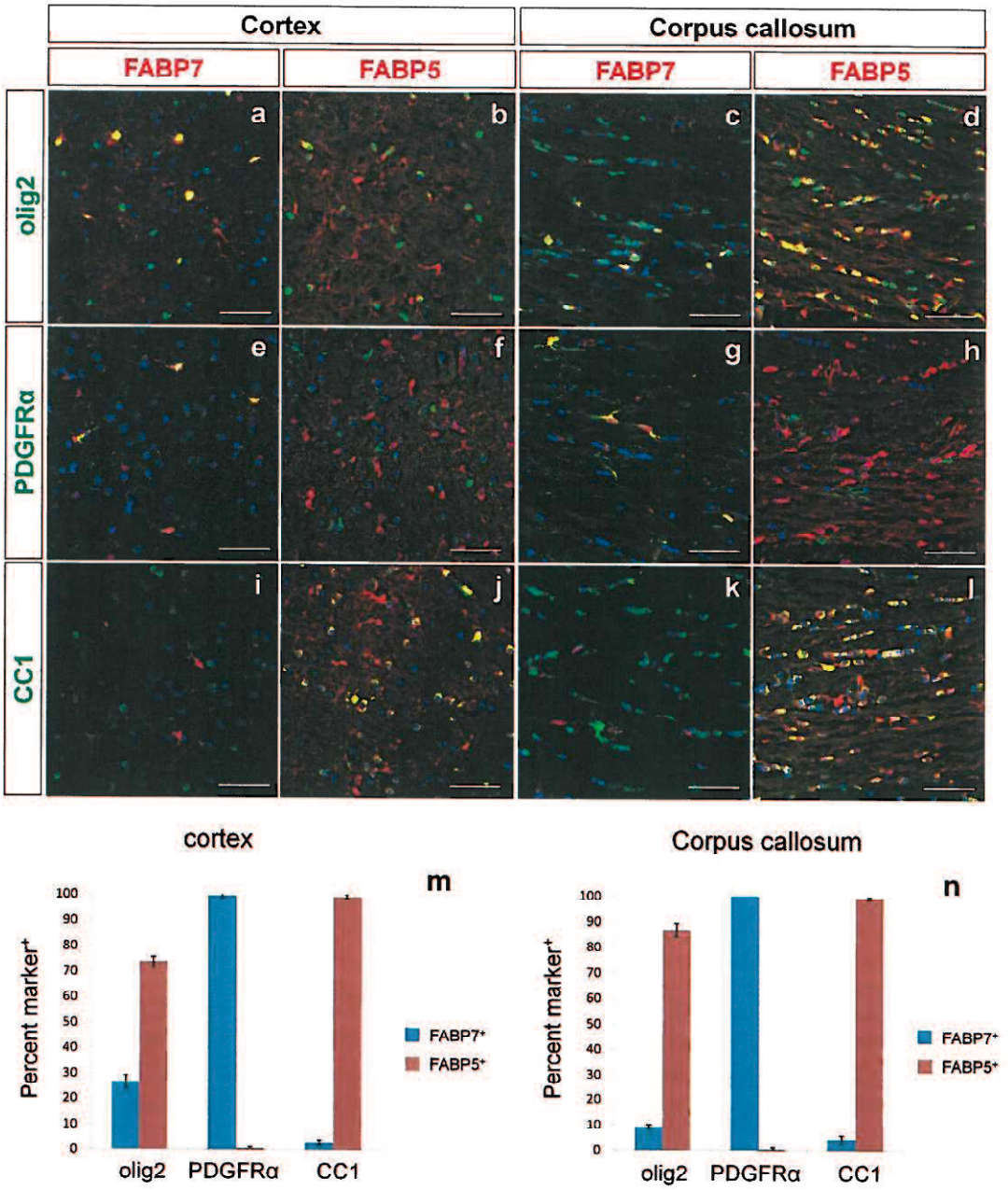

Fig. 3.3 Reciprocal *in vivo* expression of FABP5 and FABP7 in oligodendrocyte lineage of adult mouse brain.

a-l) Immunofluorescence micrographs showing expression of FABP5 and FABP7 in oligodendrocyte lineage in the cortex and corpus callosum. A minor subpopulation of oligodendrocyte lineage cells (olig2$^+$ cells) express FABP7 and a major subpopulation of them express FABP5(a-d). Almost all OPCs (PDGFRα$^+$ cells) express FABP7 and do not express FABP5 (e-h). Almost all CC1$^+$ oligodendrocytes express FABP5 and do not express FABP7 (i- l). **m,n)** Bar graphs showing percentage of FABP5 and FABP7 expression in oligodendrocyte lineage cells in the cortex (m) and corpus callosum (n). Blue: DAPI; Scale bars= 50 µm

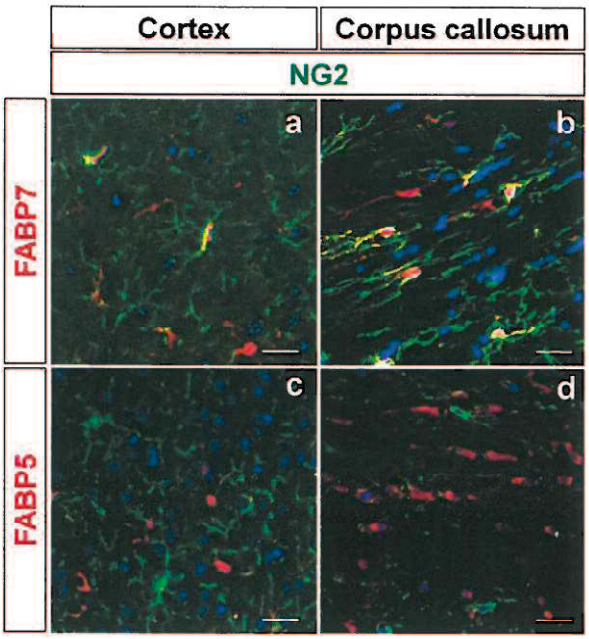

Fig. 3.4 Immunofluorescence micrographs showing expression of FABP5 and FABP7 in NG2$^+$ OPCs in the cortex and corpus callosum. **a,b)** NG2$^+$ OPCs express FABP7. **c,d)** NG2$^+$ cells do not express FABP5. Blue: DAPI; Scale bars= 20 μm

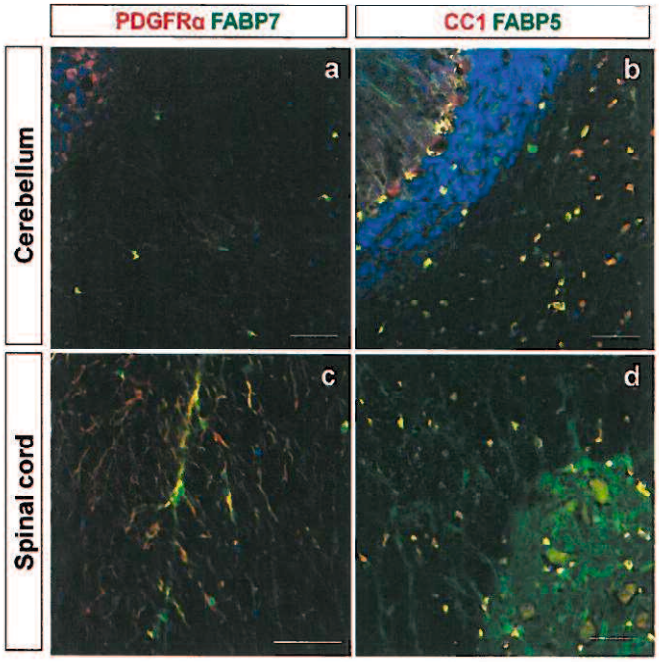

Fig. 3.5 Immunofluorescence micrographs showing expression of FABP7 in OPCs and expression of FABP5 in oligodendrocytes in cerebellum (a,b) and spinal cord (c,d). **a,c)** Expression of FABP7 in PDGFRα$^+$ OPCs in cerebellum (a) and spinal cord (c; anterior median fissure , lumbar level). **b,d)** Expression of FABP5 in CC1+ oligodendrocytes in cerebellum (b) and spinal cord (d; dorsal horn, lumbar level). Blue: DAPI; Scale bars= 50 μm

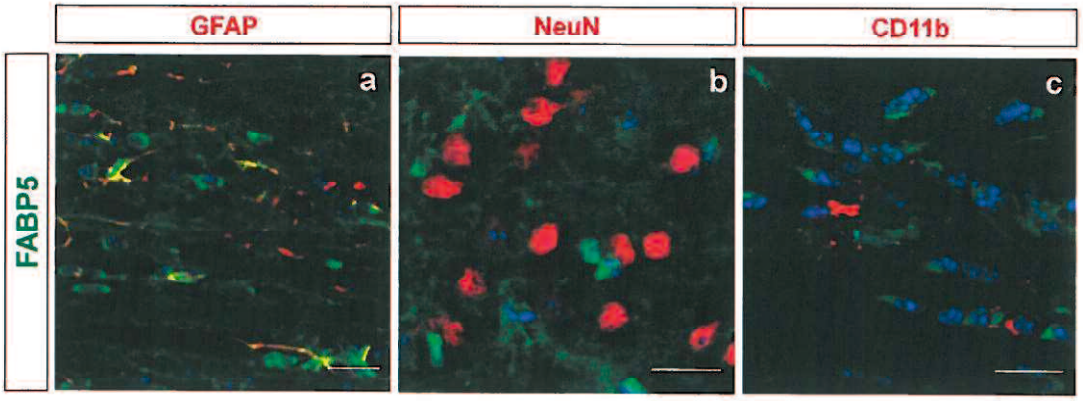

Fig. 3.6 Immunofluorescence micrographs showing expression pattern of FABP5 in astrocytes, neurons and microglia. **a)** FABP5 is expressed in GFAP$^+$ astrocytes (corpus callosum). **b)** FABP5 is not expressed in NeuN$^+$ neurons (cortex). **c** FABP5 is not expressed in CD11b$^+$ microglia (corpus callosum). Blue: DAPI; Sclae bars= 20 μm

Expression of FABP5 and FABP7 in primary cultured oligodendrocyte lineage

To examine the expression of FABPs in the oligodendrocyte lineage *in vitro*, we analyzed primary cultured OPCs and oligodendrocytes in mixed cortical culture. In agreement with our *in vivo* results, in the mouse primary OPC culture, most olig2$^+$ cells (i.e., PDGFRα$^+$ NG2$^+$ OPCs) were FABP7$^+$ while no FABP7 expression was detected in CC1$^+$ oligodendrocytes (Fig. 3.7 a-d). In addition, FABP5 expression was detected in a minor subpopulation of olig2$^+$ cells (i.e., CC1$^+$ mature oligodendrocytes) whereas PDGFRα$^+$ NG2$^+$ OPCs did not express FABP5 (Fig. 3.7 e-h).

To evaluate the roles of FABPs in oligodendrocyte lineage, a tractable culture system was needed. For this, we used the oligosphere method by which a replenishable source of OPCs derived from embryonic neural stem cells could be maintained in proliferation medium or differentiated into oligodendrocytes through the selective use of synthetic growth factors. Prior to functional analysis, the FABPs expression pattern was confirmed using this culture system. Consistent with our previous results, in this culture system, OPCs were FABP7$^+$ FABP5$^-$ (Fig. 3.7 i, j, m, n), whereas mature oligodendrocytes were FABP7$^-$ FABP5$^+$ (Fig. 3.7 k, l, o, p). the reproducibility of these data was confirmed by three independent experiments.

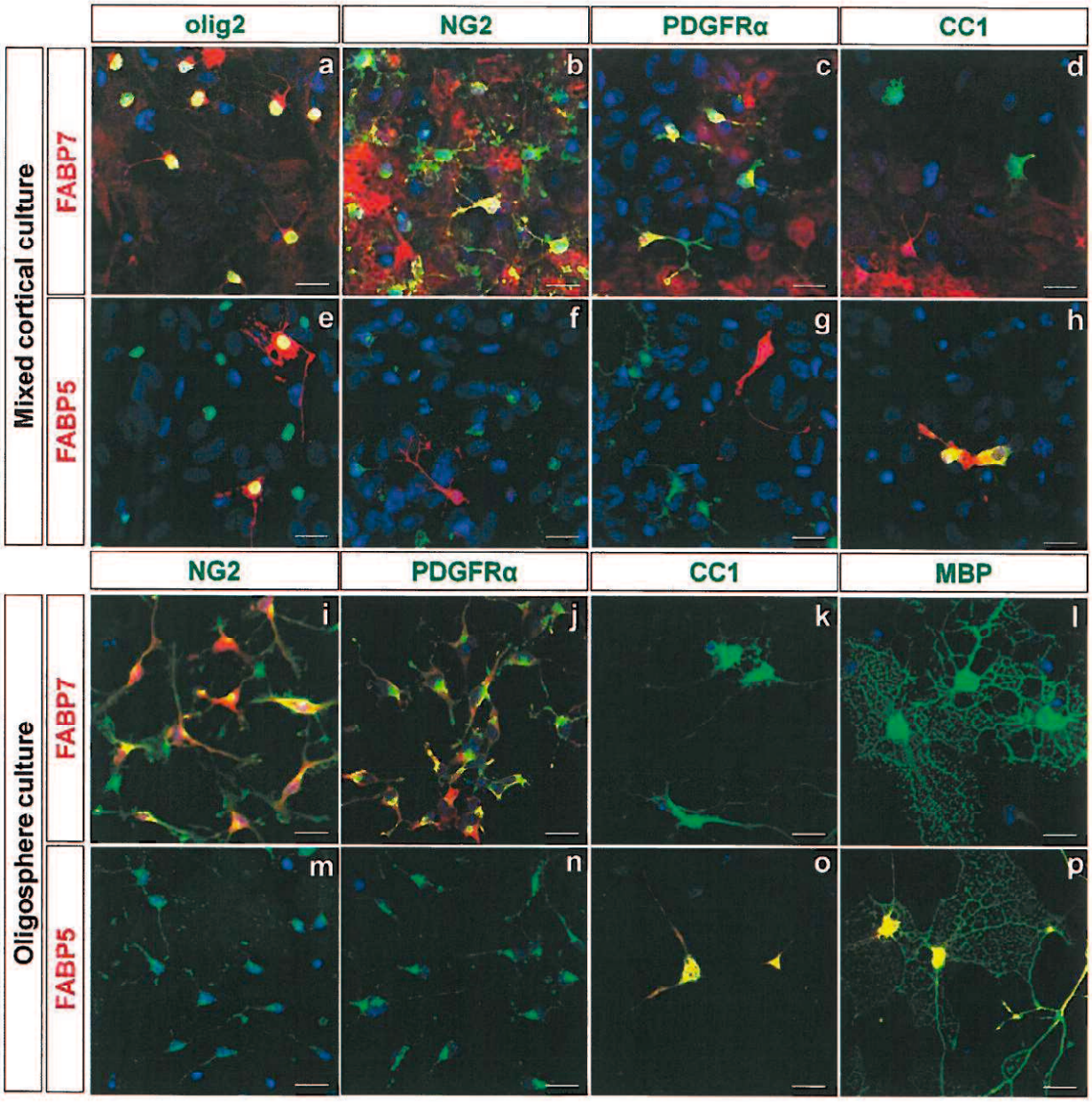

Fig. 3.7 Reciprocal *in vitro* expression of FABP5 and FABP7 in oligodendrocyte lineage.
Immunofluorescence micrographs showing expression of FABP5 and FABP7 in primary cultured oligodendrocyte lineage from mixed cortical culture (a-h) or oligosphere method (i-p). **a, e)** Most oligodendrocyte lineage (olig2$^+$) cells in the mixed cortical culture express FABP7, whereas a minor subpopulation express FABP5. **b, c, i, j)** OPCs characterized by expression of NG2 and PDGFRα, express FABP7. **d, k, l)** FABP7 is not expressed in mature oligodendrocytes labeled by CC1 and MBP. **f, g, m, n)**FABP5 is not expressed in NG2$^+$ PDGFRα$^+$ OPCs. **h, o, p)** FABP5 is expressed in CC1$^+$ MBP$^+$ mature oligodendrocytes. Blue: DAPI; Scale bars= 20 µm

Gradient reciprocal enrichment of FABP5 and FABP7 in oligodendrocyte lineage

To evaluate the expression of FABPs in immature oligodendrocytes, OPCs were incubated in differentiation medium for 2 days untill some of them differentiated into $O4^+$ MBP oligodendrocytes. The intensity of FABPs expression in immature oligodendrocytes was immunocytochemically compared with that of OPCs and mature oligodendrocytes. FABP7 was enriched in OPCs and was gradually downregulated upon differentiation (Fig. 3.8 a-c). In addition, FABP5 was gradually upregulated during differentiation of OPCs into oligodendrocytes (Fig. 3.8 d-f).

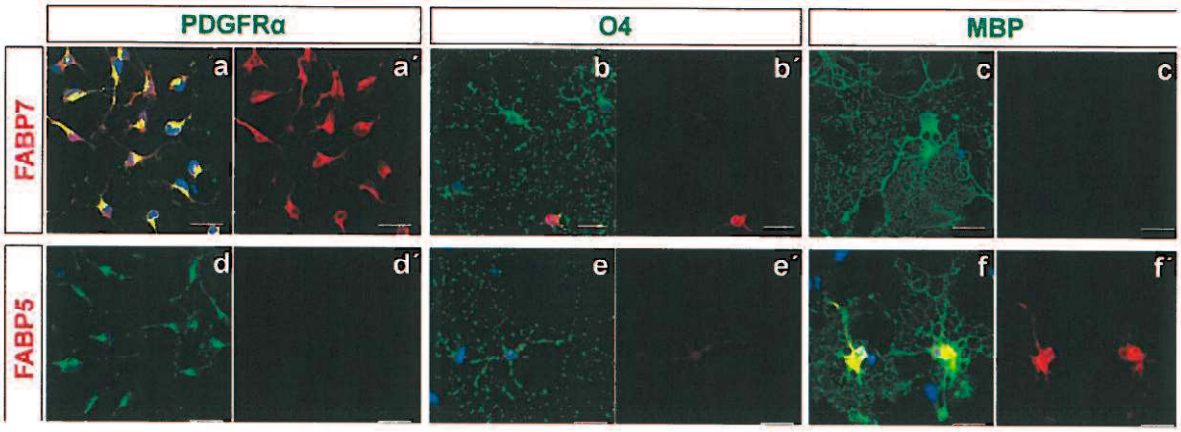

Fig. 3.8 Gradient reciprocal enrichment of FABP5 and FABP7 in oligodendrocyte lineage. Immunofluorescence micrographs showing expression of FABP5 and FABP7 in OPCs, immature oligodendrocytes, and mature oligodendrocytes. **a-c, a´-c´)** FABP7 expression is gradually downregulated following differentiation of $PDGFR\alpha^+$ OPCs to MBP^+ mature oligodendrocytes. Note the weak expression of FABP7 in $O4^+$ immature oligodendrocytes (b, b´). **d-f, d´-f´)** FABP5 expression is gradually upregulated upon differentiation of $PDGFR\alpha^+$ OPCs into MBP^+ mature oligodendrocytes. Note the weak expression of FABP5 in $O4^+$ immature oligodendrocytes (e, e´). Blue: DAPI; Scale bars =20 μm

FABP7 regulates proliferation of OPCs

To evaluate the role of FABPs on OPC proliferation, OPCs from WT, FABP5-KO, and FABP7-KO mice were seeded at equal seeding densities ($5X10^4$ cells/ml/well). After 5 days of incubation in OPC proliferation medium, population density of FABP7-KO OPCs was significantly lower compared with WT OPCs (86.4±1.9 vs. 190.9±27.6 / 0.4 mm^2 respectively; Fig. 3.9 a, b, d, e, j).

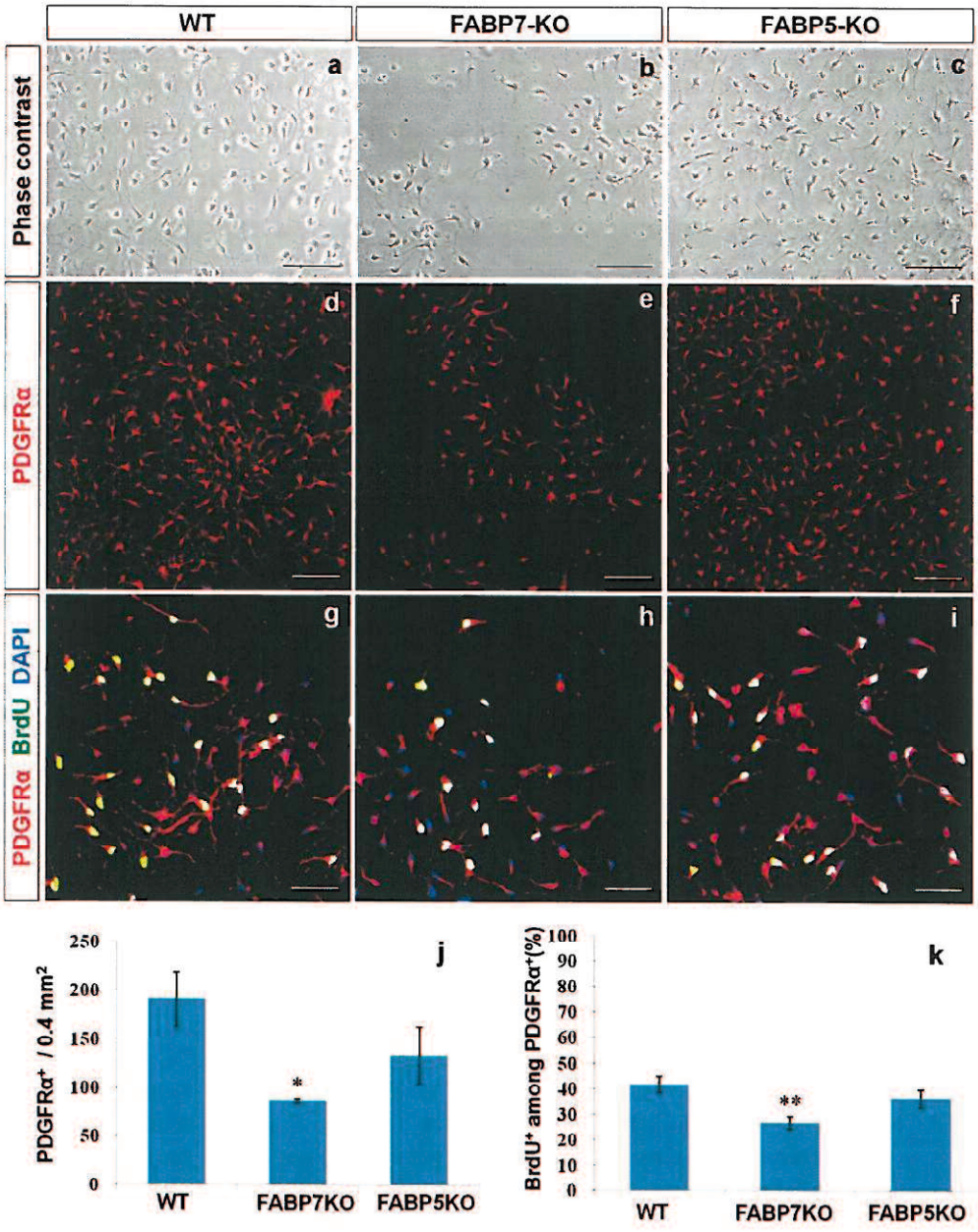

Fig. 3.9 FABP7 regulates proliferation of OPCs.

a-c) Representative phase contrast micrographs showing WT (a), FABP7-KO (b), and FABP5-KO (c) OPCs after 5 days of incubation in proliferation medium. Note the low population density of FABP7-KO OPCs (b). **d-i)** Representative immunofluorescent micrographs showing WT (d, g), FABP7-KO (e, h) and FABP5-KO (f, i) OPCs labeled by PDGFRα and their $BrdU^+$ subpopulations. **j)** Bar graph quantifying the population density of WT, FABP7-KO, and FABP5-KO OPCs. Note the low population density of FABP7-KO OPCs compared to WT OPCs. **k)** Bar graph quantifying the percentage of BrdU-labeled cells among all OPCs. Note the decreased BrdU uptake in FABP7-KO OPCs compared to WT OPCs. $^{**}P < 0.01$; Scale bars= 100 µm (a-f), 50 µm (g-i)

FABP7-KO OPCs showed a 37% decrease in BrdU uptake (proportion of $BrdU^+$ cells among $PDGFR\alpha^+$ cells) compared with WT OPCs (26.67±2.3% vs. 41.67±3.2%; Fig. 3.9 g, h, k). FABP5-KO OPCs did not show significant differences in terms of population density (Fig. 3.9 a, c, d, f, j) and BrdU uptake (Fig. 3.9 g, I, k). To investigate the possible effect of apoptosis in lower population density of FABP7-KO OPCs, the ratio of apoptotic cells ($Caspase3^+$ cells among $PDGFR\alpha^+$ cells) was evaluated in WT and FABP7-KO OPCs which showed no significant difference (3.43±2.48% Vs 2.8±0.17%; Fig.3.10). These data suggest that FABP7 may positively regulate OPC proliferation.

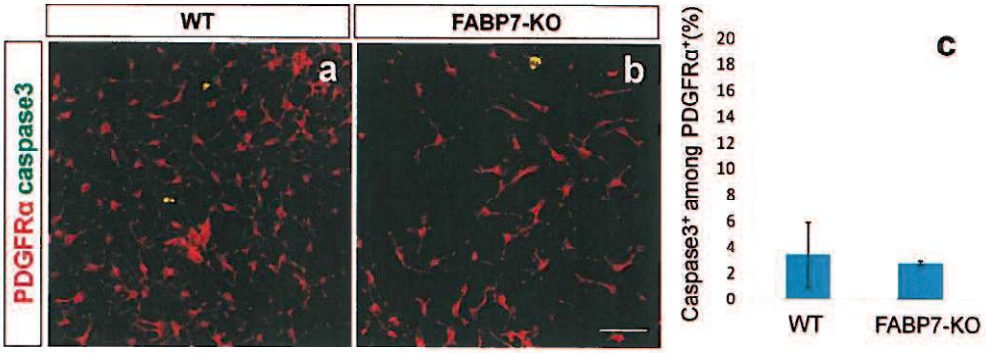

Fig. 3.10 a-b) Representative immunofluorescent micrographs showing $caspase3^+$ cells among WT (a) and FABP7-KO (b) OPCs. **c)** Bar graph quantifying the percentage of $caspase3^+$ cells among all OPCs. Scale bars= 50 µm

FABP5 and FABP7 control oligodendrocyte differentiation

To evaluate the role of FABPs in oligodendrocyte differentiation, OPCs from WT, FABP5-KO, and FABP7-KO mice were incubated in oligodendrocyte differentiation medium for 5 days. The percentage of $O4^+$ cells among whole oligodendrocyte lineage cells (total population of cells positive for either NG2 or O4) was significantly lower in FABP7-KO than in WT cells (3.2±1.1% in FABP7-KO vs. 19.4±1.7% in WT; Fig. 3.11 a-c, g, Fig. 3.12), suggesting that FABP7 positively regulates the differentiation of OPCs into oligodendrocytes. In addition, the population of MBP^+ cells among $O4^+/MBP^+$ cells showed a significant decrease in FABP7-KO and FABP5-KO cells compared with WT cells (18.4±5.1% in FABP7-KO and 16.3±1.5 FABP5-KO vs. 33.8%±3.7% in WT; Fig. 3.11 d-f, h). Furthermore, we morphologically classified oligodendrocytes as "simple", "complex", or "membrane sheet" and found a significantly higher percentage of

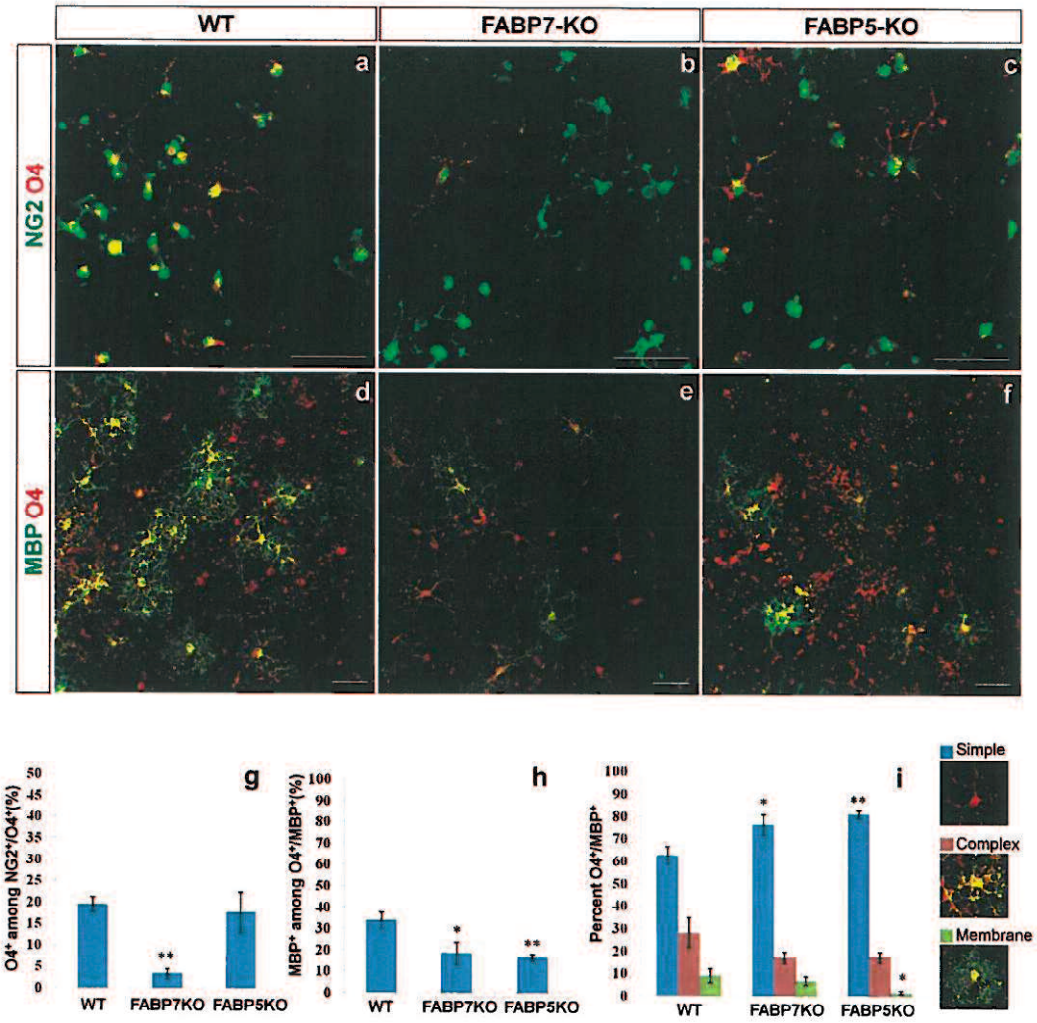

Fig. 3.11 FABP5 and FABP7 regulate oligodendrocyte differentiation.

a-c) Representative immunofluorescent micrographs showing undifferentiated $NG2^+$ OPCs and $O4^+$ oligodendrocytes in WT (a), FABP7-KO (b), and FABP5-KO (c). **d-f)** Representative immunofluorescent micrographs showing the population density and morphology of $O4^+/MBP^+$ oligodendrocytes from WT (d), FABP7-KO (e), and FABP5-KO (f). **g)** Bar graph quantifying the percentage of $O4^+$ oligodendrocytes among whole lineage cells (cells positive for either NG2 or O4). Note the lower percentage of $O4^+$ cells in FABP7-KO cells compared to WT cells. **h)** Bar graph quantifying the percentage of mature MBP^+ oligodendrocytes among MBP^+ or $O4^+$ oligodendrocytes. Note the lower percentage of MBP^+ cells in FABP7-KO and FABP5-KO compared to that of WT cells. **i** Bar graph showing the percentage of $O4^+/MBP^+$ cells with simple, complex, or membrane sheet morphology as an index of differentiation and maturation of oligodendrocytes. Note the higher percentage of simple morphology in FABP5-KO and FABP7-KO and the lower percentage of membrane sheet morphology in FABP5-KO compared to those in WT cells. *P < 0.05, **P < 0.01; Scale bars= 50 µm

oligodendrocytes having "simple" morphology in FABP7-KO and FABP5-KO cells (76.2±4.4% in FABP7-KO and 81±1.7% in FABP5-KO vs. 62.6±3.9% in WT) as well as a significantly lower percentage of oligodendrocytes having "membrane sheet" morphology in FABP5-KO cells compared with WT cells (1.5± 0.75% in FABP5-KO vs. 9.06± 3.09% in WT; Fig. 3.11 i). These findings suggest FABP7 as a positive regulator of early oligodendrocyte differentiation and FABP5 as a positive regulator of terminal oligodendrocyte differentiation and maturation.

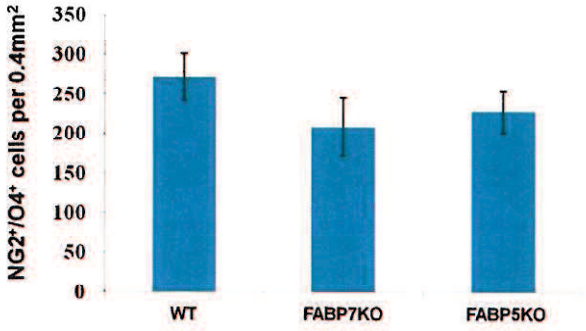

Fig. 3.12 Bar graph quantifying the population density of WT, FABP7-KO and FABP5-KO oligodendrocyte lineage cells (cells positive for either NG2 or O4) in the end of differentiation assay.

DISCUSSION

The expression and roles of FABPs in the oligodendrocyte lineage is poorly understood. In this study, we showed that two different FABPs, namely FABP7 and FABP5, are expressed by OPCs and mature oligodendrocytes, respectively, both *in vivo* and *in vitro*. We also showed that reciprocal enrichment of FABP5 and FABP7 in the oligodendrocyte lineage follows a gradient pattern. Purification of OPCs and oligodendrocytes is necessary for biochemical evaluation of our immunohistochemical data. However, based on our data, which are consistent with the results of published transcriptome databases of oligodendrocyte lineage [23-24], FABP5 and FABP7 may serve as cytosolic markers that can be used to identify oligodendrocyte lineage cells in combination with known oligodendrocyte lineage markers.

The underlying mechanisms that switch expression of FABP7 to FABP5 in oligodendrocyte lineage should be further studied. Several factors have been previously suggested as regulators of FABP7 expression in the brain, including the NFI gene family, notch 1, DAB1, Pax6 [25], and ApoE [26]. Interestingly, we found all of these factors to be enriched in

OPCs over mature oligodendrocytes in the transcriptome database of neural cells [23] suggesting them as possible regulators of FABP7 enrichment in OPCs. Differential enrichment of FABP7 and FABP5 in oligodendrocyte lineage cells may also reflect different fatty acid (FA) or lipid requirements in different stages of the lineage, since FABP7 and FABP5 show differential FA-binding preferences towards long chain fatty acids. While FABP7 has a high affinity for n-3 PUFAs, FABP5 shows a preference for more saturated fatty acids [25, 27].

In this study, we observed significantly lower proliferation of FABP7-deficient OPCs compared to that of WT OPCs, suggesting that FABP7 may positively regulate OPC proliferation (self-renewal). This is consistent with the known function of FABP7 as a positive regulator of the proliferation in neural stem cells [11, 28-29] and astrocytes [15]. Moreover, we detected that a significantly lower percentage of FABP7-deficient OPCs differentiated into $O4^+$oligodendrocytes compared with WT OPCs. Since OPCs differentiate to oligodendrocytes by "asymmetric division" or "proliferative differentiation" [7], our data suggest FABP7 as a regulator of the asymmetric division in OPCs. In this context, FABP7 may be an effector downstream target for asymmetry regulators and cell fate determinants such as notch1 [30]; interestingly, FABP7 expression is partly dependent on the notch-signaling pathway [25]. Proliferation of OPCs and their differentiation into oligodendrocytes are major events in spontaneous remyalination. Of interest, we found FABP7 as one of upregulated genes in transcriptome database of spontaneous remyelination [21], which is consistent with our results . Further studies in our laboratory are ongoing in order to unravel the roles of FABP7 on the proliferation and differentiation of OPCs under normal conditions or following demyelination. Notably, our preliminary results using cuprizone-induced demyelination model, showed alterations of spontaneous remyelination in FABP7-KO mice (not shown).

In contrast to FABP7, lack of FABP5 did not show any significant effect on the proliferation of OPCs or their differentiation to $O4^+$ oligodendrocytes; these findings are in agreement with the expression pattern of FABP5 and FABP7 in oligodendrocyte lineage cells. On the other hand, FABP5-deficient oligodendrocytes showed defects in their terminal differentiation and maturation since lower percentage of them expressed MBP and exhibited the "membrane sheet" morphology. However, *in vivo* and *in vitro* studies are in progress to

verify the current data by evaluating the impact of both FABP5 and FABP7 in myelination and remyelination.

Preliminary immunohistochemical assessments of FABP5-KO and FABP7-KO mice in normal condition did not show significant defects regarding oligodendrocyte lineage (not shown). Though this makes our FABP-KO mice more appropriate for *in vivo* demyelination/ remyelination studies, further proliferation, differentiation, and myelination assays in our mice are underway. Several mechanisms including other molecules related to fatty acid metabolisms may compensate FABP deficiency under normal conditions which may not be sufficient to overcome rapidly increasing demands under pathological conditions. On the other hand, the biology of the oligodendrocyte lineage is dependent on interactions with other neural cells through humoral factors [1]. Expression of FABP5 and FABP7 in astrocytes may lead to alterations in such humoral factors, which may indirectly influence the OPC biology. It has been reported that FABP7 regulates the expression of bFGF, PDGF AA and SPP1 in U87 astrocytoma cell line [31]. Therefore we are careful to distinguish direct and indirect roles of FABPs on oigodendrocyte lineage while using our FABP-KO mice for *in vivo* studies. In addition, possible impacts of contaminating astrocytes on our *in vitro* results cannot be excluded. Our culture system has a relatively high content of astrocytes, which could not be eliminated due to technical limitations. Although proliferation and differentiation of OPCs in the current culture system is mainly controlled by supplemented synthetic growth factors, humoral factors secreted by the contaminating astrocytes might have influenced the biology of oligodendrocyte lineage cells. Specifically, FABP deficient astrocytes might have altered the production of such humoral factors and indirectly impacted our data. To overcome such deficit, OPC culture systems with a higher purity are needed to confirm our current results.

Further studies may explore the underlying mechanism of FABPs roles in the oligodendrocyte lineage. However, studies in other cell types have shown that FABPs exert their functions through modulation of FA homeostasis, including the uptake, transportation, and metabolism of FAs as well as mediating their gene regulation activities. Particularly, FABPs can regulate gene expression by activating nuclear receptors such as PPARs and RXRs, possibly by targeting their ligands towards the nuclei [32-33]. Interestingly, activation of RXRγ has been

reported to stimulate differentiation of OPCs, thereby enhancing remyelination [21], and PPARγ has been shown to accelerate oligodendrocyte maturation [34-35]. Moreover, FABP7 has been shown to control the uptake of n-3 PUFAs in astrocytes [15]. Knockdown of FABP5 reportedly decreases cellular cholesterol levels and decreases apoB100 secretion and triglyceride accumulation in ARPE-19 cells [36]. On the other hand, FA supplementation and lipid metabolism are critical in the biology of oligodendrocytes. Supplementation of n-3 PUFAs has been shown to enhance oligodendrocyte differentiation [14]. Intracellular cholesterol content has also been shown to regulate lipid raft formation, differentiation of oligodendrocytes, and myelination [37-38]. These pieces of evidence indicate that FABPs may perform their regulatory roles in the oligodendrocyte lineage by controlling lipid homeostasis and gene regulation; however, further evaluation is needed to verify this.

In addition to the hypothetic roles of FABPs in the pathophysiology of demyelinating disorders, our data suggest new explanations for the known association between FABP7 and glioma. Notably, OPCs have recently been introduced as tumor cell of origin in glioma [6]. Asymmetry-defective OPCs have been shown to be glioma precursor cells [7]. Notch1, an asymmetric cell division regulator, is overexpressed in low-grade oligodendroglioma (Sugiarto et al. 2011). Accordingly, FABP7, a direct target of notch1, which mediates some aspects of notch signaling [39], is expressed in glioblastoma stem cells [40-41] and is associated with poor prognosis and invasion of malignant glioma [12, 42].

Our data also provide a new hypothesis regarding the association of brain expressed FABPs with schizophrenia. Remarkably, FABP7 has been mapped to a quantitative trait locus for prepulse inhibition (PPI), an endophenotype of schizophrenia [11]. FABP7-KO mice show impaired PPI and behavioral alterations reminiscent of schizophrenia [10-11]. n3-PUFAs, which are specific ligands of FABP7, have protective effects against schizophrenia [43-45]. Oligodendrocyte lineage is also thought to be important in the pathophysiology of schizophrenia. Schizophrenic brains have a lower volume of white matter than normal brains [4, 46-48]. White matter damage induced by cuprizone causes PPI and behavioral changes suggestive of schizophrenia in humans [49]. Microarray studies have shown that genes related

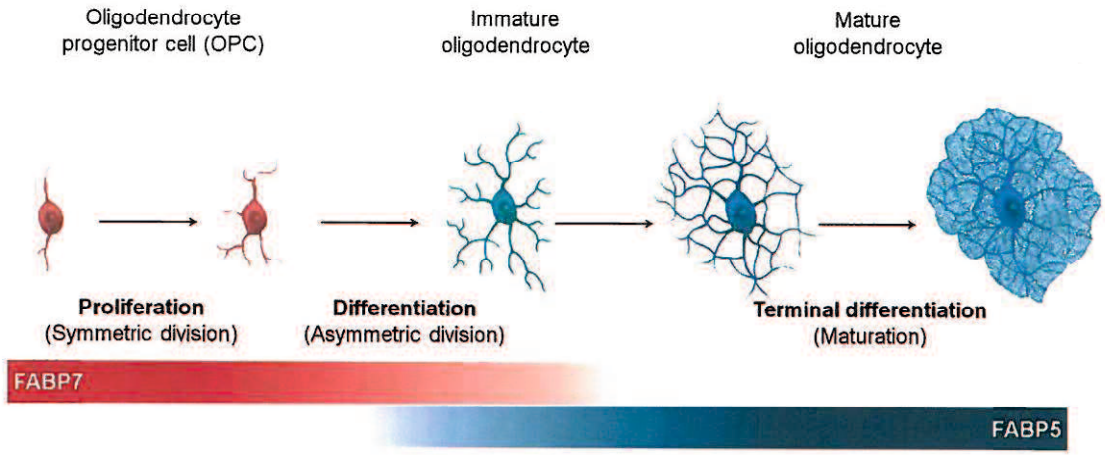

Fig. 3.13 Reciprocal enrichment of FABP5 and FABP7 regulates proliferation and differentiation of oligodendrocyte lineage cells.

Schematic illustration summarizing our results. FABP7 is enriched in OPCs and regulates proliferation of OPCs and their early differentiation into oligodendrocytes. FABP5 is enriched in mature oligodendrocytes and regulates their terminal differentiation and maturation

to oligodendrocyte function and myelination are downregulated in schizophrenic brains [5, 50-52].

Overall, our present data introduce FABP5 and FABP7 as new cytosolic markers and biological regulators of the oligodendrocyte lineage (Fig. 3.13). FABP7 may control the proliferation of OPCs and early oligodendrocyte differentiation, whereas FABP5 may control oligodendrocyte maturation. Our present findings provide new insights regarding the significance of lipid homeostasis in the differentiation of oligodendrocyte lineage cells and may support the preventive and therapeutic effects of PUFAs in neuropsychiatric and neurodegenerative diseases. Furthermore, FABP5, FABP7, and their ligands may become novel targets for the diagnosis, prevention, and treatment of major CNS diseases including multiple sclerosis, schizophrenia, and glioma.

REFERENCES

1. Moore, C.S., et al., *How factors secreted from astrocytes impact myelin repair.* J Neurosci Res, 2011. **89**(1): p. 13-21.

2. Levine, J.M., R. Reynolds, and J.W. Fawcett, *The oligodendrocyte precursor cell in health and disease.* Trends Neurosci, 2001. **24**(1): p. 39-47.
3. Franklin, R.J., *Why does remyelination fail in multiple sclerosis?* Nat Rev Neurosci, 2002. **3**(9): p. 705-14.
4. Bartzokis, G., et al., *Dysregulated brain development in adult men with schizophrenia: a magnetic resonance imaging study.* Biol Psychiatry, 2003. **53**(5): p. 412-21.
5. Katsel, P., K.L. Davis, and V. Haroutunian, *Variations in myelin and oligodendrocyte-related gene expression across multiple brain regions in schizophrenia: a gene ontology study.* Schizophr Res, 2005. **79**(2-3): p. 157-73.
6. Liu, C., et al., *Mosaic analysis with double markers reveals tumor cell of origin in glioma.* Cell, 2011. **146**(2): p. 209-21.
7. Sugiarto, S., et al., *Asymmetry-defective oligodendrocyte progenitors are glioma precursors.* Cancer Cell, 2011. **20**(3): p. 328-40.
8. Owada, Y., T. Yoshimoto, and H. Kondo, *Spatio-temporally differential expression of genes for three members of fatty acid binding proteins in developing and mature rat brains.* J Chem Neuroanat, 1996. **12**(2): p. 113-22.
9. Smathers, R.L. and D.R. Petersen, *The human fatty acid-binding protein family: evolutionary divergences and functions.* Hum Genomics, 2011. **5**(3): p. 170-91.
10. Owada, Y., et al., *Altered emotional behavioral responses in mice lacking brain-type fatty acid-binding protein gene.* Eur J Neurosci, 2006. **24**(1): p. 175-87.
11. Watanabe, A., et al., *Fabp7 maps to a quantitative trait locus for a schizophrenia endophenotype.* PLoS Biol, 2007. **5**(11): p. e297.
12. Kaloshi, G., et al., *FABP7 expression in glioblastomas: relation to prognosis, invasion and EGFR status.* J Neurooncol, 2007. **84**(3): p. 245-8.
13. Salvati, S., et al., *Eicosapentaenoic acid stimulates the expression of myelin proteins in rat brain.* J Neurosci Res, 2008. **86**(4): p. 776-84.
14. van Meeteren, M.E., et al., *Polyunsaturated fatty acid supplementation stimulates differentiation of oligodendroglia cells.* Dev Neurosci, 2006. **28**(3): p. 196-208.
15. Sharifi, K., et al., *FABP7 expression in normal and stab-injured brain cortex and its role in astrocyte proliferation.* Histochem Cell Biol, 2011. **136**(5): p. 501-13.
16. Owada, Y., et al., *Altered water barrier function in epidermal-type fatty acid binding protein-deficient mice.* J Invest Dermatol, 2002. **118**(3): p. 430-5.
17. McTigue, D.M., P. Wei, and B.T. Stokes, *Proliferation of NG2-positive cells and altered oligodendrocyte numbers in the contused rat spinal cord.* J Neurosci, 2001. **21**(10): p. 3392-400.
18. Nishiyama, A., R. Suzuki, H. Zuo and X. Zhu, *Oligodendrocyte progenitor cell culture*, in *Protocols for neural cell culture*, L.C.Doering, Editor. 4th ed. 2010, Humana Press: New York. p. 217-230.
19. Pedraza, C.E., et al., *Production, characterization, and efficient transfection of highly pure oligodendrocyte precursor cultures from mouse embryonic neural progenitors.* Glia, 2008. **56**(12): p. 1339-52.
20. Hatch, M.N., G. Nistor, and H.S. Keirstead, *Derivation of high-purity oligodendroglial progenitors.* Methods Mol Biol, 2009. **549**: p. 59-75.
21. Huang, J.K., et al., *Retinoid X receptor gamma signaling accelerates CNS remyelination.* Nat Neurosci, 2011. **14**(1): p. 45-53.
22. Stock, K., et al., *Transcription factor-based modulation of neural stem cell differentiation using direct protein transduction.* Cell Mol Life Sci, 2010. **67**(14): p. 2439-49.
23. Cahoy, J.D., et al., *A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function.* J Neurosci, 2008. **28**(1): p. 264-78.

24. Najm, F.J., et al., *Rapid and robust generation of functional oligodendrocyte progenitor cells from epiblast stem cells.* Nat Methods, 2011. **8**(11): p. 957-62.
25. Liu, R.Z., et al., *Fatty acid binding proteins in brain development and disease.* Int J Dev Biol, 2010. **54**(8-9): p. 1229-39.
26. Hung, M.C., et al., *Cerebral protein kinase C and its mRNA level in apolipoprotein E-deficient mice.* Life Sci, 2001. **69**(12): p. 1419-27.
27. Hanhoff, T., C. Lucke, and F. Spener, *Insights into binding of fatty acids by fatty acid binding proteins.* Mol Cell Biochem, 2002. **239**(1-2): p. 45-54.
28. Arai, Y., et al., *Role of Fabp7, a downstream gene of Pax6, in the maintenance of neuroepithelial cells during early embryonic development of the rat cortex.* J Neurosci, 2005. **25**(42): p. 9752-61.
29. Matsumata, M., et al., *The effects of Fabp7 and Fabp5 on postnatal hippocampal neurogenesis in the mouse.* Stem Cells, 2012. **30**(7): p. 1532-43.
30. Egger, B., K.S. Gold, and A.H. Brand, *Regulating the balance between symmetric and asymmetric stem cell division in the developing brain.* Fly (Austin), 2011. **5**(3): p. 237-41.
31. Kipp, M., et al., *BLBP-expression in astrocytes during experimental demyelination and in human multiple sclerosis lesions.* Brain Behav Immun, 2011.
32. Kitajka, K., et al., *Effects of dietary omega-3 polyunsaturated fatty acids on brain gene expression.* Proc Natl Acad Sci U S A, 2004. **101**(30): p. 10931-6.
33. Schroeder, F., et al., *Role of fatty acid binding proteins and long chain fatty acids in modulating nuclear receptors and gene transcription.* Lipids, 2008. **43**(1): p. 1-17.
34. De Nuccio, C., et al., *Peroxisome proliferator-activated receptor gamma agonists accelerate oligodendrocyte maturation and influence mitochondrial functions and oscillatory Ca(2+) waves.* J Neuropathol Exp Neurol, 2011. **70**(10): p. 900-12.
35. Bernardo, A., et al., *Peroxisome proliferator-activated receptor-gamma agonists promote differentiation and antioxidant defenses of oligodendrocyte progenitor cells.* J Neuropathol Exp Neurol, 2009. **68**(7): p. 797-808.
36. Wu, T., et al., *Knockdown of FABP5 mRNA decreases cellular cholesterol levels and results in decreased apoB100 secretion and triglyceride accumulation in ARPE-19 cells.* Lab Invest, 2010. **90**(6): p. 963-5.
37. Schmitz, M., et al., *Oligodendroglial Process Formation is Differentially Affected by Modulating the Intra- and Extracellular Cholesterol Content.* J Mol Neurosci, 2012.
38. Saher, G., et al., *High cholesterol level is essential for myelin membrane growth.* Nat Neurosci, 2005. **8**(4): p. 468-75.
39. Anthony, T.E., et al., *Brain lipid-binding protein is a direct target of Notch signaling in radial glial cells.* Genes Dev, 2005. **19**(9): p. 1028-33.
40. Qiang, L., et al., *HIF-1alpha is critical for hypoxia-mediated maintenance of glioblastoma stem cells by activating Notch signaling pathway.* Cell Death Differ, 2012. **19**(2): p. 284-94.
41. Thirant, C., et al., *Clinical relevance of tumor cells with stem-like properties in pediatric brain tumors.* PLoS One, 2011. **6**(1): p. e16375.
42. Liang, Y., et al., *Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme.* Proc Natl Acad Sci U S A, 2005. **102**(16): p. 5814-9.
43. Muskiet, F.A. and R.F. Kemperman, *Folate and long-chain polyunsaturated fatty acids in psychiatric disease.* J Nutr Biochem, 2006. **17**(11): p. 717-27.
44. Hedelin, M., et al., *Dietary intake of fish, omega-3, omega-6 polyunsaturated fatty acids and vitamin D and the prevalence of psychotic-like symptoms in a cohort of 33,000 women from the general population.* BMC Psychiatry, 2010. **10**: p. 38.
45. Fedorova, I., et al., *Deficit in prepulse inhibition in mice caused by dietary n-3 fatty acid deficiency.* Behav Neurosci, 2009. **123**(6): p. 1218-25.

46. Buchanan, R.W., et al., *Structural evaluation of the prefrontal cortex in schizophrenia.* Am J Psychiatry, 1998. **155**(8): p. 1049-55.
47. Cannon, T.D., et al., *Regional gray matter, white matter, and cerebrospinal fluid distributions in schizophrenic patients, their siblings, and controls.* Arch Gen Psychiatry, 1998. **55**(12): p. 1084-91.
48. Sanfilipo, M., et al., *Volumetric measure of the frontal and temporal lobe regions in schizophrenia: relationship to negative symptoms.* Arch Gen Psychiatry, 2000. **57**(5): p. 471-80.
49. Xu, H., et al., *Behavioral and neurobiological changes in C57BL/6 mice exposed to cuprizone.* Behav Neurosci, 2009. **123**(2): p. 418-29.
50. Aberg, K., et al., *Human QKI, a potential regulator of mRNA expression of human oligodendrocyte-related genes involved in schizophrenia.* Proc Natl Acad Sci U S A, 2006. **103**(19): p. 7482-7.
51. Hakak, Y., et al., *Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia.* Proc Natl Acad Sci U S A, 2001. **98**(8): p. 4746-51.
52. Peirce, T.R., et al., *Convergent evidence for 2',3'-cyclic nucleotide 3'-phosphodiesterase as a possible susceptibility gene for schizophrenia.* Arch Gen Psychiatry, 2006. **63**(1): p. 18-24.

Concluding remarks

Our studies on expression pattern and biological roles of FABPs in macroglia have resulted in several basic and novel findings which can improve the FABP research in the field of cellular and molecular neuroscience.

Reactive astrogliosis is a common hallmark of CNS pathologies and a major component of brain regenerative program across species [1-4]. We showed that FABP7, a well known marker of neural stem/progenitor cells of the developing brain, is also upregulated in reactive astrocytes in the adult brain and positively regulates their proliferation. We also showed the role of FABP7 in proliferation and FA uptake in cultured astrocytes. These data suggests that in addition to brain development, FABP7 is important in regenerative programs of the brain likely through controlling lipid homeostasis and thereby may be involved in various CNS diseases.

Furtheromore, we studied the expression pattern of FABP7 and FABP5 in oligodendrocyte lineage. We showed that FABP7 is enriched in OPCs and is down regulated upon their differentiation while FABP5 is upregulated upon differentiation and is enriched in mature oligodendrocytes. Accordingly, we showed that FABP7 regulates the proliferation of OPCs and their early differentiation while FABP5 regulates oligodendrocyte maturation. Oligodendrogenesis, including proliferation of OPCs and their differentiation and maturation towards myelinating oligodendrocytes are important parts of brain regeneration and their dysregulation is a central part in the pathophysiology of major CNS disorders such as demyelinating disorders, neuropsychiatric disorders, neurodegenerative disorders and malignant glioma. Therefore, our present data highlight the significance of FABPs and their ligands as future diagnostic and therapeutic targets for CNS diseases.

Our data regarding roles of FABP7 in proliferation of astrocytes and OPCs, is consistent with other reports which have shown the roles of FABP7 in cell proliferation and it's therapeutic potentials in malignant glioma [5-7], breast cancer[8], renal cell carcinoma [9] and malignant melanoma [10-11]. It remains to be studied whether dysregulated overexpression of FABP7

may have oncogenic effect in astrocytes or OPCs. It is likely that development of transgenic mice which overexpress FABP7 provide novel oncomice models. In this context, therapeutic potentials of FABP7 inhibition in malignant glioma by RNA interference strategy, FABP7 antagonists or neutralizing anti FABP7 antibodies, should be evaluated both in vivo and in vitro. Therapeutic potentials of FABP7 inhibition in glioma are also being studied using our FABP7-KO mice.

Several biochemical screening studies have shown the altered levels of brain FABPs in CSF or plasma following various CNS injuries as diagnostic or prognostic biomarkers [12]. Our data on identification of cells which express FABP7 and FABP5 in the adult brain provide valuable cues for interpretation of such biochemical findings regarding the involvement of FABPs and reaction of FABP expressing cells in various CNS pathologies. FABPs have been so far believed as intracellular proteins and their presence in plasma or CSF have been considered as a result of their release after cell damage. Very recently active secretion of FABP4 and FABP5 from adipocytes has been shown[13]. This suggests possible secretion of other FABPs from their cells of origin and provides several new hypotheses. For example, increased plasma or CSF levels of FABP7 after brain injury might be due to increased secretion of FABP7 as a result of its increased expression by rapidly proliferating reactive astrocytes or OPCs according to the type of injury. Possible secretion of brain FABPs from neural cells and biological functions of the secreted FABPs in autocrine or paracrine manner remain to be studied.

Our data suggest the rational for development of new materials or use of more advanced technologies for the future studies on brain FABPs. Based on our results, FABP7 is shared between astrocytes and OPCS. Similarly, FABP5 is shared between astrocytes and oligodendrocytes. Therefore, our FABP-KO mice show some limitations to study the direct roles of FABPs on oligodendrocyte lineage in vivo. Similar limitations have arisen in all FABP studies. To overcome such limitations, in addition to cell transplantation studies, development of cell type specific conditional KO mice may be necessary and may result in significant improvement in FABP research. Furthermore, most of FABP-KO mice so far have shown few defects in steady state and show impairments only under stress conditions. On the other hand, upregulation of

FABPs in several disease models have been shown. This suggests the rational for development of FABP overexpressing animal s which may provide new disease models. In addition, further studies on roles of FABPs on macroglia, are greatly dependent on several high throughput studies using brain samples or purified isolated neural cells from FABP-KO mice. Development of agonists or antagonists for brain FABPs are other technical requirements for the future of studies on brain FABPs, which are now being studied by several research groups [14].

The present data provided the basic evidence and new hypotheses for several studies in our lab. Roles of FABP7 and FABP5 and their ligands in glioblastoma stem cells, mechanistic basis and detailed roles of FABP7 during astrocyte reactivation, roles of FABPs in myelination and spontaneous remyelination, as well as general molecular phenotypes of FABP-deficient macroglia are being studied in our lab.

Taken together, our studies presented here, provide novel important data on expression and biological roles of FABPs in macroglia, which suggest new insights towards significance of lipid metabolism in brain regeneration and provide basic evidence and hypotheses for several future studies with implications in molecular and regenerative medicine, targeting the potential therapeutic or diagnostic significance of FABPs and their ligands in association with various CNS diseases.

REFERENCES

1. BUFFO, A., ET AL., *ORIGIN AND PROGENY OF REACTIVE GLIOSIS: A SOURCE OF MULTIPOTENT CELLS IN THE INJURED BRAIN.* PROC NATL ACAD SCI U S A, 2008. **105**(9): P. 3581-6.
2. SHIMADA, I.S., ET AL., *SELF-RENEWAL AND DIFFERENTIATION OF REACTIVE ASTROCYTE-DERIVED NEURAL STEM/PROGENITOR CELLS ISOLATED FROM THE CORTICAL PERI-INFARCT AREA AFTER STROKE.* J NEUROSCI, 2012. **32**(23): P. 7926-40.
3. SIRKO, S., ET AL., *REACTIVE GLIA IN THE INJURED BRAIN ACQUIRE STEM CELL PROPERTIES IN RESPONSE TO SONIC HEDGEHOG. [CORRECTED].* CELL STEM CELL, 2013. **12**(4): P. 426-39.
4. BAUMGART, E.V., ET AL., *STAB WOUND INJURY OF THE ZEBRAFISH TELENCEPHALON: A MODEL FOR COMPARATIVE ANALYSIS OF REACTIVE GLIOSIS.* GLIA, 2012. **60**(3): P. 343-57.
5. DE ROSA, A., ET AL., *A RADIAL GLIA GENE MARKER, FATTY ACID BINDING PROTEIN 7 (FABP7), IS INVOLVED IN PROLIFERATION AND INVASION OF GLIOBLASTOMA CELLS.* PLOS ONE, 2012. **7**(12): P. E52113.
6. KALOSHI, G., ET AL., *FABP7 EXPRESSION IN GLIOBLASTOMAS: RELATION TO PROGNOSIS, INVASION AND EGFR STATUS.* J NEUROONCOL, 2007. **84**(3): P. 245-8.

7. LIANG, Y., ET AL., *GENE EXPRESSION PROFILING REVEALS MOLECULARLY AND CLINICALLY DISTINCT SUBTYPES OF GLIOBLASTOMA MULTIFORME.* PROC NATL ACAD SCI U S A, 2005. **102**(16): P. 5814-9.
8. LIU, R.Z., ET AL., *A FATTY ACID-BINDING PROTEIN 7/RXRBETA PATHWAY ENHANCES SURVIVAL AND PROLIFERATION IN TRIPLE-NEGATIVE BREAST CANCER.* J PATHOL, 2012. **228**(3): P. 310-21.
9. TOLLE, A., ET AL., *IMPORTANCE OF BRAINTYPE FATTY ACID BINDING PROTEIN FOR CELL-BIOLOGICAL PROCESSES IN HUMAN RENAL CARCINOMA CELLS.* ONCOL REP, 2011. **25**(5): P. 1307-12.
10. SLIPICEVIC, A., ET AL., *THE FATTY ACID BINDING PROTEIN 7 (FABP7) IS INVOLVED IN PROLIFERATION AND INVASION OF MELANOMA CELLS.* BMC CANCER, 2008. **8**: P. 276.
11. GOTO, Y., ET AL., *ABERRANT FATTY ACID-BINDING PROTEIN-7 GENE EXPRESSION IN CUTANEOUS MALIGNANT MELANOMA.* J INVEST DERMATOL, 2010. **130**(1): P. 221-9.
12. PELSERS, M.M., W.T. HERMENS, AND J.F. GLATZ, *FATTY ACID-BINDING PROTEINS AS PLASMA MARKERS OF TISSUE INJURY.* CLIN CHIM ACTA, 2005. **352**(1-2): P. 15-35.
13. CAO, H., ET AL., *ADIPOCYTE LIPID CHAPERONE AP2 IS A SECRETED ADIPOKINE REGULATING HEPATIC GLUCOSE PRODUCTION.* CELL METAB, 2013. **17**(5): P. 768-78.
14. BERGER, W.T., ET AL., *TARGETING FATTY ACID BINDING PROTEIN (FABP) ANANDAMIDE TRANSPORTERS - A NOVEL STRATEGY FOR DEVELOPMENT OF ANTI-INFLAMMATORY AND ANTI-NOCICEPTIVE DRUGS.* PLOS ONE, 2012. **7**(12): P. E50968.