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An invited review following *the Soujinkai Young Investigator Award*: Heat Shock Factor 1 could be a Promising New Therapeutic Target for Melanoma

Yoshitaka Nakamura,[1] *Akira Nakai*[2] *and Masahiko Muto*[1]

[1] Department of Dermatology, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
[2] Department of Biochemistry and Molecular Biology, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
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Correspondence to Yoshitaka Nakamura, MD, PhD. E-mail: naka629@yamaguchi-u.ac.jp

Abstract Heat shock factor 1 (HSF1) has recently been reported to promote malignant transformation and growth. Furthermore, HSF1 was identified as a potent pro-invasion oncogene in human melanomas. However, the biological functions of HSF1 in human melanoma remain poorly understood. In this study, we found that silencing HSF1 suppressed proliferation, migration and invasiveness of human melanoma cells *in vitro* and HSF1 is required for melanoma invasion and metastasis, as well as tumorigenic potential *in vivo*. Furthermore, we demonstrated that these decreased functions by HSF1 knockdown were restored by overexpression of wild-type HSF1 both *in vitro* and *vivo*. Our findings suggest that HSF1 could be a promising new therapeutic target for melanoma.

This article is an invited review by the awardee of *the Soujinkai Young Investigator Award*, based on his original paper published in Cancer Letters in 2014.

Key words: human melanoma, heat shock factor 1 (hsf1), proliferation, migration, invasion

Introduction

The heat shock response is a fundamental adaptive response that maintains protein homeostasis in all organisms, from bacteria to humans, and is characterized by the induced expression of genes coding for heat shock proteins (HSPs) and non-HSP proteins.[1,2] It is regulated mainly at the level of transcription by heat shock factors (HSFs) in eukaryotes. HSF1 is a master regulator of heat shock genes in mammalian cells. It has long been established that HSPs levels are increased in a wide range of tumors. Moreover, previous research has shown that HSF1 expression is elevated in a wide range of human cancer tissues, and that high HSF1 expression is associated with a poor prognosis.[3-5] Recent studies have revealed that the HSF1-mediated stress response is a powerful multifaceted modifier of carcinogenesis.[6] HSF1 greatly enhances the efficacy of oncogenic transformation by orchestrating a broad network of core cellular functions, including proliferation, survival, protein synthesis and glucose metabolism.[7,8] Furthermore, in a study integrating genetically engineered mouse models, cross-species cancer genomics, and functional screens, HSF1 was identified as a potent pro-invasion oncogene in human melanomas;[9] however, little is known about the biological functions of HSF1 in melanoma progression and metastasis.

HSF1 knockdown by shRNA in human melanoma cells

To suppress HSF1 gene expression in human melanoma cells, we first identified two 19-nucleotide gene-specific sequences and then generated adenovirus-expressing shRNA for HSF1 (HSF1-1 and HSF1-2 shRNA). Human melanoma cells (HMV-I, MeWo and HMV-II) were infected with HSF1-1, HSF1-2, or scrambled shRNA for 2 hr and maintained with normal medium for 70 hr. HSF1 protein and mRNA levels were determined by Western blot and RT-PCR analysis, respectively, in whole cell extracts of melanoma cells infected with scrambled, HSF1-1, or HSF1-2 shRNA-expressing adenovirus. HSF1 protein and mRNA levels were significantly reduced in melanoma cells with either HSF1-1 or HSF1-2 shRNA, but not in melanoma cells with scrambled shRNA

Proliferation of melanoma cells infected with HSF1 shRNA adenovirus is markedly reduced

To examine the proliferation of human melanoma cells in which HSF1 was knocked down by shRNA, cell numbers were counted each day from 72 hr after viral infection. Surprisingly, the proliferation of melanoma cells infected with HSF1 shRNA was markedly reduced compared to cells infected with scramble shRNA. Suppression of cell proliferation was not observed at all in normal human keratinocyte HaCat cells. This is the first demonstration that HSF1 plays a critical role in the proliferation of human melanoma cells.[10]

Silencing HSF1 in human melanoma cells led to delayed wound healing

To examine the biological functions of HSF1 in melanoma cells, we performed a wound-healing assay after transfection with shRNA. HSF1 knockdown resulted in decreased motility in both HSF1-1 and HSF1-2 shRNA-transfected melanoma cells. These findings revealed an essential role of HSF1 in human melanoma cell motility.

HSF1 knockdown caused a marked reduction in migration and invasive ability of human melanoma cells.

The acquisition of migration and invasive ability is a critical step for tumor progression and metastasis. We next examined whether knockdown of HSF1 affected migration and invasive ability of melanoma cells. Transwell migration and invasion assay revealed marked reduction in migration and invasive ability in melanoma cells transfected with either HSF1-1 or HSF1-2 shRNA. Meanwhile, silencing HSF1 by shRNA did not affect normal human keratinocyte HaCat cell motility, migration and invasive ability. These results demonstrate that HSF1 is required for melanoma cell migration and invasion.

Reduced migration and invasive ability by silencing HSF1 is restored by overexpression of wild-type HSF1.

We next examined whether the reduced ability to migrate and invade due to knockdown of HSF1 recovers after wild-type HSF1 overexpression. The decrease in migration and invasive ability by silencing HSF1 were normalized by overexpression of wild-type HSF1. These results suggest that HSF1 helps to maintain migratory and invasive abilities of human melanoma cells, and promotes melanoma progression.

HSF1 is indispensable for melanoma progression and metastasis *in vivo*

To confirm the *in vitro* data, we performed xenograft experiments in which athymic nude mice were inoculated subcutaneously with melanoma cells infected with adenovirus. In this study, we confirmed that the tumorigenic potential of melanoma cells was significantly reduced by HSF1 knockdown *in vivo*. Furthermore, we showed that the incidence of intraperitoneal invasion and lung metastasis was significantly decreased by silencing HSF1 in xenograft experiments. In addition, we confirmed that the reduced invasiveness and metastatic ability, as well as the reduced tumorigenic potential, were restored by overexpressing wild-type HSF1. These results strongly suggest that HSF1 both promotes melanoma cell migration and invasiveness and plays a critical role in melanoma progression and metastasis.

Conclusion

To the best our knowledge, our study showed

for the first time that silencing HSF1 suppressed proliferation, migration and invasiveness of human melanoma cells *in vitro* (Fig. 1).[11] Furthermore, we demonstrated that HSF1 is required for melanoma invasion and metastasis, as well as tumorigenic potential *in vivo*. Taken together, these results suggest that HSF1 could be a promising new therapeutic target for melanoma patients.

Conflict of Interest

The authors state no conflict of interest.

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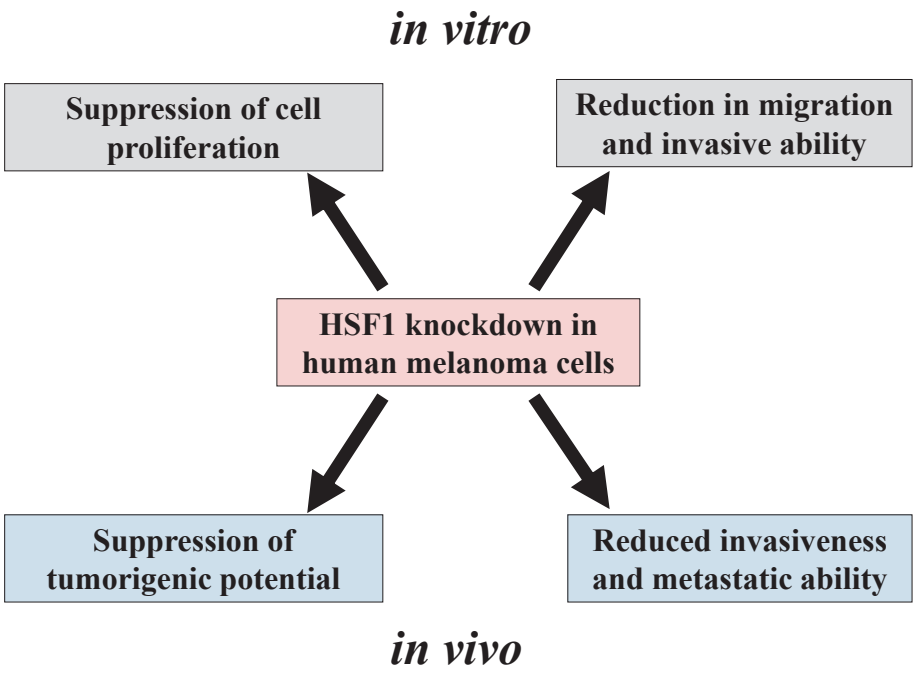

Fig. 1 Silencing HSF1 suppressed proliferation, migration and invasiveness of human melanoma cells *in vitro*. Furthermore, HSF1 is required for melanoma invasion and metastasis, as well as tumorigenic potential *in vivo*.

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