Bull Yamaguchi Med Sch 43(3-4) : 1996

Flow-Cytometric FISH (Fluorescence In Situ Hybridization) in Human Gliomas

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(Received August 29, 1996)

Cytogenetic analyses can be performed, even in interphase nuclei. by fluorescence in situ hybridization (FISH) using chromosome-specific DNA probes[1)2)]. While FISH with probes for DNA has been used for the detection of chromosomal aberrations in solid tumors on slides, there have been very few previous studies about flow analysis of FISH on nuclei in suspension[3)4)5)6)]. we have attempted the flow-cytometric quantification of chromosomal aberrations by FISH to know how these changes contribute to the DNA ploidy. Cell suspensions prepared from freshly -frozen tissue specimens were used to examine aberrations in the numbers of chromosome 7 signals in 10 human gliomas. Nuclear DNA was hybridized in vitro with an alpha satellite DNA probe specific for the centromeric regions of chromosome 7. using FISH. The intensity of the fluorescence signal from the hybridized probe was measured, together with the nuclear DNA content by flow cytometry. The mean probe fluorescence of all nuclei was compared to the mean copy number per nucleus by microscopic scoring. Moreover, the mean probe fluorescence ratio of DNA aneuploid nuclei relative to DNA diploid nuclei (FISH*a*/FISH*d*) was calculated to know how the numerical aberration of chromosome 7 signals contribute to the DNA ploidy of the sample.

The results from the flow-cytometric analysis and from microscopic evaluation were compatible. One of 4 tumors with DNA diploidy had a higher average intensity of FISH signal and a broader coefficient of variation in FISH signal than normal brain tissue, and was shown to be due to the gain of chromosome 7 signals. Although FISH*a*/FISH*d* correlated with DNA indices ($p < 0.01$), there were some disparities, probably due to other complex genotypic associations involving several gains or losses of chromosomes. Thus, gain of chromosome 7 in gliomas is related to both DNA ploidy change and chromosome specific gain. It is concluded that flow-cytometric quantification of FISH is useful in investigating numerical aberrations of chromosomes and nuclear DNA content simultaneously.

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