A Novel General Strategy for Cloning Tumor Suppressor Genes Using Radiation-Reduced Chromosomal Superfragments

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KOI, M., JOHNSON, L.A. and FEINBERG, A.P. *A Novel General Strategy for Cloning Tumor Suppressor Genes Using Radiation-Reduced Chromosomal Superfragments*. Tohoku J. Exp. Med., 1992, **168** (2), 167-168 — To identify the tumor suppressor gene on human chromosome 11p15, we generated mouse microcell hybrids containing small transferable chromosome 11p15 fragments, which we have termed "DNA superfragments". These hybrids will be used to identify which fragments contain a tumor suppressor gene by direct transfer of the fragments to tumor cells via microcell fusion. ——— DNA superfragments ; tumor suppressor gene ; microcell fusion

We have developed a novel strategy which uses direct functional cloning for isolation of tumor suppressor genes. Our specific strategy was applied to the human chromosome 11p15 tumor suppressor because the loss of heterozygosity at this region has been demonstrated in many childhood cancers involving the kidney, liver, adrenal gland and muscle, and also in adult tumors involving bladder, lung, breast and ovary, suggesting the epidemiological importance of the 11p15 gene. Furthermore, the presence of a functional tumor suppressor gene on 11p15 has been suggested by chromosome transfer experiments. We have developed a method for generating small chromosomal fragments which can be transferred from one cell to another.

RESULTS

Mouse A9 cells containing a single human chromosome with a translocation between chromosome 11 and a small portion of the X chromosome (Xq26-Xqter) were constructed (Bonetta et al. 1990). The cells were transfected with PSV2 neo plasmid DNA and G418-resistant clones were isolated and pooled. Then microcells were prepared from the pooled clones and fused to A9 cells. The fused cultures were selected against HAT plus G418 and double resistant hybrids were

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isolated. The microcells were again prepared from double resistant clones, irradiated with 10 K rad of γ -ray and fused to A9 cells. The microcell hybrids that contained chromosomal fragments harboring neo from chromosome X/11 were isolated in G418 medium. In this way, 85 irradiated microcell clones were isolated and the presence of 5 markers on 11p15 and 2 markers on 11p13 was screened by standard Southern blot analysis. Twenty-six hybrids were positive for the 11p15 markers and twelve hybrids were positive for 11p13. We are now transferring these fragments to several kinds of tumors, including Wilms tumor, rhabdomyosarcoma, cervical carcinoma and non-small cell lung carcinoma cells.

Reference

- 1) Bonetta, L., Kuehn, S.E., Huang, A., Law, D.J., Kalikin, L., Koi, M., Reeve, A.A., Yeger, H., Williams, B.R.G. & Feinberg, A.P. (1990) Wilms tumor locus on 11p13 defined by multiple CpG island-associated transcripts. *Science*, **250**, 994-997.
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