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Chromosomal Localization of Putative Tumor-Suppressor Genes in Several Human Cancers

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Restriction-fragment-length polymorphism analysis was performed on several different types of human cancers, including carcinoma of the uterine cervix, neuroblastoma, hepatocellular carcinoma, pheochromocytoma, stomach cancer, and small-cell lung carcinoma (SCLC), to determine the chromosomal loci of putative tumor-suppressor genes in each type of tumor because loss of heterozygosity (LOH) is supposed to unmask the recessive mutation of tumor-suppressor gene in the remaining allele. Chromosomal loci showing frequent LOH differed among these tumors, suggesting that there are several tumor-suppressor genes in the human genome and that critical genes for the development of each type of tumor are different. In some cases LOH was observed in the early stage of tumor such as chromosome 3p loss in carcinoma of the uterine cervix, and in other cases it was observed only in the advanced stage of tumor such as chromosomes 4 and 16q loss in hepatocellular carcinoma. These results suggest that there are two different types of tumor-suppressor genes: one is the gene whose inactivation is responsible for malignant transformation of a normal cell and the other is the gene whose inactivation is responsible for the progression of a tumor cell. In SCLC, LOH at three different chromosomal loci, 3p, 13q, and 17p, was simultaneously observed in nearly 100% of tumors. It was observed even in stage I tumors and an untreated tumor, and it occurred prior to N-*myc* amplification. These results may imply that at least six genetic alterations are necessary to convert a normal cell into a fully malignant cancer cell in SCLC.

Introduction

Tumor-suppressor genes can be inactivated by either mutation or loss, and loss of heterozygosity (LOH) is believed to be a critical chromosomal mechanism to lose one of the allelic genes. Recent molecular genetic studies have shown that LOH at specific chromosomal loci occurs very frequently in certain types of human tumors, suggesting that inactivation of tumor-suppressor genes is involved in the development of a wide variety of human cancers. Molecular analysis of genetic alterations with reference to the clinical and biological behavior of cancer cells will give us valuable information for understanding the molecular mechanisms involved in human carcinogenesis. We have performed a restriction-fragment-length polymorphism (RFLP) analysis for several different types of human cancers to determine the chromosomal loci of putative tumor-suppressor genes in each type of tumor. LOH at specific chromosomal loci was detected frequently and specifically in each type of cancer. The results of our RFLP analysis will be summarized and described here with specific emphasis on those of small-cell lung carcinoma (SCLC).

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Loss of Heterozygosity in Several Human Cancers

An RFLP analysis was performed for several different types of human cancers, including carcinoma of the uterine cervix, neuroblastoma, hepatocellular carcinoma, pheochromocytoma, stomach cancer, and SCLC. LOH was observed at specific loci with high frequency in these cancers, and the loci showing frequent allele loss differed in each type of cancer (Table 1).

In carcinoma of the uterine cervix, LOH at the *D3S2* locus on chromosome 3p was observed in all nine patients who could be evaluated (*1*). Human papilloma virus type 16 and 18 were present in 7 and 3 of 18 tumors, respectively, while no amplification of 13 oncogenes, including c-*myc* and c-H-*ras*, was detected in these tumors. These results suggest that recessive genetic changes on chromosome 3p are one of the important genetic alterations for the development of carcinoma of the uterine cervix. Since this locus is also lost commonly in lung cancer and in renal cell carcinoma, it is possible that these three different types of adult tumors result from mutations of the same tumor-suppressor gene on chromosome 3p.

In neuroblastoma, the incidence of LOH was high at the *D14S1* locus on chromosome 14q, being detected in 6 of 12 patients (50%) (*2*). LOH on 14q was detected in

Table 1. Loss of heterozygosity in human cancers.

Type of tumor	Site of allele loss	Incidence	(%)	Reference
Small-cell lung carcinoma	3p	15/15	(100%)	(*10,11*)
	13q	15/16	(94%)	
	17p	16/16	(100%)	
Carcinoma of the uterine cervix	3p	9/9	(100%)	(*1*)
Neuroblastoma	1p	2/9	(22%)	(*2*)
	14q	6/12	(50%)	
Hepatocellular carcinoma	4	8/16	(50%)	(*3*)
	16q	8/14	(57%)	
Pheochromocytoma	1p	3/4	(75%)	(*4*)
	11p	3/5	(60%)	
Stomach cancer	Nonspecific and infrequent	—		(*5*)

two stage-I patients, who had received no chemotherapy or radiotherapy before the surgical removal of the tumors. In spite of the cytogenetic finding suggesting high frequency of chromosome 1p deletion, LOH at the *MYCL* locus on 1p32 was detected only in two of nine patients (22%). These results indicate that the tumor-suppressor gene for neuroblastoma is located on the long arm of chromosome 14, and inactivation of this gene is critical for the malignant transformation of cells. The results also indicate that chromosome 1p deletion, as well as N-*myc* amplification, may be a secondary event occurring during the progression of neuroblastoma.

In hepatocellular carcinoma, LOH at the *HP* locus on 16q22 and at the *MT2P1* on 4p11-q21 was detected in 57% (8/14) and 50% (8/16) of the tumors, respectively (*3*). These losses were predominantly detected in the advanced stage of tumors and seem to be common in both hepatocellular carcinogenesis associated with hepatitis B virus infection and with non-A non-B hepatitis. On the other hand, incidence of LOH was low at other chromosomal loci, e.g., chromosomes 1p, 3p, 11p, 13q, or 17p, where LOH occurs frequently in other types of cancers such as carcinomas of the lung, kidney, bladder, and uterine cervix. Since there is no report showing frequent LOH on chromosome 16 or 4 in cancers other than hepatocellular carcinoma, recessive genetic changes involving sequences on these chromosomes seem to be specifically associated with development and/or progression of hepatocellular carcinoma.

We have performed an RFLP analysis for several other types of human tumors. One interesting observation is that LOH was detected even in a benign tumor. In pheochromocytoma, LOH on chromosomes 1p and 11p was detected in 75% (3 of 4 patients) and 60% (3 of 5 patients), respectively (*4*). In spite of our effort to determine the chromosomal loci of putative tumor-suppressor genes for stomach cancers by a similar approach, common chromosomal loci showing frequent LOH have not yet been determined in stomach cancer (*5*).

Loss of Heterozygosity on Chromosomes 3p, 13q, and 17p in Small-Cell Lung Carcinoma

In SCLC, two different types of genetic alterations have been reported: one is the deletion of chromosome 3 (*6,7*) and the other is the amplification of *myc* family oncogenes (*8,9*). We performed an RFLP analysis for 16 fresh tumors and 9 cell lines derived from SCLCs (*10,11*). As suggested by the cytogenetic studies, LOH on the short arm of chromosome 3 was observed in 100% (15/15) of the informative cases. However, an RFLP analysis revealed additional chromosomal changes, namely, the frequent occurrence of LOH on chromosomes 13q and 17p. LOH at chromosome 13q loci was observed in 15 of 16 cases (94%) and that on chromosome 17p loci occurred in all 16 cases analyzed (100%). The common regions of LOH on chromosomes 3, 13, and 17 reside between *D3S2* (3p14–p21) and *ErbA* (3p22–p24.1), between *D13S1* (13q12) and *D13S2* (13q22), and distal to *MYH2* (17p13.1), respectively. LOH on these chromosomes was simultaneously observed in four cases of stage I tumors and one case of an untreated tumor, and it occurred prior to N-*myc* amplification in the case of a stage IV tumor. These results suggest that LOH on chromosomes 3p, 13q, and 17p occurs relatively early in the development of SCLCs and may be critical for the genesis of the tumor rather than being a secondary event that takes place during the progression of the tumor, such as N-*myc* amplification.

LOH on chromosome 3p was also frequently observed in carcinoma of the uterine cervix (*1*), as described above, and in renal cell carcinoma (*12*). Thus, it is highly possible that inactivation of the same tumor-suppressor gene is involved in the development of these three types of tumors. It is also possible that three different genes for each type of tumor are located on the same region of chromosome 3p. The common region of LOH for chromosome 13q in SCLC contains the *Rb* locus at 13q14, reminiscent of retinoblastoma. We and others previously described abnormal transcripts and the absence of normal Rb protein in SCLC, suggesting that the remaining allele of the *Rb* gene is inactivated in these tumors (*13,14*). Similar abnormalities of the *Rb* gene have been observed in osteosarcoma and breast carcinoma, in which the frequent occurrence of LOH on 13q has also been reported (*15,16*). These findings strongly suggest that complete inactivation of the *Rb* gene is frequently the net result of LOH on chromosome 13q in several types of human tumors. Frequent LOH on the short arm of chromosome 17 has been reported in colorectal carcinoma, osteosarcoma, and breast carcinoma, and the p53 gene is supposed to be a target gene on this chromosome in these cancers (*15,17*). These re-

sults strongly suggested that at least three different tumor-suppressor genes are involved in the development of SCLC and may imply that at least six genetic alterations are necessary to convert a normal cell into a fully malignant cancer cell in the case of SCLC.

Two Different Types of Tumor-Suppressor Genes

In summary, it is concluded that chromosomal loci showing frequent LOH differ among each type of tumor. This result suggests that there are many different tumor-suppressor genes in the human genome that are located on several different chromosomes and that critical genes for each type of tumor are different among these tumors. In some cases LOH was detected even in the early stage of tumor, and in other cases LOH was observed only in the advanced stage of tumor. Therefore, we can propose that there are two different types of tumor-suppressor genes in the human genome. One is the tumor-suppressor genes whose inactivation is responsible for malignant formation of normal cells, and the other is the tumor-suppressor genes whose inactivation is responsible for progression of tumor cells. Future studies should focus on the cloning of critical genes from the chromosomal regions showing LOH in tumors. Characterization of these genes will provide us much useful information for understanding the biological role of LOH in tumor development.

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REFERENCES

1. Yokota, J., Tsukada, Y., Nakajima, T., Gotoh, M., Shimosato, Y., Mori, N., Tsunokawa, Y., Sugimura, T., and Terada, M. Loss of heterozygosity on the short arm of chromosome 3 in carcinoma of the uterine cervix. Cancer Res. 49: 3598–3601 (1989).
2. Suzuki, T., Yokota, J., Mugishima, H., Okabe, I., Ookuni, M., Sugimura, T., and Terada, M. Frequent loss of heterozygosity on chromosome 14q in neuroblastoma. Cancer Res. 49: 1095–1098 (1989).
3. Zhang, W., Hirohashi, S., Tsuda, H., Shimosato, Y., Yokota, J., Terada, M., and Sugimura, T. Frequent loss of heterozygosity on chromosome 4 and 16q in human hepatocellular carcinoma. Jpn. J. Cancer Res. 81: 108–111 (1990).
4. Tsutsumi, M., Yokota, J., Kakizoe, T., Koiso, K., Sugimura, T., and Terada, M. Loss of heterozygosity on chromosomes 1p and 11p in sporadic pheochromocytoma. J. Natl. Cancer Inst. 81: 367–370 (1989).
5. Wada, M., Yokota, J., Mizoguchi, H., Sugimura, T., and Terada, M. Infrequent loss of chromosomal heterozygosity in human stomach cancer. Cancer Res. 48: 2988–2992 (1988).
6. Little, C. D., Nau, M. M., Carney, D. N., Gazdar, A. F., and Minna, J. D. Amplification and expression of the c-*myc* oncogene in human lung cancer cell line. Nature 306: 194–196 (1983).
7. Nau, M. M., Brooks, B. J., Battey, J., Sauville, E., Gazdar, A. F., and Minna, J. D. L-*myc*, a new *myc*-related gene amplified and expressed in human small cell lung cancer. Nature 318: 69–73 (1985).
8. Whang-Peng, J., Kao-Shan, C. S., Lee, E. C., Bunn, P. A., Carney, D. N., Gazdar, A. F., and Minna, J. D. Specific chromosome defect associated with human small-cell lung cancer: deletion 3p(14–23). Science 215: 181–182 (1982).
9. Yokota, J., Wada, M., Yoshida, T., Noguchi, M., Terasaki, T., Shimosato, Y., Sugimura, T., and Terada, M. Heterogeneity of lung cancer cells with respect to the amplification and rearrangement of *myc* family oncogenes. Oncogene 2: 607–611 (1988).
10. Yokota, J., Wada, M., Shimosato, Y., Terada, M., and Sugimura, T. Loss of heterozygosity on chromosome 3, 13, and 17 in small-cell carcinoma and on chromosome 3 in adenocarcinoma of the lung. Proc. Natl. Acad. Sci. U.S.A. 84: 9252–9256 (1987).
11. Mori, N., Yokota, J., Oshimura, M., Cavenee, W. K., Mizoguchi, H., Noguchi, M., Shimosato, Y., Sugimura, T., and Terada, M. Concordant deletions of chromosome 3p and loss of heterozygosity for chromosome 13 and 17 in small cell lung carcinoma. Cancer Res. 49: 5130–5135 (1989).
12. Zbar, B., Braugh, H., Talmadge, C., and Linehan, M. Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. Nature 327: 721–724 (1987).
13. Yokota, J., Akiyama, T., Fung, Y. -K. T., Benedict, W. F., Namba, Y., Hanaoka, M., Wada, M., Terasaki, T., Shimosato, Y., Sugimura, T., and Terada, M. Altered expression of the retinoblastoma (RB) gene in small-cell carcinoma of the lung. Oncogene 3: 471–475 (1988).
14. Harbour, J. W., Lai, S. -L., Whang-Peng, J., Gazdar, A. F., Minna, J. D., and Kaye, F. J. Abnormalities in structure and expression of the human retinoblastoma gene in SCLC. Science 241: 353–357 (1988).
15. Toguchida, J., Ishizaki, K., Sasaki, M. S., Ikenaga, M., Sugimoto, M., Kotoura, Y., and Yamamuro, T. Chromosomal reorganization for the expression of recessive mutation of retinoblastoma susceptibility gene in the development of osteosarcoma. Cancer Res. 48: 3939–3943 (1988).
16. Lee, E. Y. -H. P., To, H., Shew, J. -Y., Brookstein, R., Scully, P., and Lee, W. -H. Inactivation of the retinoblastoma susceptibility gene in human breast cancers. Science 241: 218–221 (1988).
17. Nigro, J. M., Baker, S. J., Preisinger, A. C., Jessup, J. M., Richard, H., Clearly, K., Bigner, S. H., Davidson, N., Baylin, S., Devilee, P., Glover, T., Collins, F. S., Weston, A., Modali, R., Harris, C. C., and Vogelstein, B. Mutations in the p53 gene occur in diverse human tumour types. Nature 342: 705–708 (1989).