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Advances in Brief

Frequent Somatic Mutation of the *MTS1/CDK4I* (Multiple Tumor Suppressor/Cyclin-dependent Kinase 4 Inhibitor) Gene in Esophageal Squamous Cell Carcinoma[1]

Takahiro Mori, Koh Miura, Takahisa Aoki, Tetsuro Nishihira, Shozo Mori, and Yusuke Nakamura[2]

Department of Biochemistry, Cancer Institute, 1-37-1 Kami-Ikebukuro, Toshima-ku, Tokyo 170 [T. M., K. M., T. A., Y. N.], and Second Department of Surgery, Tohoku University School of Medicine, Sendai 980 [T. M., T. N., S. M.], Japan

Abstract

We previously reported frequent loss of heterozygosity on chromosome 9p in esophageal carcinomas and suggested that a tumor suppressor gene located on this chromosomal arm might be involved in development of these cancers. Since recently published studies have shown that a gene mapped on chromosome 9p21, *MTS1/CDK4I* (multiple tumor suppressor 1/cyclin-dependent kinase 4 inhibitor), is frequently mutated in various types of tumors, we chose to examine esophageal squamous cell carcinomas for mutations in this candidate gene. DNA sequence analyses revealed somatic mutations of *MTS1/CDK4I* in 14 of 27 tumors examined; 8 were frame-shift mutations and 6 were missense mutations. These results suggested that the *MTS1/CDK4I* gene is a tumor suppressor the inactivation of which plays an important role during carcinogenesis of the squamous cell type of esophageal carcinoma.

Introduction

Recent advances in molecular biology have revealed that the genesis and/or progression of tumors is due to accumulation of multiple genetic alterations, including inactivation of tumor suppressor genes and/or activation of protooncogenes (1–3). However, the molecular features of ESC[3] have remained unclear; the somatic mutations found thus far in this type of tumor have been limited to inactivation of the *p53* gene (4) and amplification of the *cyclin D* gene (5).

We previously reported an allelotype study of ESC which indicated that putative tumor suppressor genes on chromosomal arms 3p, 5q, 9p, 9q, 10p, 13q, 17p, 17q, 18q, 19q, and 21q might be associated with carcinogenesis in the esophagus (6). We were especially interested in 3p, 9p, and 9q with reference to ESC, because cytogenetic and molecular abnormalities in these chromosomal regions are frequently noted in squamous cell tumors of the esophagus, lung, head, and neck (7–9).

A putative tumor suppressor gene, *MTS1/CDK4I,* was isolated recently and mapped on one of these candidate loci, at 9p21 (10, 11). Mutations of *MTS1/CDK4I* have been reported in melanoma cell lines and in lymphoblastoid cell lines derived from dysplastic nevus syndrome (10, 11). Therefore, we considered it a candidate gene for ESC and looked for somatic mutations in 27 ESCs. Here we present evidence that inactivation of *MTS1/CDK4I* does play a significant role during esophageal carcinogenesis.

Materials and Methods

Tumor Samples. Genomic DNAs from esophageal squamous cell carcinomas and their corresponding normal tissues were extracted from frozen tissues (6).

Mutation Analysis. We looked for *MTS1/CDK4I* mutations in 27 ESCs by sequencing the DNA of exon 2 of this gene. In brief, this exon was amplified by polymerase chain reaction with the primers: 5′-TATAAGCTTGGCTCTACACAAGCTTCCTT-3′ and 5′-TATTCTAGATGAGCTTTGGAAGCTCTCAG-3′. Polymerase chain reaction products were subcloned into pBluescript SK(−) (Stratagene, La Jolla, CA) and a mixture containing at least 50 subclones was used as template for DNA sequencing as described elsewhere (12). Sequencing primers were as follows:

5′-TACAAATTCTCAGATCAT-3′;
5′-CCGGCCCCCACCCTGGCT-3′;
5′-ACACGCTGGTGGTGCTGC-3′;
and 5′-CCAGGTCCACGGGCAGA-3′.

Results and Discussion

We have examined exon 2 of the *MTS1/CDK4I* gene, which covers the majority of the coding region, in esophageal tumors by the DNA-sequencing method. Fig. 1 shows two examples of results that revealed *MTS1/CDK4I* mutations; in case 111, a missense mutation at codon 66 resulting in a change from aspartic acid to asparagine is clearly observed; in case 117, extra bands indicate deletion of one base at codon 97. The experiments were repeated to confirm these genetic alterations. Comparisons of these DNA sequences with DNA from corresponding normal tissues confirmed that the changes were somatic events. A total of 14 somatic mutations of the *MTS1/CDK4I* gene were detected among 27 tumors examined as shown in Table 1. Among them, 8 were frame-shift mutations due to deletion of 1, 2, or 50 base pairs, and 6 were missense mutations. The results clearly indicated that inactivation of the *MTS1/CDK4I* gene plays an important role in development or progression of ESC.

We venture to predict that allelic deletions on 9p21 and on 17p at the *p53* locus occur during transformation of precancerous dysplastic cells to cancer cells in the esophagus,[4] as *p53* mutations do in colorectal carcinoma (3). Because the protein encoded by *MTS1/CDK4I,* p16, has been proposed as a general inhibitor of cdk4 (10, 11) and *p53* is thought to regulate S-phase entry through interaction with $p21^{CIP1/WAF1}$ (13–17), loss of function with respect to G_1 arrest seems to be necessary for progression of a precancerous lesion to malignancy. Inasmuch as other reported molecular aberrations in ESC include amplification of the *cyclin D1* locus *(PRAD1)* (5, 18) and alteration of *Rb1* mRNA (19), accumulation of mutations among cell cycle regulators may be responsible for carcinogenesis and/or progression of ESC.

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[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: ESC, esophageal squamous cell carcinoma; *MTS1,* multiple tumor suppressor 1; *CDK4I,* cyclin-dependent kinase 4 inhibitor.

[4] T. Mori and Y. Nakamura, unpublished data.

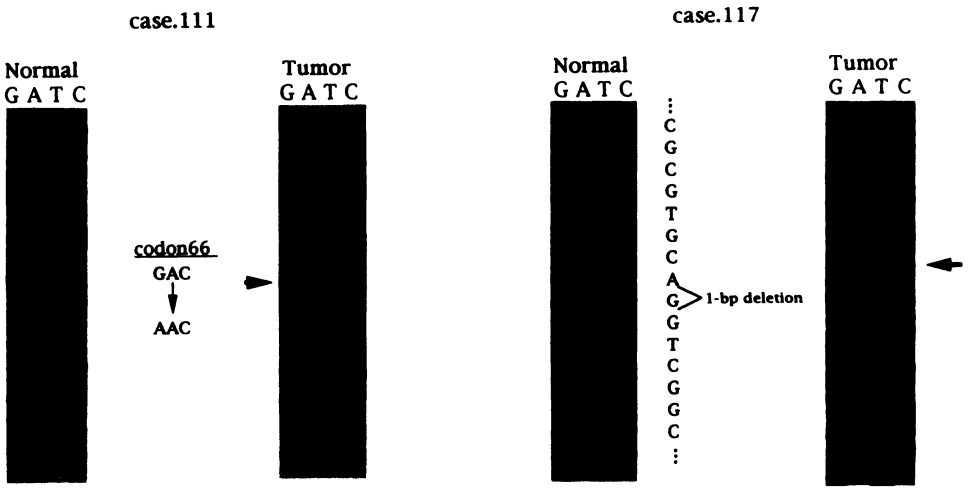

Fig. 1. Examples of DNA sequence which reveal mutations of the *MTS1/CDK4I* gene in ESC patients: case 111, base substitution from guanidine to adenine, resulting in amino acid change from aspartic acid to aspargine; case 117, deletion of guanidine at codon 97 *(arrow)*.

Table 1 *Mutations of MTS1/CDK4I in ESCs*

Case	Nucleotide change	Codon	Coding change
19	ATGGGCAGC → ATGGCAGC	47	1-base pair deletion
44	CTGCTC . . . CCCGTG → CTGCGTG	57–73	50-base pair deletion
114	GCG → ACG	60	Ala → Thr
36	TGC → GGC	64	Cys → Gly
111	GAC → AAC	66	Asp → Asn
37	ACCCGACCC → ACCCACCC	72	1-base pair deletion
28	GAC → AAC	76	Asp → Asn
48	CGGCTGGAC → CGGTGGAC	96	1-base pair deletion
110	CGGCTGGAC → CGGTGGAC	96	1-base pair deletion
117	CTGGACGTG → CTGACGTG	97	1-base pair deletion
11	CGC → CAC	116	Arg → His
23	GGGGGCACC → GGGCACC	128	2-base pair deletion
22	CGC → TGC	136	Arg → Cys
46	GCCCGCATA → GCCGCATA	136	1-base pair deletion

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References

1. Fearon, E. R., and Vogelstein, B. A genetic model of colorectal tumorigenesis. Cell, *61:* 759–767, 1990.
2. Knudson, A. G. All in the (cancer) family (review). Nat. Genet., *5:* 103–104, 1993.
3. Ichii, S., Takeda, S., Horii, A., Nakatsuru, S., Miyoshi, Y., Emi, M., Fujiwara, Y., Koyama, K., Furuyama, J., Utsunomiya, J., and Nakamura, Y. Detailed analysis of genetic alterations in colorectal tumors from patients with and without familial adenomatous polyposis (FAP). Oncogene, *8:* 2399–2405, 1993.
4. Hollstein, M. C., Metcalf, R. A., Welsh, J. A., Montesano, R., and Harris, C. C. Frequent mutation of the *p53* gene in human esophageal cancer. Proc. Natl. Acad. Sci. USA, *87:* 9958–9961, 1990.
5. Jiang, W., Kahn, S. M., Tomita, N., Zhang, Y. J., Lu, S. H., and Weinstein, I. B. Amplification and expression of the human cyclin D gene in esophageal cancer. Cancer Res., *52:* 2980–2983, 1992.
6. Aoki, T., Mori, T., XiQun, D., Nishihira, T., Matsubara, T., and Nakamura, Y. Allelotype study of esophageal carcinoma. Genes Chromosomes Cancer, in press, 1994.
7. Lukeis, R., Irving, L., Garson, M., and Hasthorpe, S. Cytogenetics of non-small cell lung cancer: analysis of consistent non-random abnormalities. Genes Chromosomes Cancer, *2:* 116–124, 1990.
8. Ah-See, K. W., Cooke, T. G., Pickford, I. R., Soutar, D., and Balmain, A. An allelotype of squamous carcinoma of the head and neck using microsatellite markers. Cancer Res., *54:* 1617–1621, 1994.
9. van der Riet, P., Nawroz, H., Hruban, R. H., Corio, R., Tokino, K., Koch, W., and Sidransky, D. Frequent loss of chromosome 9p21–22 early in head and neck cancer progression. Cancer Res., *54:* 1156–1158, 1994.
10. Kamb, A., Gruis, N. A., Weaver-Feldhaus, J., Liu, Q., Harshman, K., Tavtigian, S. V., Stockert, E., Day, R. S., III, Johnson, B. E., and Skolnick, M. H. A cell cycle regulator potentially involved in genesis of many tumor types. Science (Washington DC), *264:* 436–440, 1994.
11. Nobori, T., Miura, K., Wu, D. J., Lois, A., Takabayashi, K., and Carson, D. A. Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature (Lond.), *368:* 753–756, 1994.
12. Nigro, J. M., Baker, S. J., Preisinger, A. C., Jessup, J. M., Hostetter, R., Cleary, K., Binger, 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 tumor types. Nature (Lond.), *342:* 705–708, 1989.
13. Serrano, M., Hanson, G. J., and Beach, D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature (Lond.), *366:* 704–707, 1993.
14. Xiong, Y., Hannon, G. J., Zhang, H., Casso, D., Kobayashi, R., and Beach, D. p21 is a universal inhibitor of cyclin kinases. Nature (Lond.), *366:* 701–704, 1993.
15. Gu, Y., Turck, C. W., and Morgan, D. O. Inhibition of CDK2 activity *in vivo* by an associated 20K regulatory subunit. Nature (Lond.), *366:* 707–710, 1993.
16. El-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. WAF1, a potential mediator of p53 tumor suppression. Cell, *75:* 817–825, 1993.
17. Dulić, V., Kaufmann, W. K., Wilson, S. J., Tlsty, T. D., Lees, E., Harper, J. W., Elledge, S. J., and Reed, S. I. p53-dependent inhibition of cyclin-dependent kinase activities in human fibroblasts during radiation-induced G_1 arrest. Cell, *76:* 1013–1023, 1994.
18. Motokura, T., Bloom, T., Kim, H. G., Jüppner, H., Ruderman, J. V., Kronenberg, H. M., and Arnold, A. A novel cyclin encoded by a bcll-linked candidate oncogene. Nature (Lond.), *350:* 512–515, 1991.
19. Huang, Y., Meltzer, S. J., Yin, J., Tong, Y., Chang, E. H., Srivastava, S., McDaniel, T., Boynton, R. F., and Zou, Z-Q. Altered messenger RNA and unique mutational profiles of *p53* and Rb in human esophageal carcinomas. Cancer Res., *53:* 1889–1894, 1993.