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FREQUENT DELETION OF MTS1/p16 GENE AND CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS IN ENDOMETRIAL CARCINOMA

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Objective: To investigate the possible relationship between deletion of MTS/p16 gene and progression of endometrial carcinoma. Methods: Forty-six primary endometrial carcinoma, 7 tumor-adjacent endometrial tissue, 10 normal endometrial tissue specimen and 5 xenografts from patients with endometrial carcinoma were examined for homozygous deletion of MTS/p16 gene by polymerase chain reaction-based analysis. Results: Of 46 endometrial cancer specimens, 9 showed homozygous deletion, no deletion was detected in the tumor-adjacent and normal endometial tissues. Nor was it detected in well-differentiated endometrial carcinoma and all xenografts. Conclusions: Deletion of MTS1/p16 gene might contribute to the progression of endometrial carcinoma and could be served as indicator for predicting prognosis.

Key words: Endometrial carcinoma, MTS1/p16 gene, Gene deletion, Polymerase chain reaction.

Endometrial carcinoma is one of the most common gynecological malignant tumors in China. The tumorigenesis of endometrial carcinoma is thought to be a multistep process and involves multistep genetic changes.[1] Thus far, not much is known about the genetic changes involved in development of this disease. There are reports on alteration of several proto-oncogene and tumor suppressor, such as p53, Rb, c-*myc*, c-*fos* and *ras*. Recent, one highly related member of the cyclin-dependent kinase inhibitor family, termed p16 (MTS1) is isolated from the chromosome band 9p21–22.[2,3] The high frequency of p16 gene alterations initially reported in tumor- derived cell lines and in some tumor types made this gene an excellent candidate for a tumor suppresser. Emerging evidence suggests that homozygous deletion of the p16 gene are involved in the progression of certain tumor types. However, there has been no reported in literature at home on the deletion of the p16 gene in endometrial carcinoma. The present study aimed to investigate whether homozygous deletion of the p16 gene has taken place in endometrial carcinoma and attempt to further elucidate the relationship between the p16 gene and the development of the disease.

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MATERIALS AND METHODS

Tissue Samples and Xenografts

Forty-six cases of primary endometrial carcinoma and 7 adjacent tissues were obtained from Department of Gynecological Oncology at the Cancer Hospital, Chinese Academy of Medical Sciences. Tissues were fresh frozen in liquid nitrogen followed pathological examination and kept in -70ºC. None of the patients had been treated previous with radiotherapy or chemotherapy. 5 xenografts were involved in this study. Tumor modes named SL-1, SL-2, SL-3, SL-4 and SL-5, which had been xenografted subcutaneously with 2 mm^2 fresh surgical samples of

human endometrial carcinoma into nude mice, and the tumor maintained in nude mice exceeding 10 passage were used before our experiment. Nude mice were bred and maintained incircumastances free of specific pathogen.

DNA Extraction

Genomic DNA was extracted by using the proteinase K-phenol-chloroform method. Concentrations of DNA stocks were estimated by spectrophotometer.

Detection of Homozygous Deletion in p16 Gene by PCR

All xenografts, 46 primary tumor samples 7 adjacent and 10 normal endometrial tissue specimens were examined for homozygous deletion of the p16 gene by PCR amplification. The primers used to amplify exon2 of the p16 gene were:

pst1:5'-GGCTCTACACAAGCTTCCCTT-3',

pst2:5'-TGAGCTTTGGAAGCTCTCCAG-3'.

The primers of β-actin as an inner control were presented:

Sense:5'-GCCGCCACCA CCATGTACCCT-3'

Antisense:5'AGGGGCCGGACTCGTCATACT-3'

The PCR amplification consisted of 30 cycles, under the following conditions: 94c for 50 sec, 56c for 45sec 72c for 1 min after the initial denotation step (95c for 5 min). The expected sizes of the amplification products were 312 base pairs for p16 and 421 base pairs for β-actin gene. PCR products were electrophonsed on a 1.5% agrose gel containing 1 mg/ml ethidium bromide and visualized by UV. The signals of the p16 gene in tumor and adjacent samples were compared when the signals of the β-actin gene were identical. Homozygous deletion of the p16 was scored if the signal of the p16 missing or highly reduced in the tumor samples when compared to the β-actin.

RESULTS

Homozygous Deletion of the p16 Gene in Endometrial Carcinoma

No homozygous deletion of p16 gene was detected in any of the 5 endometrial carcinoma xenografts, adjacent tissues and normal tissues. For the 46 cases of primary tumors, 9 showed homozygous deletion of the p16 gene. The signal intensities of the p16 were highly reduced or missed in tumor samples of these cases, while the control marker showed normal intensities in tumor DNA (Figure 1).

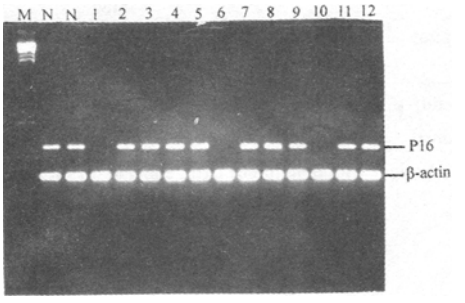

Fig. 1. PCR analysis of deletion of the p16 gene in endometrial carcinoma. M: molecular weight marker. N: normal tissue. Lanes 1–11: endometrial carcinoma. Lanes 12: adjacent tissue.

Homozygous Deletion of the p16 and its Relation to Pathology

The relationship between pathological findings of 46 patients and the deletion of the p16 gene is summarized in Table 1. To clarify the meaning of the difference in the p16 gene deletion between primary tumors and xenografts, 5 low passage nude mice modes were examined. We found that there were not homozygous deletion of p16 gene in 5 xenografts when compared with their original tumors.

Table 1. p16 gene deletion in 46 cases of endometrial carcinoma and pathological differentiation

Pathology	No.	Grade		
		G1	G2	G3
Adenocarcinoma	35	0/16	4/13	2/6
Adenosquamous	5	0/2	1/2	1/1
Clear cell	3	-	1/2	0/1
Papillary	3	0/2	0/1	-

Homozygous Deletion of the p16 and its Relation to Clinical and Surgical Stage

Among 9 cases of homozygous deletion of the p16, 6 patients dealt with surgical stage were II–IV and 2 for stage I respective (Table 2).

Table 2. p16 deletion in 46 cases of endometrial carcinoma and surgical and clinical stage

Staging	No.	Stage			
		I	II	III	IV
Operative*	40	2/19	3/9	1/7	2/5
Clinical	46	6/31	3/11	0/3	1/1

*One patient with deletion of p16 gene had no surgical stage

DISCUSSION

Chromosomal loss at 9p21–22 has been implicated in the genesis of several different tumors. p16 gene isolated from this region was found by Kamb and Noborl in 1994. Its intact encoding area includes 3 exon and 2 intron, encoding a 16 KD protein. This p16 protein is one of the cyclin-dependent kinase inhibitors, and inhibits the catalytic activity of CDK4 and CDK6 complexes. It can regulate the G1/S transition of cell cycle and control cell growth. Deletion of the p16 gene has been reported in a vaiety of human cancers, including leukemia, lung cancer, glioma, esophageal cancer, gastric cancer and melanoma. After wild-type p16 gene infects to tumor cell lines with deletion of the p16, it can suppress tumor cell growth.[3,4] Therefore, the p16 gene appears to be a multiple tumor suppresser gene involved in the oncogensis of human cancer.

In has proved that inactivity of the p16 gene mainly includes deletion, mutation, rearrangement and methylation.[5,6] High frequency of homozygous deletion has reported in human cancer cell lines and some solid tumors. However, most studies have shown that deletion of the p16 gene was less common in primary tumors than in cell lines, suggesting that the p16 gene alterations might provide some advantage associated with the growth of tumor cells and maintained the malignant phenotype of tumor cells *in vitro*.[4,7] In contrast to previously identified the suppresser gene such as p53, it appears that homozygous deletion rather than point mutation is predominant alteration at p16 gene.[3,8]

Frequent deletion of the p16 gene is not the same in tumor of different type and the same type with different malignancy. Kinoshita[9] et al. reported that p16 gene deletion may be associated with the progression and metastases of tumor, suggesting that the presence of p16 gene may involve in the development and progression of tumor. We examined genomic DNAs derived from 46 patients with endometrial carcinoma as well as 5 xenografts originating from 5 patients in this study, which a homozygous deletion of p16 gene was detected in 9 of 46 primary endometrial carcinoma (19.56%). No deletion of the p16 were fund in adjacent tissues, normal tissues, grade 1 tumors and 5 xenografts, showing a clear specificity in the affected histological types. Our observation is consistent with the results of recent molecular studies, in which a high frequency of homozygous deletion in the p16 gene was reported in various human cancers. The reason that adjacent tissues have no deletion of the p16 is that the adjacent tissues could contain a great deal of normal tissues and DNA amplification using PCR is very sensitive method. The incidence of homozygous deletion in the p16 gene may be an underestimation in non-cultured samples using PCR.

Table 1 shows that the p16 gene homozygous deletion was detected in 6/35 adenocarcinomas, 1/3 clear cell carcinoma, and 2/5 adenosquamous carcinoma, in which histological classification engage as 3 grade 3, 6 grade 2. According to FIGO surgical stage, 6 patients with p16 deletion contributed to stage II–IV, 2 for stage I (Table 2). Because of few cases in each group and short of long-term follow up, this difference was not statistically significant in early or late stage. In addition, no deletion of the p16 gene was observed in 5 low passage xenografts when compared with their original tumor. On the base of results of present study, however, deletion of the p16 gene may be related to the late stage, low differentiation, and high malignancy. This observation supports the notion that deletion of the p16 gene is a relatively late event in tumorigensis, possible associated with the progression and prognosis of endometrial carcinoma. We postulate that deletion of the p16 could potentially be used as one of the predicting factors of the progression.

Taking into account all of our data in the present and those of others, homozygous deletion of p16 gene appears to be one of the most frequent genetic events in endometrial carcinoma and may play a crucial role associated with progression of tumor. Further studies need to be performed to characterize mutation, rearrangement, and methylation of the p16 gene in

genesis and development of this disease. This will be a subject of our major concern.

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