The Mutation of Nm23–H1 in Patient with Ovarian Carcinoma

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Abstract **Object** The mutation of nm23-H1 gene was studied in our research in order to look for an index for monitoring mutation and instructing treatment of ovarian carcinoma. **Method** A safe and simple method, polymerase chain reaction (PCR)-single strand conformational polymorphism (SSCP) analysis , was performed in our study to examine mutation in 5 exons of nm23-H1 gene. **Results** Mutation was found in 9 cases out of 32 cases malignant ovarian tumors(28.1%), while no mutation was found in normal ovaries, benign ovarian tumors and borderline ovarian tumors. The rate of mutation in malignant cases was significantly higher than in the other three types. In pathological grade III was higher than those in grade I, II. Among ovarian carcinomas with different histological types, The rate of mutation of nm23-H1 gene in serous carcinoma was higher than in the other three types, Among different clinical stages , the rates of mutation of nm23-H1 gene in stages III, IV were higher than those in stages I, II. In cases with lymph node mestatases was higher than in the cases without lymph nodal mestatases. **Conclusion** These results suggested that mutation of nm23-H1 gene tend to exist in advanced, serous ovarian carcinoma with lymph node mestatases and in the grade III differentiated tissues.

Key Words Ovarian Carcinoma; nm23-H1 gene; mutation; PCR-SSCP

According to lately researches, there are complicated genetic changes in human neoplasma. These changes exist in many stages of tumor development and are associated with various gene changes. But exactly genetic changes are still unknown. The mortality of ovarian carcinoma is the highest among all kinds of gynecological tumors. It is hard to discover an ovarian tumor because of the anatomical position, so most of the cases are advanced ones. Furthermore, it is still effective that treating the patients with combined twice exploration and chemical therapy which is different from other tumors. So, Finding out indicators to supervise the metastasis of ovarian cancer and guide therapy becomes a important project .

Among numerous metastasis suppressor, nm23-H1 is most noticeable while little research has been performed in the mutation of nm23-H1 gene in ovarian carcinomar[1]. In 1989, Orita[2] reported SSCP (single strand conformation polymorphism) was applied to detect changes of bases in product of PCR (polymerace chain reation). In this study, to decide the correlation between mutation of nm23-H1 and histological types, pathological degree, clinical stages and lymphatic metastasis, 32 samples from patients with ovarian carcinoma were assayed. We applied SSCP technique, stained by EB, to detect product of PCR for 5 exons of nm23-H1gene.

MATERIAL AND METHODS

Material

Samples Mutation in 5 exons of nm23-H1 gene was examined in samples from 6 normal ovaries, 15 benign ovarian tumors, 6 borderline ovarian tumors and 32 malignant ovarian tumors. 32 malignant ovarian tumors were constituted by 16 serous carcinoma, 9 mucinous carcinoma, 6 endometrioid carcinoma and 1 clear cell carcinoma. The clinical stages of ovarian carcinoma were decided by criterions of FIGO, 5 cases in stage I, 3 cases in stage II, 11 cases in stage III and 13 cases in stage IV. As to degree of differentiation, there were 20 cases in grade I, 6 cases in grade II and 6 cases in grade III. 6 cases of normal ovary were also examined as control group.

Instruments Thermal cycler (PE480), ultraviolet spectrophotometre (753B model Ⅱ), electrophoresis apparatus (DY-600) and vertical electrophoresis tank (V16).

Reagents

The sequences of primer was according to reference

[3] and was designed and synthesized by Bao Biological Company, estimating primer's concentration with high pressure liquid chromatograph (table 1).

Protease K was bought from Sigma Corporation, Taq DNA polymerase, dNTPs, d.d. H_2O, 10×buffer, solution of $MgCl_2$ were bought (15mmol/L) from Bao Biological Company, RNA hydrolase from Promega and DNA standard molecular weight from Huamei Bioengineer Company.

METHODS

Extraction of DNA and estimation of its concentration and pureness

Method of DNA extraction refered to[4], and stored at 4℃.

Estimated the concentration and pureness of DNA with ultraviolet spectrophotometre. OD260 ranged from 0.2 to 0.6, discarding those less than 0.1.

Estimating DNA pureness with value of OD260/OD280, the outcoming should be >1.8.

Amplified the 5 exons of nm23 –H1 gene with PCR

Reaction system of PCR: 0.5ul template \5ul 10× buffer \4ul (200uM) 4×dNTP \7.5pmol primer each\ 1.2mM Mg2+ \1.25U Tag polymerase, added d.d. H_2O to 50ul, slightly centrifugated and put in 50ul paraffin oil.

Condition of amplification

First cycle: 94℃ 5min\57℃ 1min\72℃ 1min Followed 35 cycles: 94℃ 1min\57℃ 1min\72℃ 1min

Last cycle: 94℃ 1min\57℃ 1min\72℃ 5min Carried on electrophoresis on 1.5% sepharose at 80V voltage for 45min (0.5×TBE), visualized the amplificated products by ultraviolet illumination.

Retrieved target lanes and performed 2nd PCR

The retrieved DNA was electrophoresed once more to assure specific extracting product retrieved[4]. To reaction system retrieved product was added, deciding the volume of template according to the brightness of lanes. Reaction system as followed:

1ul-6ul template\8ul 4 ×dNTPs\5ul 10 ×Buffer \ 6-9pmol primer each\ 1.2mM Mg2+\1.25U Taq polymerase, added d.d. H_2O to 50ul. The program of amplifying was the same as former.

Carried on electrophoresis on 1.5% sepharose at 80V voltage for 45min (0.5×TBE), visualized the amplificated products by ultraviolet illumination and took

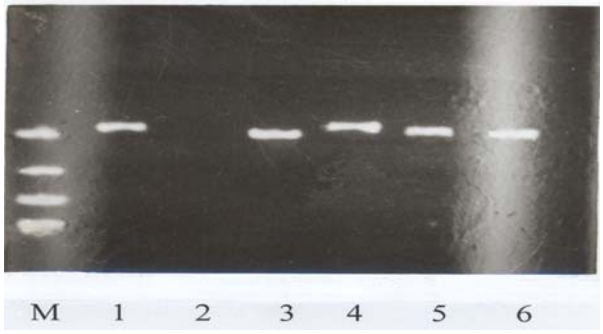

Fig.1 Some PCR extracted product from 5 exons of nm23-H1 gene in ovarian carcinoma
M: standard molecular weight (PBR322/BS+NI)
Lane 1, 3, 4, 5, 6 are PCR extracted product;
Lane 2 is negative control

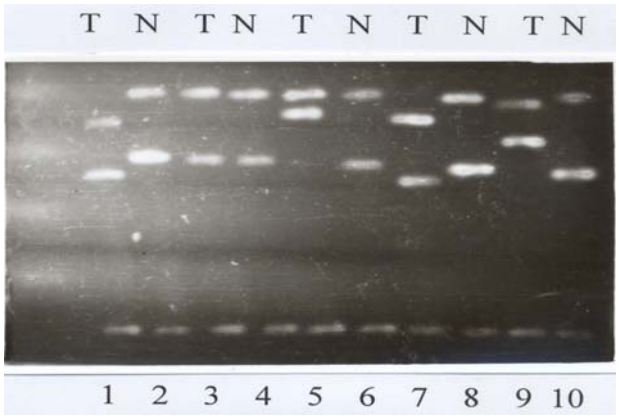

Fig.2 Analysis 5 exons of nm23-H1 gene for samples from ovarian carcinoma by means of SSCP Mutation was found in Sample 1, 5, 7, 9; in sample 1,5, mutation on exon 2; in sample 7, 9 mutation on exon 3
T: sample for tumor
N: normal tissue of the same patients as control

Fig.3 Analysis 5 exons of nm23-H1 gene for sample of ovarian carcinoma by SSCP Mutation was found in sample 1, 3, 5, 9, 11; in sample 3, 5, mutation on exon 4; in sample 9, 11, mutation on exon 5
T: sample for tumor
N: normal tissue of the same patients as control

pictures.

Analyse mutation of nm23-H1 by SSCP

Filled gel as described by Huang Shangzhi [5] and analysed mutation in 10ul product of 2nd PCR by means of SSCP. Observed with 254nm ultraviolet transmission and took picture.

Statistics: X2 test

RESULTS

The mutation rate of nm23-H1 gene in ovarian tissue

5 exons of nm23-H1 gene were amplified by PCR (see Fig.1) and examined the mutation by SSCP (Fig.2). The mutation rate was 28.1% (9/32), while no mutation was found in 6 normal ovaries, 15 benign ovarian tu-

Table 1 The sequences of 5 exons of nm23-H1 gene

Exon	The sequence of primers in PCR
No.1 exon	5'-CAACAGTGAGGCGTACCTTCA-3'
	5'-ATCCAGTTCTGAGCACAGCT-3'
No.2 exon	5'-TGGATCCTCTTGCAGCAGCC-3'
	5'-AACCCTTGTCCTTACCAGAA-3'
No.3 exon	5'-TGCAGCCGGAGTTCAAACCT-3'
	5'-GCGGAATCCTTTCTGCTACG-3'
No.4 exon	5'-TCTCCTACAGCCACCTGAAG-3'
	5'-CTGACGCACACCTATTGCAA-3'
No.5 exon	5'-TGTTGCTGCAGATCCGTGGG-3'
	5'-GAGGTCACTCACCTGGAGTG-3'

Table 2 The relationship between mutation of nm23-H1 gene and pathological data of ovarian carcinoma

Pathological classification	nm23-H1$^+$ (case number)	nm23-H1$^-$ (case number)	Rate(%)	Value of P
Ovarian samples				
norma	0	6	0	
benign tumor	0	15	0	
borderline tumor	0	6	0	
ovarian cancer	9	23	28.1	<0.05(compared with former three)
Histological type				
serous cancer	7	9	43.8	
mucinous cancer	1	8	11	
endometrioid cancer	1	5	16.7	
clear cell cancer	0	1	0	<0.01(compared serous ones with others)
Degree of differentiation				
Ⅰ	2	18	10	
Ⅱ	2	4	33.3	
Ⅲ	5	1	83.3	<0.01(compared with former two)
Clinical stages				
Ⅰ	0	5	0	
Ⅱ	0	3	0	
Ⅲ	4	7	36.3	
Ⅳ	5	8	38.5	<0.05(compared stages Ⅲ,Ⅳ and stages Ⅰ,Ⅱ)
Metastases of lymph node				
Positive	9	16	36.0	<0.05
negative	0	7	0	

mors and 6 borderline ovarian tumors. The rate of malignant was significantly higer than the other three types (P<0.05)(table 2). As for the site of mutation, it was found on no.2 exon for 2 cases, on no.3 exon for 3 cases, on no.4 exon for 2 cases and on no.5 exon for 2 cases, while no mutation was found on no. 1 exon.

The correlation between mutation of nm23 -H1 gene and histological types (table 2)

The mutation rate of serous carcinoma, mucinous carcinoma, endometrioid carcinoma and clear cell carcinoma was 43.8% (7/16), 11.1%(1/9), 16.7% (1/6) and 0 (0/1), respectively. The mutation rate of serous carcinoma was significantly higher than those of the other three types(P<0.01).

The correlation between mutation of nm23-H1 gene and differentiating degree of ovarian carcinoma (table 2)

The mutation rates of nm23-H1 gene were 10% (2/20), 33.3% (2/6) and 83.3% (5/6) respectively in differentiate grade I, II and III. It was significantly different when the later was compared with the former two types (P<0.01).

The correlation between mutation of nm23-H1 gene and clinical stages of ovarian carcinoma (table 2).

No nm23-H1 gene mutation was found in cases of stage I and II ovarian carcinoma; while the mutation rates in stage III and IV were 36.3% and 38.5% respectively, which were significantly higher than those in stage I and II (P<0.05).

The correlation between mutation of nm23-H1 gene and metastases of ovarian carcinoma (table 2).

The mutation rate was 36% (9/25) in cases conbined with metastases.

DISCUSSION

The mutation rate of nm23-H1 gene in ovarian carcinoma

It has been confirmed by many researchers that nm23-H1 gene is a inhibiting gene for cancer metastases. The mutation rates of nm23-H1 gene were 71.4%, 64% and 16.7% respectively in neuroblastma, cancer of breast and colon. But few gynecologic researches had been done, especially, much less research about ovarian carcinoma. In our research, no mutation was found in normal ovaries, benign ovarian tumors, borderline ovarian tumors and stage I, II ovarian carcinoma, which was consistent with Mandai's conclusion[6]. The mutation rate was 28.1% (9/32) in stage III and IV ovarian carcinoma here, while the result of Mandai's research was 2.9% (1/35). The reason of this inconsistency might be that the incidence of ovarian carcinoma in Japan is lower than that in China; furthermore, only 2 kinds of cancer were choosed by Mandai, serous and endometrioid carcinoma, most of which were in early stages and only 11 cases (31.4%) combined with metastasis. In our research, all histological types were chosen, serous, mucinous, endometrioid and clear cell carcinoma; what's more, there were 24 cases (75%) combinedwith metastasis. So, the differences of the ases and the number of cases in late stages might come to different results.

Mutation of nm23-H1 gene and histological types

We found that the mutation rate of nm23-H1 gene in serous carcinoma was 43.8%, which was much higher than those in other types. Many researchers had reported that the lymph metastasis on rate of serous carcinoma was significantly higher than other ovarian carcinoma [8-10], the results suggested that the mutation of nm23-H1 gene might be a mechanism causing metastatsis of cancer.

Mutation of nm23 -H1 gene and differentiative degree of ovarian carcinoma

Hennessy[11] reported that the higher degree of differentiation of breast cancer, the more expression of nm23-H1 mRNA had been detected. We found that the mutation rate of nm23-H1 gene in ovarian carcinoma with low differentiation was higher than that of high and median differetiantion. The rate of metastasis in low differetiation was significantly higher than other[9]. This reconfirmed that the mutation of nm23-H1 gene might play a role in metastasis by influencing the expression of gene.

Mutation of nm23-H1 gene and clinical stages of ovarian carcinoma

It was reported that the expression of nm23-H1 gene is related with clinical stage in ovarian carcinoma, i.e. it was lower in stage III and IV than that in stage I and II[12, 13]. Mandai[14] also got the same result by mRNA assay. In our results, the mutation rate of nm23-H1 gene in stage III and IV was higher than that in stage I and II, which was consistent with the results of Mandai[6] and Leary[15].

Mutation of nm23-H1 gene and metastases of ovarian carcinoma

Nm23-H1 gene is a generally accepted inhibiting gene for metastasis of several kinds of human cancer. Liu Deshun found that the expression of nm23 gene in ovarian carcinoma with lymph node metastasis was less than that without metastasis by means of immunohisto-

chemistry [13]. Mandai [16] got the same conclusion by means of RT-PCR. In our research, the mutation rate of nm23-H1 gene was 37.5% (9/24) among cases with lymph metastasis; while none was found in stage I and I-I. It shows that the mutation of nm23-H1 gene is related with metastasis of ovarian carcinoma, and supports that nm23-H1 is a metastasis inhibiting gene for cancer. It was reported that metastasis was found in stage I and II [8-10], while no mutation of nm23-H1 gene was detected in stage I and II in our research. The reason might be short of cases in stage I and II there are some other genes involved in metastasis of cancer.

These results suggested that mutation of nm23-H1 gene tend to exist in advanced, serous ovarian carcinoma with lymph node metastasis and grade III differentiation, i.e. it is related with histological types, degree of differentiation and clinical stages, which supports that nm23-H1 gene is a inhibiting gene of cancer metastasis.

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