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Evaluation of the Tyrosine Kinase Domain of the *Met* Proto-oncogene in Sporadic Ovarian Carcinomas*

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Most of the ovarian cancers originate from the ovarian surface epithelium derived from the coelomic mesothelium. The *Met* proto-oncogene encodes a transmembrane tyrosine kinase receptor (Met) that has the capacity to regulate cell proliferation and differentiation and it is activated by hepatocyte growth factor. Trisomy of chromosome 7 and *Met* protein overexpression have been observed in ovarian carcinomas, the papillary renal cancers and other solid tumors. Frequent mutations of *Met* proto-oncogene have been found in hereditary papillary renal cancer (HPRC) and most of the muta-

tions are located in the tyrosine kinase domain. The aim of this study to perform a mutation analysis of exons 17–19 of *Met* proto-oncogene in epithelial ovarian tumors (EOTs). We have examined 24 tumor samples from patients, operated with EOTs. Mutation was detected in exon 18 in only one sample of 24 EOTs. Our results indicate that mutations located in the *Met* proto-oncogene is not a common event in EOT. It is not clear whether the mutation plays a role in the tumorigenesis or progression of EOT or not. (Pathology Oncology Research Vol 5, No 3, 187–191, 1999)

Keywords: *Met* proto-oncogene, epithelial ovarian tumor, tyrosine kinase domain, mutation

Introduction

The *Met* proto-oncogene product (Met) is the high-affinity receptor for hepatocyte growth factor/scatter factor (HGF/SF) belonging to the tyrosine kinase growth factor receptors family.³ The HGF/Met pathway has remarkably diverse biologic functions in different tissues and has been implicated in mitogenic responses, cellular motility, proliferation, morphogenic differentiation, and development of numerous organs, invasion, and wound healing.^{2,10,15,25} The HGF/Met system acts as a mediator between the mesenchymal and epithelial tissues because HGF is produced by mesenchymal cells and c-Met is mainly expressed on various epithelial cells.²³ Ovarian

cancers originate, in approximately 90% of cases, from the ovarian surface epithelium. This epithelium is derived from the coelomic mesothelium.¹⁶ The *Met* proto-oncogene has been found to be activated in gastric carcinoma, where it is activated and overexpressed, and overexpressed without amplification in primary thyroid carcinomas derived from the follicular epithelium.^{6,19} Missense mutations were observed in the tyrosine kinase domain of *Met* in patients suffering from hereditary papillary renal carcinomas. Trisomy 7 is the characteristic genetic alteration in papillary renal cancer and it has been discovered that the duplicated chromosomes harbour the mutated *Met* allele.^{1,11} Overexpression and mutation of *Met* together in various tumors suggest that it may have an important role in the tumor progression and metastasis.²¹

Increased expression of *Met* has also been observed in human epithelial ovarian tumors.⁵ Trisomy of chromosome 7 is a characteristic cytogenetic alteration in ovarian carcinomas.^{16,18}

Because the potential importance in tumor progression and metastasis of the *Met*, and to gain further information

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about the possible involvement of the *Met* in ovarian tumorigenesis, in the present study we have analysed mutation of the exons of *Met* proto-oncogene.

Material and Methods

Tumor specimens

Tumor tissues from 24 consecutive patients, operated with EOT were analysed in this study. All tumor samples were sporadic, no evidence of ovarian or other tumor types occurred in their medical histories. No chemotherapy or radiation therapy was used before operation. The 24 EOT included 13 serous adenocarcinomas, three mucinous adenocarcinomas, six endometrioid adenocarcinomas, and two other epithelial type malignancies. Histological classification was performed by criteria of the World Health Organization.²² The clinical stage was evaluated according to the International Federation of Gynecology and Obstetrics (FIGO) staging system.²² DNA was isolated from sections of frozen tumor tissues and blood samples by QIAamp kit (Qiagen). All patients underwent debulking surgery followed by paclitaxel based chemotherapy.

Mutation analysis

Mutation analysis of exons 17–19 of *Met* proto-oncogene was performed by PCR-SSCP (Polymerase Chain Reaction, Single Strand Conformation Polymorphism) and direct sequencing Methods. Exons were amplified individually. The following, published primers were used in PCR-SSCP analysis²¹:

Exon	Sequence 5'-3'	Position
17	GTATTCACGTGTTCCATAATGAAGT	3550-3528
	GATGGCTGGCTTACAGCTAGTT	3548-3527
18	AACAGTAGATGCTTAGTTTATGCT	3746-3723
	AACAGATTCCTCCTTGTCACCT	3756-3735
19	TTCTATTTACAGCCACGGGTAAT	3844-3823
	ATGAAAGTAAAAGAGGAGAAACTC	3844-3820

25 µl PCR reaction mixture consisted of 10 nM Tris-HCl (pH 8.3), 1.75 mM MgCl₂, 50 mM KCl, 0.01% gelatin, 200 µM each of four deoxynucleotide triphosphates, 0.25 mM of each primer, 0.5 U Taq DNA polymerase (Boehringer Mannheim Biochemica, Germany), and 200 ng DNA. The PCR reaction was carried out as follows: initial denaturation, 3 min at 94°C, then 30 cycles of 94°C for 30 sec, 55°C 30 sec, 72°C 30 min. PCR products were diluted in 5 excess volumes of formamide dye solution (95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol, 20 mM EDTA, 10 mM NaOH) and were loaded on a 0.5x MDE sequencing gel, immediately after heating for 2 min at 95°C and chilling on ice. Electrophoresis was performed at 15C with 6 W constant power for 20 hours. DNA was then trans-

ferred to Hybond N membrane (Amersham, Germany) and was fixed in an oven at 80°C for 2 hours. After hybridization with digoxigenin end-labelled corresponding primers in Church buffer at 37°C for 1 hour, DNA was visualized by a digoxigenin detection system (Boehringer Mannheim Biochemicals, Germany) using BCIP/NBT substrates. Bands with altered mobility were recovered and subjected to sequencing. Sequencing reaction was performed with PRISM dyedeoxy cycle sequencing reagent (Perkin-Elmer, USA) according to the manufacturer's instructions. Sequence was evaluated on an ABI-373 DNA analysis system (Perkin-Elmer, USA).

Results

The main clinico-pathological data of 24 tumors are seen in *Table 1*. We tested both tumor and blood samples from the patients for mutation of 17–19 exons of *Met* proto-oncogene. We found only a single mutation in exon 18 in one sample of a mucinous carcinoma. This mutation was not detectable in the blood sample of the patient. Cytosine located in position of 3820 was substituted to guanine, and guanine in position of 3821 was changed to cytosine. Consequently codon 1209 was changed from alanin to glycine. (*Table 2*.) We were only able to examine one close relative of this patient, her only son, and no mutation was found in his blood cells. No mutation was detected in either blood or tumor samples in exon 17 and 19.

Discussion

The *Met* tyrosine kinase is a receptor for hepatocyte growth factor/scatter factor (HGF/SF) and is encoded by the *Met* proto-oncogene.¹³ The *Met* receptor tyrosine kinase transduces motility, proliferation and morphogenic signals of hepatocyte growth factor in epithelial cell.²⁵ HGF is also known as a scatter factor, because of its ability to cause cell migration and scattering.²⁵ Among other proto-oncogenes encoding growth factor receptors, the *Met* oncogene has been linked to the neoplastic process.

Overexpression of *Met* has been found with and without amplification of c-*Met*, in gastric carcinoma cell lines as well, and in thyroid carcinomas, respectively.^{6,19} Moreover increased coexpression of *Met* proto-oncogene and HGF was observed in human pancreatic cancer.⁷ *Met* was shown to be expressed in very early stage of kidney development and blocking HGF activity with anti-HGF antibodies interferes with epithelial cell differentiation.²⁶ The results of Natali et al. suggest that expression of the *Met*/HGF receptor may be involved in the onset and progression of renal cell carcinomas.¹⁴ But the strongest evidence that *Met* is implicated in the development of renal carcinoma was obtained by Schmidt et al.²¹ They found that *Met* is frequently mutated in hereditary forms of pap-

Table 1. The main clinicopathologic data of tumor samples

Case (No.)	Age (year)	Diagnosis	Stage	Grade	Menopausal status	Follow up (months)
1.	45	Serous	II/A	I	Pre	26 (alive, CR)
2.	56	Serous	II/C	II	Post	18 (alive, PR)
3.	54	Serous	III/A	II	Post	7 (alive, PR)
4.	61	Serous	III/A	II	Post	54 (deceased)
5.	76	Serous	III/C	II	Post	31 (deceased)
6.	48	Serous	III/C	III	Pre	8 (deceased)
7.	73	Serous	III/C	III	Post	11 (alive, PR)
8.	52	Serous	III/C	III	Pre	25 (alive, PR)
9.	59	Serous	III/C	III	Post	17 (deceased)
10.	61	Serous	III/C	IV	Post	65 (alive, PR)
11.	68	Serous	III/C	III	Post	9 (alive, PR)
12.	72	Serous	IV	III	Post	19 (deceased)
13.	59	Serous	IV	IV	Post	26 (deceased)
14.	62	Mucinous	II/A	I	Post	37 (alive, CR)
15.	67	Mucinous	III/A	II	Post	29 (alive, CR)
16.	55	Mucinous	III/C	II	Post	18 (alive, PR)
17.	63	Endometrioid	II/A	II	Post	27 (alive, CR)
18.	70	Endometrioid	II/B	II	Post	43 (alive, CR)
19.	67	Endometrioid	III/A	II	Post	31 (alive, CR)
20.	61	Endometrioid	III/C	III	Post	19 (alive, PR)
21.	53	Endometrioid	III/C	III	Pre	6 (alive, PR)
22.	60	Endometrioid	III/C	IV	Post	8 (alive, CR)
23.	62	Clear cell	III/C	III	Post	24 (deceased)
24.	57	Clear cell	IV	IV	Post	31 (alive, PR)

CR: complete response to therapy, PR: partial response to therapy

illary renal cancer.²¹ Somatic *Met* mutations also were found in some sporadic cases, but less frequently than in the familiar form of the disease.²¹ All of the mutations were located in the tyrosine kinase domain of *Met*. Papillary renal cancer usually presents with trisomy 7 and it was reasonable to suppose that the duplication of the chromosome is not random since it effects the chromosome that harbours the mutated *Met*. The extra dosage of the mutated allele may have functional significance. Later on this prediction was proved by Zhuang and co-workers.²⁷

Di Renzo et al. reported that *Met* was detectable in the surface epithelium of the normal ovary and the level of expression was unchanged in benign ovarian tumors of various origin.⁵ Overexpression of the *Met* protein was found in 28% of EOT belonging to different histotypic variants, but showed a well-differentiated phenotype.⁵

Table 2. A somatic mutation in the tyrosine kinase domain of the *Met* proto-oncogene in an epithelial ovarian carcinoma

Diagnosis	Mutation	Location	Amino-acid change
Mucinous adenocarcinoma Stage III/A, Grade II.	C3820→G G3821→C	Exon 18 Exon 18	Alanin-Glycin codon 1209 Alanin-Glycin codon 1209

Liang et al. found elevated expression of p21ras with the trisomy of chromosome 12, on which the K-ras-2 oncogene is located.¹² K-ras mutation has been detected at high frequency in mucinous tumors of borderline malignancies, but less frequently in well-differentiated serous tumors of ovary.⁴

Trisomy of chromosomes 7, 8, and 12 were found in ovarian epithelial tumors by FISH Method.^{16,18} The presence of trisomy of chromosome 7 and overexpression of *Met* forced us to analyse the integrity of *Met* in EOTs. Because all of the detected *Met* mutations were localised for the tyrosine kinase domain of the gene we focused our attention on this region only.

The mutations in the tyrosine kinase domain found by Schmidt et al. were homologous to the mutations in the *Ret* proto-oncogene tyrosine kinase domain that causes multiple endocrine neoplasia type

2B and sporadic medullary carcinoma of the thyroid gland.^{9,21} Codon substitution in the tyrosine kinase domain of the *Ret* and *Met* proto-oncogenes existing in familiar cancer syndromes sug-

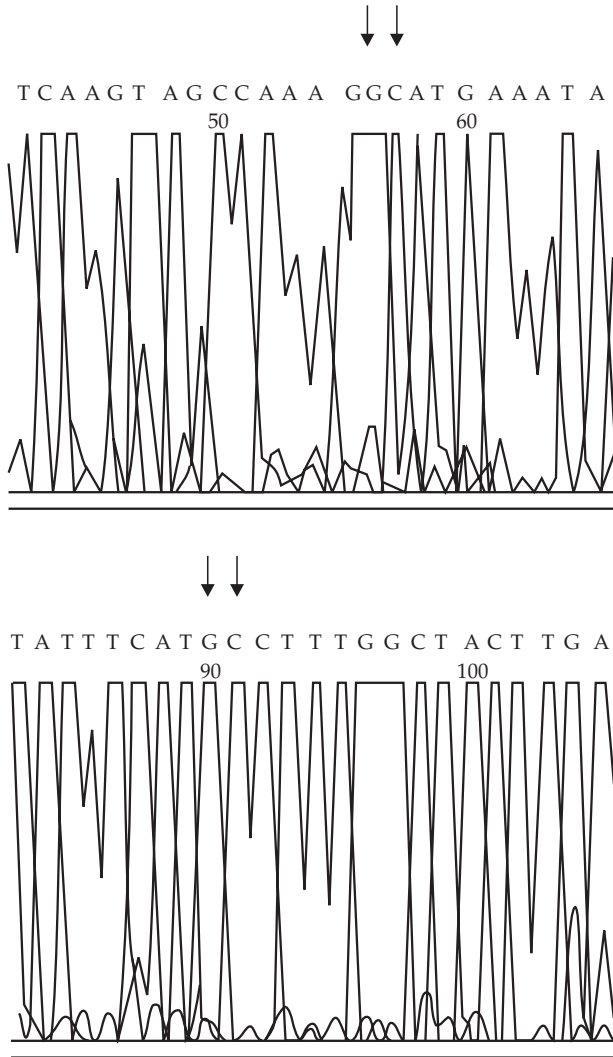


Figure 1. The forward and reverse DNA sequence of the tyrosine kinase domain of MET proto-oncogene from the tumor 15 from 3806 to 3829. Mutations are indicated with arrows.

gests that there are codons in the tyrosine kinase domain of receptor tyrosine kinases that, in mutant form may lead to uncontrolled cell growth.²⁰ However, only one mutation was found in 24 EOTs. This sample was a mucinous grade II tumor in stage III/A.²⁴ The patient was postmenopausal. This mutation was somatic because it was not found in the patient's or her son's blood cells. The patient underwent a paclitaxel-based chemotherapy after total abdominal hysterectomy, bilateral salpingo-oophorectomy and total resection of the omentum maius. There was no sign of recurrence after 37 months following the operation.

One more genetic change is added into the list of alterations take place in ovarian carcinogenesis in this study. Our results indicate that mutation in the tyrosine kinase domain of c-Met is not a common event in EOT. It affected only one of 24 tumors and it has a little practical value.

It is not clear whether the mutation plays a role in the tumorigenesis or progression of EOT or not. The conclusion can be that the scatter factor may be of more importance in metastatic potential than in actual carcinogenesis. Further study and a larger number of EOTs and their metastases are needed to compare non-metastatic ovarian carcinomas (stage I) with metastatic tumors, in the latter studying the gene expression in both the primary and secondary lesions.

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