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ARTICLE

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 differentation and it is activated by hepatocyte growth factor. Trisomy of chromosome 7 and Met protein overexpression have been were 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 mutations 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 analysis[21]:

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

25 µl PCR reaction mixture consisted of 10 nM Tris-HCl (pH 8.3), 1.75 mM $MgCl_2$, 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 transferred 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 glycin. (*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]

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-

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	C3820→G	Exon 18	Alanin-Glycin codon 1209
Stage III/A, Grade II.	G3821→C	Exon 18	Alanin-Glycin codon 1209

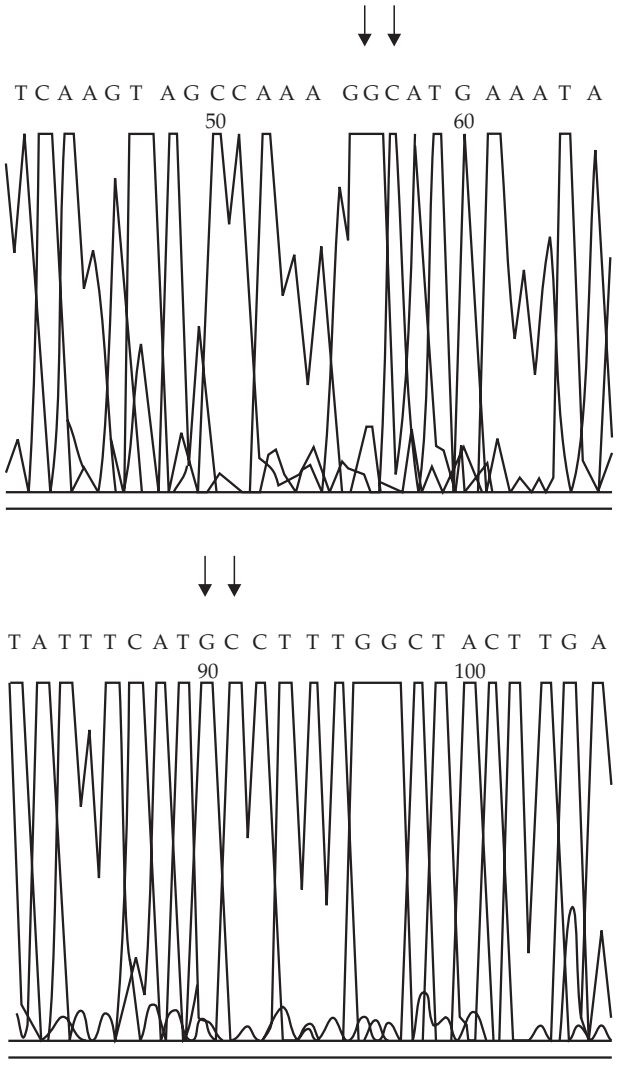

Figure 1. *The forward and reverse DNA sequence of the tyrosine kinase domain of MET proto-oncogene from the tumor 15 from 3806 to 3829. Mutations are indicated with arrows.*

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.

References

1. *Bergerheim USR, et al*: Chromosomal gains and losses in sporadic and hereditary papillary renal carcinomas by comperative genomic hybridization. J Urol 155:543A, 1996.
2. *Bladt F, Rietmacher D, Isenmann S, et al*: Essential role for the c-Met receptor in the migration of myogenic precursor cells into the limb bud. Nature 376:768-771, 1995.
3. *Bottaro DP, Rubin JS, Faletto DL, et al*: Identification of the hepatocyte growth factor receptor as the c-Met proto-oncogene product. Science 251:802-804, 1991.
4. *Chi-Ho Mok S, Bell DA, Knapp RC, et al:* Mutation of K-ras protooncogene in human ovarian tumors of borderline malignancy. Cancer Res 53:1489-1492, 1993.
5. *Di Renzo MF, Olivero M, Katsaros D, et al:* Overexpression of the Met/HGF receptor in ovarian cancer. Int J Cancer 58:658-662, 1994.
6. *Di Renzo MF, Olivero M, Ferro S, et al*.: Overexpression of the c-Met/Hgf receptor gene in human thyroid carcinomas. Oncogene 7:2549-53, 1992.
7. *Ebert M, Yokohama M, Friess H, et al*: Coexpression of the c-Met proto-oncogene and hepatocyta growth factor in human pancreatic cancer. Cancer Research 54:5775-5778, 1994.
8. *Giordano S, Ponzetto C, Di Renzo MF et al*: Tyrosine kinase receptor indistinguishable from the c-Met protein. Nature 339:155-156, 1989.
9. *Hofstra RM, Landsvater RM, Ceccherini I, et al.:* A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature 367:375-376, 1994.
10. *Jeffers M, Rong S, Vande Woude G.*: Enhanced tumorigenicity and invasion-Metastasis by hepatocyte growth factor/scatter factor-Met signalling in human cells concomittant with induction of the urokinase proteolysis network. Mol Cell Biol 16:1115-1125, 1996.
11. *Kovacs G:* Molecular cytogenetics of renal tumors. Adv Cancer Res 62:89-124, 1993.
12. *Liang JC, Kurzrock R, Gutterman JU, et al:* Trisomy 12 correlates with elevated expression of p21ras in human adenosquamosus carcinoma of the lung. Cancer 23:183-188, 1986.
13. *Naldini L, Weidner KM, Vigna E, et al:* Scatter factor and hepatocyte growth factor are indistinguishable ligands for the MET receptor. EMBO 10:2867-2878, 1991.
14. *Natali PG, Prat M, Nicotra MR, et al:* Overexpression of the Met/HGF receptor in renal cell carcinomas. Int J Cancer 69:212-217, 1996.
15. *Nusrat A, Parkos CA, Bacarra AE, et al:* Hepatocyte growth factor/scatter factor effects on epithelia. Regulation of intercellular junctions in transformed and nontransformed cell lines, basolateral polarisation of c-Met receptor in transformed and natural intestinal epithelia, and induction of rapid wound repair in a transformed model epithelium. J Clin Invest 93:2056-2065, 1994.

16. *Pejovic T, Iosif Cs, Mitelman F, et al:* Karyotypic characteristics of borderline malignant tumors of the ovary. Cancer Genet Cytogenet 92:95-98, 1996.
17. *Perez RP, Godwin AK, Hamilton TC, et al:* Ovarian cancer biology. Semin Oncol 18:186-204, 1991.
18. *Persons DL, Hartmann LC, Herath JF, et al:* Interphase molecular cytogenetic analysis of epithelial ovarian carcinomas. Am J Pathol 142:733-741, 1993.
19. *Prat M, Narsimhan RP, Crepladi T, et al:* The receptor encoded by the human c-Met oncogene is expressed in hepatocytes, epithelial cells and solid tumors. Int J Cancer 49:323-328, 1991.
20. *Santoro M, Carlomagno F, Romano A, et al:* Activation of RET as a dominant transforming gene by germline mutations of MEN2A and MEN2B. Science 267:381-383, 1995.
21. *Schmidt L, Fuh-Mei Duh, Fan C, et al*: Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nature Genet 16:68-73, 1997.
22. *Serov SF, Scully RE, Sobin LH:* International histological classification and staging of tumors. No. 9. In: Histologic typing of ovarian tumors. Geneva: World Health Organization, 1973.
23. *Sonnenberg E, Meyer D, Weidner KM, rt al:* Scatter factor/hepatocyte growth factor and its receptor, the c-Met tyrosine, can mediate a signal exchange between mesenchyme and epithelial cell during mouse development. J Cell Biol 123:223-235, 1993.
24. *Tanyi J, Tory K, Rigó J, et al:*Mutation in the tyrosine kinase domain of MET proto-oncogene in a sporadic Epithelial ovarian cancer. Submitted to J Reprod Med.
25. *Weidner KM, Sachs M, Birchmeier WJ*: The Met receptor tyrosine kinase transduces motility, proliferation, and morphogenic signals of scatter factor/hepatocyte growth factor in epithelial cells. Cell Biol 121:145-154, 1993.
26. *Woolf AS, Kolatsi-Joannou M, Hardman P, et al:* Roles of hepatocyte growth factor/scatter factor and the Met receptor in the early development of the Metanephros. J Cell Biol 128:171-184, 1995.
27. *Zhuang Z, Park WS, Pack S, et al:* Trisomy 7-harbouring nonrandom duplication of the mutatnt MET allele in hereditary papillary renal carcinomas. Nat Genet 20:66-69, 1998.