Novel Somatic Mutations of the MET Oncogene in Human Carcinoma Metastases Activating Cell Motility and Invasion

Annalisa Lorenzato, Martina Olivero, Salvatore Patanè, Edoardo Rosso, Alberto Oliaro, Paolo M. Comoglio, and Maria Flavia Di Renzo

ABSTRACT

Several gene mutations responsible for human cancer initiation have been discovered, whereas only a few have been identified in association with the progression to metastasis. In this study, we screened a large panel of human sporadic cancers, metastases, and tumor cell lines for mutations in the tyrosine kinase domain of the MET receptor, crucially involved in invasive cell growth and motility during embryogenesis. MET activating mutations have been described previously in hereditary papillary renal cell carcinoma and in a few sporadic tumors. Summarizing results of this and our previous studies, we did not detect mutations in the MET kinase domain from 153 sporadic human cancers and 25 cancer cell lines, whereas we found somatic MET mutations in 10 of 46 lymph nodal and 2 of 14 pulmonary metastases. We identified four MET mutations in metastases. Two were known as MET germ-line mutations (H1112R and Y1248C), which predispose to hereditary renal cell carcinoma. One of the two novel mutations (N1118Y) changed an asparagine in the region of the glycine-rich ATP binding site, which is highly conserved in all of the kinases. The other (Y1253D) changed a critical tyrosine, known to regulate MET kinase activity, to a negatively charged residue.

The MET receptors carrying either the N1118Y or the Y1253D mutation were constitutively active and conferred a motile-invasive phenotype on transduced carcinoma cells. The latter phenotype was additionally stimulated by the MET receptor ligand scatter factor/hepatocyte growth factor. These data suggest that MET might be one of the long sought oncogenes controlling progression of primary cancers to metastasis.

INTRODUCTION

Tumor cell subpopulations emerge during cancer progression, which have acquired the multifaceted invasive-metastatic phenotype. Metastatic cells must undergo loss of adhesion, show enhanced motility, secrete proteolytic enzymes, survive, and proliferate in a hostile environment. Whereas many genetic changes have been associated with tumor initiation and early steps of progression, genetic lesions specifically and consistently related to metastases are still elusive. Only recently, amplification of a tyrosine phosphatase gene has been associated consistently with colorectal cancer metastasis to the liver (1).

The MET proto-oncogene encodes the tyrosine kinase receptor (2) for SF1/HGF (3, 4), a multifunctional cytokine stimulating cell proliferation, dissociation, motility, and extracellular matrix invasion in vitro, and during embryonal development (5, 6). A role for MET in human tumor formation has been shown. The MET oncogene is overexpressed in tumors of specific histotypes, including thyroid (7) and pancreatic carcinomas (8), and is amplified in liver metastases of colorectal carcinomas (9). Germ-line missense mutations in the tyrosine kinase domain were detected in the majority of HPRCCs (10, 11) and in a single gastric cancer (12), whereas somatic mutations have been found in a small proportion of sporadic papillary kidney carcinomas (13) and in some childhood hepatocellular carcinomas (14).

The link between MET oncogene activation and metastasis has been suggested repeatedly. In vitro and in vivo MET receptor elicits a unique biological program leading to “invasive growth,” resulting from the activation of proliferation, motility, cell dissociation, and protection from apoptosis (reviewed in Ref. 15). In physiological conditions this program elicits the formation of epithelial and endothelial tubular structures (the so called “branched morphogenesis”), myoblast migration, and neurite branching. The deregulated activation of the invasive growth might confer metastatic and invasive properties to transformed cells. In cell culture, it was demonstrated that mutated MET receptors identified in papillary renal cell carcinoma transform transfect cell lines and enable them to invade extracellular matrix through the interaction with specific signal transducers (16). In the mouse model, metastases were obtained in animals transplanted with cells coexpressing the ligand SF1/HGF and the MET receptor, and in transgenic animals expressing the MET kinase activated by mutation or translocation (17, 18). In patients with invasive breast carcinoma, tumor expression of either MET (19) or its ligand SF1/HGF (20) was an independent predictor of decreased survival, suggesting a role for the MET receptor in human tumor aggressive behavior and progression.

We have identified previously MET gene somatic mutations in lymph node metastases of HNSCCs (21). In the previous study we harvested primary HNSCCs together with >100 either metastatic or clinically unaffected lymph nodes from the same patients. Using RT-PCR, MET-specific sequences were amplified from all of the primary HNSCCs and their lymph node metastases. We took advantage of the fact that the MET proto-oncogene is not expressed in normal lymph nodes (22) and, thus, that MET mRNAs detected in nodes must be from metastatic tumor cells. By sequencing RT-PCR products, we identified MET gene somatic mutations in a number of lymph node metastases. We also demonstrated that cells carrying MET mutations were selected during metastatic spread: transcripts of the mutant MET alleles were highly represented in metastases but barely detectable in the corresponding primary tumors. In this paper we show that MET mutations are also detectable in human lung metastases, and that the MET mutations found in lymph node and pulmonary metastases constitutively activate the MET kinase, and confer an invasive phenotype on human epithelial cells.

MATERIALS AND METHODS

Tissue Samples, Cell Lines, and Reagents. Primary carcinomas and metastasis samples from patients not previously subjected to chemo- or radiotherapy were analyzed. Tissue samples removed at surgery were dissected by
the pathologist. Normal and neoplastic tissues were immediately frozen and subsequently pulverized using a MM200 apparatus (Retsch) in the presence of liquid nitrogen. All of the mutated but not the wt sequences were sequenced by the Applied Type Culture Collection and were cultured using Isco’s modified Dulbecco’s medium plus 10% FCS. The GTL-16 gastric carcinoma cell line has been described previously (2). Recombinant SF1/HGF was obtained from culture supernatant of S9 cells infected with the Baculovirus vector containing the full-size human factor. SF1/HGF was titrated in scatter assay as S.U. Pure human recombinant SF1/HGF was purchased from R&D Systems.

RNA and DNA Extraction. Total cellular RNA and DNA were isolated using the TriReagent (Sigma Chemical) kit. Total RNA (1 μg) was used as a template for synthesis of oligodeoxynucleotidyl acid-primed double stranded cDNA, using Moloney murine leukemia virus reverse transcription from Life Technologies, Inc. (Inchinnan, United Kingdom). The suitability of the reverse transcription reaction product for PCR amplification was first checked by performing a PCR reaction for glyceraldehyde-3-phosphate dehydrogenase cDNA. For the study of MET expression sequences corresponding to exons 16–20 were amplified.

SSCP. SSCP analysis was carried out on exons 16–19 as described (21). Intronic primers were designed on the basis of intron-exon boundaries (10). PCR and SSCP conditions are available from the authors.

Sequence Analysis. Both PCR and RT-PCR products were directly sequenced after being purified from agarose gels using a QiaGen (Hilden, Germany) PCR product purification kit. SSCP-PCR products from bands were reamplified using the same primers and directly sequenced after being purified from an agarose gel. Automatic sequences (forward and reverse) were performed using the ABI Prism 310 (Perkin-Elmer) following the manufacturer’s protocols. Manual sequencing was carried out by cycle-sequencing using Amersham (Cleveland, OH) Thermo Sequenase with 33 P-labeled di-deoxynucleotides. Human MET residues were numbered according to the Human Gene Mutation Database (Cardiff, United Kingdom)5 and Online Mendelian Inheritance in Man,6 which refer to the Accession J02958. This numbering, which is the widely adopted, corresponds to an alternatively spliced minor transcript containing an insertion of 54 bp in position from nucleotide 2264 to nucleotide 2318. This transcript encodes a tyrosine kinase protein that is not correctly processed and is not located in the membrane (23). As an example, the Tyr1253 is the same as the residue Tyr1235 in the correct numbering of the cDNA sequence corresponding to the major transcript of the MET gene (24).

MASA. PCR reaction was carried out using 20–25 bp long oligonucleotide with the MET gene mutations at the 3’ end as specific primer and the corresponding primer without mutation as control (21). The mutated nucleotide at the 3’ end annealed with the wt but not with the wt sequence, because the single base mismatch at the 3’ end abolishes annealing of the primer. End point MASA was performed in either 30 or 60 cycles for 1 min at 94°C, 30 s at 60°C, and 30 s at 72°C.

Western Blot Analysis. Western blot analysis was carried out to detect MET protein as described previously (22). Briefly, proteins were solubilized in SDS-containing buffer in the presence of the reducing agent β-mercaptoethanol. Equal amounts of proteins (200 μg) were loaded in each lane. Proteins were separated by PAGE and transferred to nitrocellulose sheets. Blots were probed with the anti-MET receptor polyclonal antibody C-12 (Santa Cruz), and then with horseradish peroxidase-conjugated antirabbit immunoglobulins revealed by Enhanced Chemiluminescence (Amersham, Amersham, United Kingdom).

Immunoprecipitation. For immunoprecipitation cell monolayers were dissolved in HEPES buffer (25 mM [pH 7.4]) containing 10% glycerol, 150 mM NaCl, 1% NP40, 5 mM EDTA, 1 mM EGTA, and protease and phosphatase inhibitors at 0°C. Extracts were clarified, and proteins were immunoprecipitated using a MET monoclonal antibody DO24 raised against the extracellular MET domain. Proteins were labeled with monoclonal antiphosphotyrosine antibodies (Upstate) in Western blot analysis.

Cell Transduction with Lentiviral Vectors. Cells were transduced using third generation Lentiviral vectors with the polyuridine tract sequence (25). As transfer vector we used the pRRL.sin.PPT.ICMV.GFP.pre, where the full-length MET cDNA (4284 bp) was subcloned as NotI-XhoI fragment. Mutations were

MET SOMATIC MUTATIONS IN METASTASES OF CARCINOMAS

RESULTS

Mutation Analysis of the MET Oncogene in Human Primary Carcinomas, Metastases, and Cell Lines. The vast majority of both somatic and germ-line MET gene mutations thus far identified are missense mutations located in exons encoding the MET receptor tyrosine kinase domain (10–12). We performed an extensive mutation analysis of the tyrosine kinase domain of the MET gene in sporadic human primary tumors and human cancer cell lines. By sequencing RT-PCR products corresponding to exons 16–20, we screened a total of 153 samples of human primary cancers (Table 1) including 48 ovarian carcinomas, 27 squamous cell carcinomas of the head and neck (see also Ref. 21), and 40 musculoskeletal tumors (see also Ref. 27). We did not find any MET mutation in any of these tumors. In three cases we found a base change that did not give rise to amino acid change. In addition, we did not find mutations in genomic DNA from 25 human tumor cell lines, including carcinoma and sarcoma cell lines (Table 1). We concluded that MET gene mutation is an infrequent primary genetic event in human carcinogenesis.

In a previous study (21) we identified MET gene somatic mutations in lymph node metastases harvested from HNSCC patients (Table 1) but not in the corresponding primaries. By DS of RT-PCR products we found the Y1248C MET mutation in metastases of 1 patient. This mutation was identified previously in hereditary and sporadic renal cell carcinomas (13). In lymph node metastases from 3 other cases we found the same novel point mutation, which results in the substitution of a tyrosine with an aspartic acid at codon 1253 (Y1253D).

In this study, we have analyzed the genomic DNA from 2 liver and 14 pulmonary metachronous human metastases in patients previously operated for different primaries and not subjected to radio- and

5 Internet address: http://www.uwcm.ac.uk/uhwcm/mg.
MET SOMATIC MUTATIONS IN METASTASES OF CARCINOMAS

We performed mutation analysis of MET exons 16–19 from metastasis DNA, using both SSCP analysis and DS of PCR products. As shown in Table 1 and Fig. 1, by SSCP analysis we found an altered exon 16 allele coexisting with the wt one in 3 metastases examined (Fig. 1). SSCP bands showing aberrant migration were extracted from gels and sequenced. In 1 sample (sample 8 of Fig. 1) taken from a lung metastasis of a testicular germ cell tumor, we identified a G → A substitution in codon 1130 that does not give rise to any amino acid change. In a solitary lung metastasis of colorectal carcinoma (sample 15 of Fig. 1) we found an A → T substitution in codon 11118 that changes an asparagine to tyrosine (N11118Y). This mutation has never been described before. In a single lung metastasis of a HNSCC (sample 12 in Fig. 1), we found an A → G substitution in codon 11112, which changes a histidine to arginine (H11112R). The latter mutation was formerly found in a family suffering from HPRCC (10). We confirmed the presence of the novel mutations in metastatic samples using a MASA (data not shown). The base changes in codons 1112 and 11118 were not detected using PCR and DS of MET exon 16 in the same samples. In addition we did not find these changes in DNA extracted from the surrounding normal tissues of the same individuals. Thus, we concluded that these changes were somatic and that mutated MET sequences were not detectable by PCR-DS as they represented <50% of the total MET sequences amplified from the samples. This is not surprising as MET is an oncogene, and a single allele somatic mutation can be activating. Furthermore, metastasis samples also contained DNA from contaminating normal cells.

Functional Studies of the Novel MET Mutations Identified in Human Metastases. The MET gene mutations identified previously in hereditary and sporadic PRCC activate the MET receptor function, causing its constitutive activation and, to a different extent, cell transformation (11, 26, 28). The N11118Y mutation here reported is novel, but interestingly it is located near the kinase ATP binding site. The other novel mutation, identified in lymph node metastases of head and neck carcinomas (Y1253D), changes one of the two tyrosines (Y1253F, Y1253S) known as the MET receptor major autophosphorylation sites (29). To address whether these mutations activate the MET receptor and display biological activity, we introduced the N11118Y and the Y1253D mutations in the human MET cDNA, and expressed the mutated cDNAs in human carcinoma cell lines by lentiviral vectors that allow the sustained and long-term expression of integrated transgenes in any cell type (25). As controls, we transferred also the wt MET to obtain overexpression and the MET cDNA carrying the M1268T mutation identified previously as strongly activating (26, 28). With lentiviral vectors carrying the various MET cDNAs, we transduced two human breast cancer cell lines, T-47D and SK-BR-3, which in our experiments either did not express or express low levels of MET receptors, respectively (Figs. 2 and 3). We got MET cDNA random integration in the genome of both the T-47D and SK-BR-3 cells.

We obtained expression of wt and mutated MET receptors in both cell lines (Fig. 2A; Fig. 3A). Wt overexpressed receptors were found constitutively activated (i.e., phosphorylated in the absence of exogenously added ligand) in both cell lines (Fig. 2A; Fig. 3A). All three of the mutant MET receptors were constitutively phosphorylated, too, but the level of basal phosphorylation varied to some extent according to the mutation. The positive control mutant METY1253D was highly phosphorylated as also reported previously (30). The mutant METY1253D showed a slightly lower level of phosphorylation, as expected, because of the substitution of one of the major MET phosphorylation sites (29) with an aspartic acid. This did not impair its kinase activity that was even increased by the negative charge mimicking the phosphorylated tyrosine (these data are not shown, because they were reported elsewhere; Ref. 21). Addition of SF1/HGF stimulated additional phosphorylation in all of the cases (Fig. 3A).

Overexpressed MET receptors, either wt or mutated conferred on breast carcinoma cells an increased ability to move in vitro, to cross an artificially reconstituted basement membrane (Matrigel), and to invade a three-dimensional collagen gel. Mutant receptors triggered the latter properties more actively than wt ones.

As shown in Fig. 2B and Fig. 3B, in both T-47D and SK-BR-3 cells, expression of the mutant MET receptors led to an increased spontaneous ability to cross an artificially reconstituted basement membrane (Matrigel) with respect to expression of the wt. Moreover, the MET receptors carrying the Y1253D mutation conferred on cells a more active invasive phenotype than the receptors with the N11118Y mutation.

As shown in Fig. 2C, SK-BR-3 breast carcinoma cells expressing mutant MET receptors also showed an increased spontaneous motility in wound healing assay, higher than that of cells overexpressing the wt MET.

In three-dimensional collagen gel assay, we measured the invasive growth ability of cells expressing the various MET receptors. T-47D cells grown as spheroids proved to be an appropriate target as expression of MET receptors carrying the novel mutations resulted in a

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Table 1. Mutations scanning of the MET gene tyrosine kinase domain in human tumors

<table>
<thead>
<tr>
<th>Tumor</th>
<th>No. mutated/ no. examined</th>
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<tbody>
<tr>
<td>Ovarian carcinoma samples</td>
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</tr>
<tr>
<td>Colorectal carcinoma samples</td>
<td>0/0</td>
</tr>
<tr>
<td>HNSCC samples</td>
<td>0/15</td>
</tr>
<tr>
<td>Oral squamous cell carcinoma samples</td>
<td>0/12.0</td>
</tr>
<tr>
<td>Papillary thyroid carcinoma samples</td>
<td>0/12.0</td>
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<tr>
<td>Medullary thyroid carcinoma samples</td>
<td>0/20</td>
</tr>
<tr>
<td>Musculo-skeletal tumor samples</td>
<td>5/400</td>
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<tr>
<td>Osteosarcoma cell lines (MG-63, Saos-2, U-2 OS)</td>
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<tr>
<td>Gastrointestinal tract carcinoma cell lines (GT-16, DLD-1, HT29)</td>
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</tr>
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<td>Epidermoid carcinoma cell lines (A431)</td>
<td>0/1.0</td>
</tr>
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<td>Pancreatic carcinoma cell lines (SUIT-2, BxPc3, Capan-1, Capan-2, PF15)</td>
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</tr>
<tr>
<td>Prostate carcinoma cell lines (PC-3, LNCaP, DU 145)</td>
<td>0/3.0</td>
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<tr>
<td>Breast carcinoma cell lines (MCF7, SK-BR-3, ZR-75-1, T-47D)</td>
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<td>Thymidylate synthase lines (ARO, BCPAP)</td>
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<td>Leukemic cell lines (K-562, Jurkat)</td>
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<td>Glioblastoma cell line (U-138 MG)</td>
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<tr>
<td>Lymph node metastases of HNSCC</td>
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</tr>
<tr>
<td>Liver metastases of colorectal carcinomas</td>
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<tr>
<td>Lung metastases of colorectal carcinomas</td>
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</tr>
<tr>
<td>Liver metastases of breast carcinomas</td>
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<td>Liver metastasis of an urinary bladder carcinoma</td>
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<tr>
<td>Liver metastasis of a clear cell kidney carcinoma</td>
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</tr>
<tr>
<td>Liver metastasis of a squamous cell carcinoma</td>
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</tr>
<tr>
<td>Liver metastasis of a germ cell tumor</td>
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</tr>
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<td>Liver metastases of fibrosarcomas</td>
<td>0/2.0</td>
</tr>
<tr>
<td>Lung metastases of melanomas</td>
<td>0/2.0</td>
</tr>
</tbody>
</table>

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* Examined by RT-PCR and DS of mRNA corresponding to MET exons 16–19.
* These samples have been in part reported in previous studies (21, 27, 41, 42).
* Examined by PCR and DS.
* Mutated MET sequences were found in 10 metastatic nodules from 4 of 15 cases examined by RT-PCR and DS of mRNA corresponding to MET exons 16–19.
* Examined by both SSCP and PCR followed by DS of SSCP bands.

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Fig. 1. Detection of MET gene mutations in genomic DNA from human pulmonary metastases with SSCP analysis. Numbers on top indicate different metastasis cases. SSCP changes shown (arrows) were obtained by amplifying MET exon 16 with intron primers. Mutant allele conformers were visible in metastasis samples numbered 15, 8, and 12. Extraction and DS of abnormal conformers showed missense mutations in samples 15 and 12, and a silent base change in sample 8. 

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spontaneous formation of cords sprouting into the gel and whole cells moving from the spheroids toward the gel. Also in this assay, mutant receptors conferred a more active invasive phenotype on cells. In the presence of SF1/HGF, cell response was enhanced (Fig. 3C).

To test whether constitutive phosphorylation of MET receptors and spontaneous motility of MET-expressing cells were because of autocrine receptor activation we assayed the production of the ligand SF1/HGF using RT-PCR. We did not detect SF1/HGF-specific mRNA in either T-47D or SK-BR-3 expressing either wt or mutant receptors (data not shown).

**DISCUSSION**

In this paper we show that in human cancer somatic mutations of the MET oncogene are quite rare events in primary carcinogenesis but are more frequently associated with tumor progression. It is notewor-

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**Fig. 2. Expression, constitutive phosphorylation, and biological activities of mutant MET receptors in SK-BR-3 human breast carcinoma cells.**

A, MET receptor expression was evaluated with Western blot analysis of total cell extracts with MET antibody. Phosphorylation was evaluated by labeling the immunoprecipitated MET protein with anti-P-Tyr antibodies. Proteins were separated in gels under reducing conditions that allow the identification of both the receptor Mr 145,000 β chain and the Mr 170,000 precursor (arrows). Untransduced (NT) SK-BR-3 cells were compared with cells transduced with: the enhanced green fluorescent protein (GFP) gene, MET cDNAs carrying different mutations (indicated by the amino acid residue substitution) and wt MET cDNA (wt). B, invasion in vitro by cells expressing wt and mutant MET receptors as before. Invasion was measured in Transwell chambers where filter was covered with an artificial basement membrane (Matrigel) in the presence of low serum concentration. Y-axis shows average cell number of triplicate wells in a representative experiment; bars, ±SE. C, wound healing in vitro by cells expressing mutant MET receptors. The ability of SK-BR-3 cells to cover the wound made at time 0 h (top) was measured after 48-h growth in complete culture medium (bottom).

**Fig. 3. Expression, phosphorylation, and biological activities of mutant MET receptors in T-47D human breast carcinoma cell lines.**

A, expression and phosphorylation of mutant MET receptors in cells stimulated or not with SF1/HGF. T-47D cells were transduced with MET cDNAs carrying different mutations (indicated by the amino acid residue substitution) and with the wt MET cDNA (wt). As control, untransduced cells (NT) cells are also shown. MET receptor expression (top) was evaluated with Western blot analysis of immunoprecipitated MET protein. Phosphorylation of MET receptors was evaluated by labeling immunoprecipitated MET proteins with anti-P-Tyr antibodies. Serum-starved cells were treated with either supernatant of mock-transfected Sf9 cells or recombinant SF1/HGF (300 S.U/ml) for 15 min at 37 °C (bottom); B, invasion in vitro by cells expressing wt and mutant MET receptors measured as described in the legend to Fig. 2; bars, ±SE. C, invasive growth in three-dimensional collagen gel by T-47D cells expressing mutant MET receptors. T-47D cells were grown for 12 h in methylcellulose to allow the formation of cell spheroids. Then spheroids were embedded in collagen gel containing FCS alone or with recombinant SF1/HGF (400 S.U/ml). After 48 h spheroids were photographed under phase contrast microscope at low magnification.
We show here that MET mutation is rarely found in primary tumors. A consistent number of tumors has also been analyzed by other authors, and a very few somatic and germ-line mutations were found, HPRCC being an exception (10, 12, 14, 31–33). Altogether this collection of information shows that MET mutation is not a frequent initiating event in most common human cancer. We cannot rule out that mutations in the MET tyrosine kinase may be present in rare tumor types not included in our and in other panels, or that they are present in common tumors at a very low percentage. It is also possible that mutations exist in domains of the MET gene not yet examined by us and others, but this hypothesis seems unlikely. If on the other hand these MET mutations do exist, they would noticeably affect only codons outside the known regulatory regions. The striking homology between mutations activating the MET gene in HPRCC and those activating the RET, KIT, and v-erbB genes (10, 11) suggests that, in general, there are conserved codons and critical regulatory regions in tyrosine kinases, which are functionally relevant and are preferential targets for disease-producing mutations.

In metastases we found a significant number of MET mutations located in the receptor tyrosine kinase domain. All but one (12) of the MET mutations thus far described, mostly in hereditary PRCC, are located in the kinase domain, either in the NH2-terminal lobe, which includes the ATP binding site, or in the COOH-terminal lobe, which includes the catalytic domain and the activation loop. All of the mutations described previously activate the MET kinase, albeit to a different degree. Two of the mutations we found in metastases (H1112R and Y1248C) were described previously as activating (10, 34). Here we show that also the two novel mutations activate the receptor enzymatic activity. The novel N1118Y mutation results in a change in amino acid class and is located in the region of the glycine-rich ATP binding site that is highly conserved in all of the kinases. In this boundary, two other codons (V1110 and H1112) have been found mutated in PRCC, and their mutations were shown to activate the MET receptor kinase. The novel mutation identified in lymph node metastases of HNSCC (Y1253D) changes one of the two tyrosines (Y1252Y1253), also numbered as Y1253Y1254, see “Materials and Methods”) known as the MET receptor major autophosphorylation sites. Phosphorylation at these tyrosines positively regulates receptor kinase activity (29). The Y→D mutation results in a change in amino acid class. The negative charge of the aspartic acid probably mimics that of a phosphorylated tyrosine. This mechanism has been proposed to explain constitutive activation of another oncogene, B-Raf, having aspartic acid at corresponding position (35).

Here we did not describe the biological activity of the two already known mutations (H1112R and Y1248C) because they have been demonstrated previously (16, 34). In fact, the change of H1112 to either arginine or tyrosine was formerly found in families suffering from HPRCC (34). In addition, the latter codon was also found mutated to either leucine or tyrosine in sporadic papillary renal cell carcinoma (13). Therefore, the H1112 seems to be a particularly critical residue in regulating MET as three different changes all produce transforming receptors. Interestingly, HPRCC patients in families carrying H1112 to arginine substitution (the change that we found in the lung metastasis) showed not only primary multiple papillary renal cell carcinomas but also multiple metastases in various organs (34, 36). The other mutation we identified in carcinoma metastases (Y1248C) activates the MET kinase and was found to specifically hyperactivate cell invasiveness rather than proliferation (16).

To assay the biological properties of the novel MET mutations found in carcinoma metastases, we transfected human breast cancer cells with the aim of conferring them an invasive-metastatic phenotype. In mouse mammary carcinoma, it has been demonstrated that the MET-ligand SF1/HGF stimulates motility and invasion, and mediates anchorage-independent growth and survival (37). The expression in mice of activated MET genes leads primarily to the development of invasive mammary carcinomas (17, 18). In addition, in human breast carcinomas MET expression has been detected in the advancing margin (38). We show here that the MET receptor carrying the novel mutations found in metastases elicits breast cancer cell motility and invasion. Mutant receptors were by far more active than the overexpressed wt MET receptors. This capability reflects the unique ability of an activated MET receptor to trigger invasion (39).

The MET mutants analyzed maintain responsiveness to SF1/HGF, like the others thus far examined (18, 26). As other reports also show a strict ligand dependency of MET-mediated properties (30), it has been inferred that constitutive phosphorylation of mutated MET receptors and spontaneous biological properties elicited by mutations are because of production of the ligand by recipient cells. This is true for example in the case of the murine NIH-3T3 cells used for early studies. However, we show that breast carcinoma cells expressing the mutated MET receptors did not produce SF1/HGF.

In conclusion, all of the mutations identified in metastases confer cells the ability to move and to invade. Therefore, it seems reasonable to propose that specific MET mutated receptors contribute to the ability of subpopulation of carcinoma cells to establish metastatic colonies. One of the most promising outcomes of the work described here concerns its potential therapeutic implications. Most of the previously described genetic alterations in cancer in general, and in metastases in particular, involve inactivation of tumor suppressor genes. The latter are difficult to target with drug, because they are inactive or absent in tumor cells. In contrast, proteins of which the expression is elevated, and in particular enzymes of which the activity is increased, provide excellent targets for drugs. It has been shown repeatedly that interfering with either MET kinase activity (26) or ligand-dependent activation (30) might revert the MET-dependent transformed phenotype in vitro and metastasis in experimental models (40). This suggests that the MET oncogene could be a promising molecule to target with the objective of impairing tumor cell metastatic potential.

ACKNOWLEDGMENTS

We thank Vincenzo De Sio for technical help. We also thank Elaine Wright for reading the English. We are indebted to Prof. Luigi Naldini, Dr. Antonia Follenzi, and Dr. Elisa Vigna of the Laboratory of Gene Transfer and Therapy of the Institute of Cancer Research and Treatment for providing Lentiviral vectors, and for assisting us in the generation of vectors and transduction of cells.

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