Deletion of the ectodomain unleashes the transforming, invasive, and tumorigenic potential of the *MET* oncogene

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(Received October 9, 2008/Revised December 4, 2008/Accepted December 7, 2008/Online publication January 21, 2009)

The c-*MET* proto-oncogene, encoding the p190 hepatocyte growth factor tyrosine kinase receptor, can acquire oncogenic potential by multiple mechanisms, such as gene rearrangement, amplification and overexpression, point mutation, and ectopic expression, all resulting in its constitutive activation. Hepatocyte growth factor receptor truncated forms are generated by post-translational cleavage: p140 and p130 lack the kinase domain and are inactive. Their C-terminal remnant fragments are generally undetectable in normal cells, but a membrane-associated truncated form is recognized by anti-C-terminus antibodies in some human tumors, suggesting that a hepatocyte growth factor receptor lacking the ectodomain, but retaining the transmembrane and intracellular domains (Met-EC⁻), could acquire oncogenic properties. Herein we show that NIH-3T3 cells transduced with *MET*-EC⁻ expressed a membrane-associated constitutively tyrosine-phosphorylated 60-kDa protein and, similarly to NIH-3T3 cells expressing the cytosolic oncoprotein Tpr-Met, showed activated extracellular regulated kinase 1/2 mitogen-activated protein kinase and Akt downstream transducers. Compared to control NIH-3T3 cells, NIH-3T3-Met-EC⁻ cells grew faster and showed anchorage-independent growth and invasive properties in all aspects similar to cells expressing the transforming *TPR-MET*. Nude female mice injected subcutaneously with NIH-3T3-Met-EC⁻ cells developed visible tumors, displaying the typical morphology of carcinomas with polygonal cells, in contrast to sarcomas with spindle-shaped cells induced by the injection of NIH-3T3-Tpr-Met cells. It is suggested that the different subcellular localization of the oncoproteins, more than differences in signal transduction, could be responsible for the tumor phenotype. All together, these data show that deletion of the ectodomain activates the hepatocyte growth factor receptor and its downstream signaling pathways, unleashing its transforming, invasive, and tumorigenic potential. (*Cancer Sci* 2009; 100: 633–638)

Tyrosine kinase receptors, which mediate important biological responses, are normally activated in a tightly regulated ligand-dependent manner, but receptor activation can also be reached by ligand-independent mechanisms, including deletion of the receptor ectodomain. This mechanism was originally reported for human insulin receptor (HIR) and epidermal growth factor receptor.[1,2] In the case of oncogenic TKR, ectodomain deletion was shown to contribute to the onset of transformation or oncogenesis, and metastatis.[3,4] Loss of the ectodomain can be the consequence of a post-translational proteolytic cleavage or a translational or post-transcriptional splicing process.[5–8]

The Met protein, which is the receptor for HGF, mediates multiple biological functions, namely cell proliferation, motility, survival, differentiation, and morphogenetic remodeling, in a wide spectrum of tissues, including epithelial, endothelial, hematopoietic, and neuronal tissues.[9] The coordinated integration of these HGF-induced processes plays a pivotal role in organ formation during embryogenesis and in tissue homeostasis in adults.[10,11] The inappropriate activation of Met results in tumorigenesis and metastatic spreading of tumor cells. Indeed, *MET* was originally identified as an oncogene activated after chromosomal rearrangement, which gave rise to the cytosolic oncogenic variant Tpr-Met.[12] As well as amplification and overexpression, missense point mutations in defined Met domains have been reported in human cancers of specific histotypes.[9,13,14] In most cases, inappropriate receptor activation is ligand-independent, although HGF may be required for *in vitro* cell transformation triggered by some missense point-mutated Met.[15] Finally, also ectopic expression of the HGF–Met axis, activating a paracrine/autocrine loop can contribute to tumorigenesis.[16]

Apart from the normal full-sized heterodimeric p190 form of Met, consisting of the extracellular 50-kDa α chain and the transmembrane 145-kDa β chain, truncated isoforms have also been described, all of which are generated by post-transcriptional cleavage. Two of these, p140 and p130, identified originally using monoclonal antibodies directed against the ectodomain, are devoid of the TK domain and thus incompetent for transformation.[7] p140 is exposed at the cell surface anchored to the plasma membrane through its transmembrane segment; its complementary remnant is a soluble cytoplasmic isoform of Met. This soluble cytoplasmic Met isoform has constitutive kinase activity, transforming activity *in vitro*, oncogenic activity *in vivo*,[17] and can immortalize hepatocytes, but can only transform them if coexpressed with c-myc.[18] It is generally not detected, possibly because it contains a PEST motif, which directs it to degradation;[19] a molecule with this feature has recently been observed translocated into the nucleus of some human tumors and cell lines.[20] p130 corresponds to the Met ectodomain and is continuously released as a soluble molecule from the cell surface under normal conditions, in a process known as shedding. Shedding is upregulated by treatment with the protein kinase C activator 12-*O*-tetradecanoylphorbol 13 acetate or receptor agonists (HGF or agonist monoclonal antibodies),[7] indicating that it could be a mechanism to downregulate the receptor. Also, the corresponding remnant of p130, consisting of the membrane-spanning motif and the intracellular kinase domain, is not normally detected being generally degraded, after ubiquitination.[21,22] In some human tumors a truncated Met isoform was localized to the plasma membrane and recognized only by antibodies directed against the cytoplasmic receptor tail.[23] Its expression was correlated with severe outcome in patients.[24] Similarly, a truncated form of the

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Abbreviations: DMEM, Dulbecco's modified Eagle's medium; eGFP, enhanced green fluorescent protein; Erk, extracellular regulated kinase; FCS, fetal calf serum; HGF, hepatocyte growth factor; LV, lentivirus; MAPK, mitogen activated protein kinase; Met, hepatocyte growth factor receptor; Met-EC⁻, hepatocyte growth factor receptor devoid of the ectodomain; PAGE, polyacrylamide gel electrophoresis; PI3K, phosphatidylinositol 3-kinase; SDS, sodium dodecylsulfate; TK, tyrosine kinase; TKR, tyrosine kinase receptor; WT, wild type.

doi: 10.1111/j.1349-7006.2008.01079.x
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Ron TKR, which belongs to the Met family of receptors, was detected in several human cancers.[25,8] It is devoid of the extracellular domain but comprises the whole transmembrane and intracellular domains, and its expression in a human carcinoma cell line induces a transformed and invasive phenotype. All of the above findings prompted us to investigate whether a truncated Met receptor lacking the ectodomain can acquire transforming, invasive, and tumorigenic properties.

Materials and Methods

Reagents, antibodies, and cell culture. All reagents were from Sigma-Aldrich (St. Louis, MO), except when stated. The following antibodies were used: Met C-terminus (DQ-13),[26] phosphotyrosine (clone 4G10; Upstate Biotechnology, Lake Placid, NY), Met C-terminus and Erk 1/2 (C28 and sc-154; Santa Cruz Biotechnology, Santa Cruz, CA), P-Erk 1/2, Akt, and P-Akt (#9106, #9272, and #4051; Cell Signaling Technology, Danvers, MA), cytokeratins and vimentin (clones AE1-AE-3 and V9; Neomarkers, (Lab Vision Corporation, Fremont, CA). Horseradish peroxidase-coupled secondary antibodies were from Amersham Biosciences Otelfingen, CH. NIH-3T3 cells expressing Tpr-Met and Met^{WT} were kindly provided by Riccardo Taulli Dept Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy and Maria Flavia Di Renzo, Laboratory of Cancer Genetics, Institute for Cancer Research and Treatment (IRCC), University of Torino, Italy, respectively. Cells were maintained in DMEM supplemented with 10% FCS (Invitrogen, Carlsbad, CA) in a humidified atmosphere of 5% CO_2 in air. HGF was purchased from Peprotech, (Rocky Hill, NJ) Noble agar was from Difco Laboratories (Sparks, MD), Matrigel was from Collaborative Research (Becton Dickinson Italia S.p.A., Milano, Italy), and oligonucleotides were from MWG-Biotech (Ebersberg, Germany). Proteins were quantified with the BCA Protein Assay kit (Pierce Thermo Fisher Scientific Inc, Rockford, IL).

Plasmids and LV cell transduction. NIH-3T3 mouse embryo fibroblasts (CRL1658; American Type Culture Collection, Manassas, VA) were transduced with third-generation LV,[27] using the pCCLsin.PPT.hPGK.eGFP.pre vector transfer construct, where the eGFP cDNA (*Bam*HI–*Sal*I fragment) was substituted with the *MET*-leader-*MET*-EC^- cDNA construct. These fragments were obtained by polymerase chain reaction with the primers sense 5′-atgaagg cccccgctgtgctg-3′ and antisense 5′-cccattgctcctctgcaccaaggt-3′, and sense 5′-ttgattgctggtgttgtctcaatatcaacagc-3′ and antisense 5′-ctatgatgtctcccagaaggaggctggtc-3′, respectively from the full-size Met cDNA.[28] The *Bam*HI–*Age*I (72 bp) and *Age*I–*Sal*I (1379 bp) fragments were ligated and subcloned into the LV transfer construct. The pCCL.sin.PPT.hPGK.eGFP.pre vector was used as a control for transduction efficiency.

Cell lysates, immunoprecipitation, and western blot analysis. Confluent cells were lysed in RIPA buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 1% deoxycholate, 5 mM ethylenediaminetetraacetic acid, 1 mM Na_3VO_4) and a protease inhibitor cocktail.[29] Confluent NIH-3T3 cells expressing Met^{WT} were stimulated with HGF for 12 min. Clarified cell extracts were immunoprecipitated for 2 h with the different antibodies and Sepharose-protein A (GE Healthcare, Little Chalfont, UK). Washed immunoprecipitates were eluted in reducing Laemmli buffer at 95°C, resolved by SDS-PAGE, transferred onto polyvinylidene fluoride filters (Amersham Biosciences), probed with antibodies diluted in Tris-buffered saline with 3% bovine serum albumin, followed by horseradish-peroxidase conjugated secondary antibodies. Protein bands were visualized by enhanced chemoluminescence (Amersham Biosciences).

Cell fractionation. All steps were carried out on ice or at 4°C. Cells underwent swelling in hypotonic solution (10 mM Tris-HCl, pH 8, 1.5 mM $MgCl_2$, 5 mM KCl, 50 µg/mL leupeptin, 5 µg/mL pepstatin A, 2 mM phenylmethylsulfonyl fluoride), mechanical disruption with Dounce (Cole-Parmer Instrument Company, Vernon Hills, IL) and centrifugation at 34 000 *g* for 30 min. The soluble cytoplasmic and insoluble membrane fractions were then recovered and analyzed directly by western blotting after SDS-PAGE separation.

***In vitro* biological assays.** For the proliferation assay, cells were plated at low density in 96-well plates, starved in low serum, and proliferation was evaluated at fixed times in 5% serum by a colorimetric assay. After fixation, the cells were stained with crystal violet, solubilized, and the absorbance read at 595 nm, relative to a calibration curve.[29]

For anchorage-independent growth, cells (5×10^3 cells/4-cm^2 well) were seeded in soft agar (0.4% Noble agar in DMEM with 10% FCS) and colonies were stained with MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide], counted, and photographed after 21 days.

The invasion assay was carried out in blind-well Transwell chambers (Costar, Corning B.V. Life Sciences, Schiphol-Rijk, Netherlands). Cells (10^5) were seeded in DMEM with 5% FCS on the upper side of a porous polycarbonate membrane (pore size 8 µm), pre-coated with Matrigel (1.2 mg/mL). After 48 h, cells attached to the lower side of the filter were fixed and stained with crystal violet.

Tumorigenicity assay. Cells (5×10^5) were injected subcutaneously into the posterior flank of athymic $Foxn1^{nu}$ female mice. The tumor mass was measured constantly and animals were killed after 32 days. For immunohistological examination, tumors were fixed in formalin, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin–eosin or incubated with the anti-Met DQ-13, cytokeratins or vimentin monoclonal antibody, followed by goat anti-mouse peroxidase-coupled secondary antibody and reaction with 3,3′-diaminobenzidine and counterstaining with hematoxylin. All procedures were carried out in accordance with the European Community Directive for Care and Italian Laws on animal experimentation (Law by Decree 116/92).

Results

Expression of a constitutively activated ectodomain-truncated Met at the cell membrane and its effect on Erk1/2 and Akt activation. NIH-3T3 fibroblasts were transduced using third-generation LV vectors carrying Met-EC^- or eGFP as a control. Transduced cells expressed the different exogenous genes stably. Lysates prepared from confluent 60-cm^2 plates, equalized for protein amounts, were immunoprecipitated with polyclonal anti-Met antibodies, separated in two gels by SDS-PAGE, and immunoblotted with either the anti-Met or anti-phosphotyrosine monoclonal antibodies. When NIH-3T3-Met-EC^- cells were used, a band of 60 kDa was visualized in both cases (Fig. 1a). In the same kind of experiment a molecule of approximately 65 kDa was detected in immunoprecipitates from NIH-3T3-Tpr-Met by both anti-Met and anti-phosphotyrosine antibodies. When the same kind of experiment was carried out using lysates from NIH-3T3-Met^{WT}, the 145-kDa protein corresponding to the β chain of full-size Met was detected to be phosphorylated only after HGF stimulation. No specific bands were visualized from lysates from NIH-3T3-eGFP fibroblasts. It was thus shown that NIH-3T3-Met-EC^- cells express a Met molecular species of the anticipated molecular weight and that this molecule is constitutively activated.

The subcellular localization of Met-EC^- was determined by subcellular fractionation. The Met-EC^- protein was partitioned in the insoluble membrane fraction (Fig. 1b), whereas all Tpr-Met protein was localized in the cytosolic soluble fraction. This demonstrated that the Met-EC^- protein is correctly expressed at the cell membrane. Finally, when two of the main signal transduction pathways were analyzed by western blotting of total cell lysates, it was found that both Erk1/2 and Akt were activated in NIH-3T3-Met-EC^- fibroblasts, as they were in the control

doi: 10.1111/j.1349-7006.2008.01079.x
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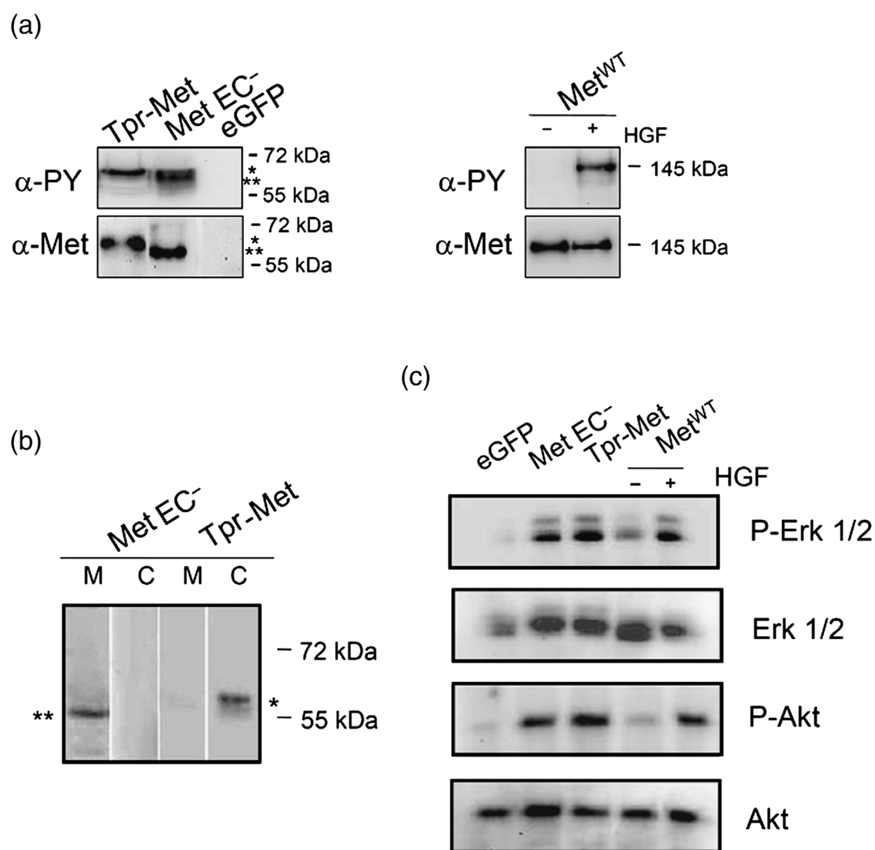
Fig. 1. Expression of a constitutively activated ectodomain-truncated hepatocyte growth factor receptor (*MET*) gene at the cell membrane and its effect on extracellular regulated kinase (Erk) 1/2 and Akt activation. (a) Clarified detergent (RIPA buffer) lysates from NIH-3T3 cells transduced with lentivirus (LV) for hepatocyte growth factor receptor devoid of the ectodomain (Met-EC⁻), Tpr-Met, wild-type Met (Met^{WT}), or enhanced green fluorescent protein (eGFP) were immunoprecipitated with anti-Met polyclonal antibodies and Sepharose protein A. Immunoprecipitates were separated in two gels by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and analysed by western blotting with monoclonal antibodies, either anti-Met (α-Met) or anti-phosphotyrosine (α-PY), followed by secondary horseradish peroxidase-labeled antibodies and processing for enhanced chemoluminescence. Quiescent NIH-3T3-Met^{WT} cells were stimulated or not with hepatocyte growth factor (HGF) for 12 min. Asteriks on the blots indicate the position of **Met-EC⁻ and *Tpr-Met. (b) NIH-3T3-Met-EC⁻ or NIH-3T3-Tpr-Met cells underwent swelling by hypotonic treatment, mechanical disruption, and centrifugation. Proteins in the different fractions were analysed by western blotting with the anti-Met DQ-13 monoclonal antibody. **Met-EC⁻ protein was recovered in the membrane particulate fraction (M), whereas *Tpr-Met was recovered in the cytoplasmic soluble fraction (C). (c) The same cells as in (a) were lysed and equal amounts of extracted protein were separated by SDS-PAGE and analysed by western blotting with Erk1/2, P-Erk1/2, Akt, and P-Akt antibodies, followed by appropriate secondary horseradish peroxidase-labeled antibodies and processing for enhanced chemoluminescence. These are representative experiments from the three independent experiments carried out.



NIH-3T3-Tpr-Met cells (Fig. 1c). In NIH-3T3-Met^{WT} cells the two transducers were phosphorylated only if cells were stimulated with HGF. Together, these experiments showed that the Met-EC⁻ protein is expressed at the plasma membrane and that deletion of the ectodomain constitutively activates the Met receptor and its downstream signaling pathways.

Deletion of the Met ectodomain enhances proliferation and confers transforming and invasive properties. We next tested whether this form of activated Met could induce a transformed and invasive phenotype. We evaluated the proliferation potential both in conventional monolayer cultures and in anchorage-independent conditions. In conventional culture conditions, NIH-3T3-Met-EC⁻ cells grew at a rate similar to that of NIH-3T3-Tpr-Met cells, which was faster than that of both NIH-3T3-Met^{WT} or NIH-3T3-eGFP, used as controls (Fig. 2a). Their doubling time was approximately twice that of the cells used as controls. Similarly, NIH-3T3-Met-EC⁻ cells displayed the same capability as NIH-3T3-Tpr-Met cells to grow in anchorage-independent conditions (Fig. 2b). In this assay, as already reported,[15] NIH-3T3 cells expressing Met^{WT} displayed only a weak ability to proliferate. HGF could only double the number of colonies, whose number, however, still remained significantly low in comparison to the number of colonies grown from NIH-3T3-Met-EC⁻. The invasive potential of Met-EC⁻-expressing cells was evaluated using the transwell system. Cells were seeded on the top of a porous filter coated with Matrigel, featuring an artificial basement membrane. The expression of Met-EC⁻ gave NIH-3T3 cells an invasive phenotype, comparable to that of NIH-3T3-Tpr-Met cells, used as a positive reference (Fig. 2c). By contrast, the expression of Met^{WT} only slightly increased the invasive potential of NIH-3T3 cells, and the addition of HGF did not even double this cellular activity, in accordance with previous reports.[30] Together, these experiments demonstrated that the deletion of the Met ectodomain confers transforming and invasive properties on transduced NIH-3T3 cells.

Deletion of the Met ectodomain confers tumorigenic properties. The strong ability of NIH-3T3-Met-EC⁻ cells to grow in soft agar suggested that these cells may also have the ability to form tumors in nude mice. To test this hypothesis, NIH-3T3 cells (5×10^5) expressing Met-EC⁻, Tpr-Met, Met^{WT}, and eGFP proteins were injected subcutaneously into nude mice (six animals/group) and the growth of tumors was monitored constantly. Only cells expressing Met-EC⁻ and Tpr-Met, used as positive controls, formed visible tumors within 14 days of injection, with cells expressing Met-EC⁻ being somehow more potent (Fig. 3a). Mice were killed after 32 days due to tumor burden, and histological examination revealed that whereas NIH-3T3-Tpr-Met cells formed sarcomas with spindle-shaped cells (Fig. 3b), cells expressing Met-EC⁻ formed tumors with a morphology typical of carcinomas, with polygonal cells (Fig. 3c). The differences in tumor cell morphology mirrored those of the corresponding transduced cells grown *in vitro* (not shown). Both tumors expressed Met, with a diffuse cytoplasmic localization in the case of NIH-3T3-Tpr-Met tumors and a membrane-vesicular pattern in the case of NIH-3T3-Met-EC⁻ tumors, similar to the one observed in most human tumor sections[26] (Fig. 3b,c). Consistent with the tumor cell morphology, immunohistochemical examination revealed that whereas the sarcoma-like cells expressing Tpr-Met were highly positive for vimentin and sporadically positive for cytokeratins, NIH-3T3-Met-EC⁻ tumors were positive for cytokeratins and negative for vimentin. The scanty matrix within the tumor did not stain with Alcian blue (data not shown). These experiments showed that deletion of the Met ectodomain confers tumorigenic properties to transduced NIH-3T3 cells.

Discussion

The data reported herein show that deletion of the ectodomain activates Met and the downstream signaling pathways ras to Erk1/2

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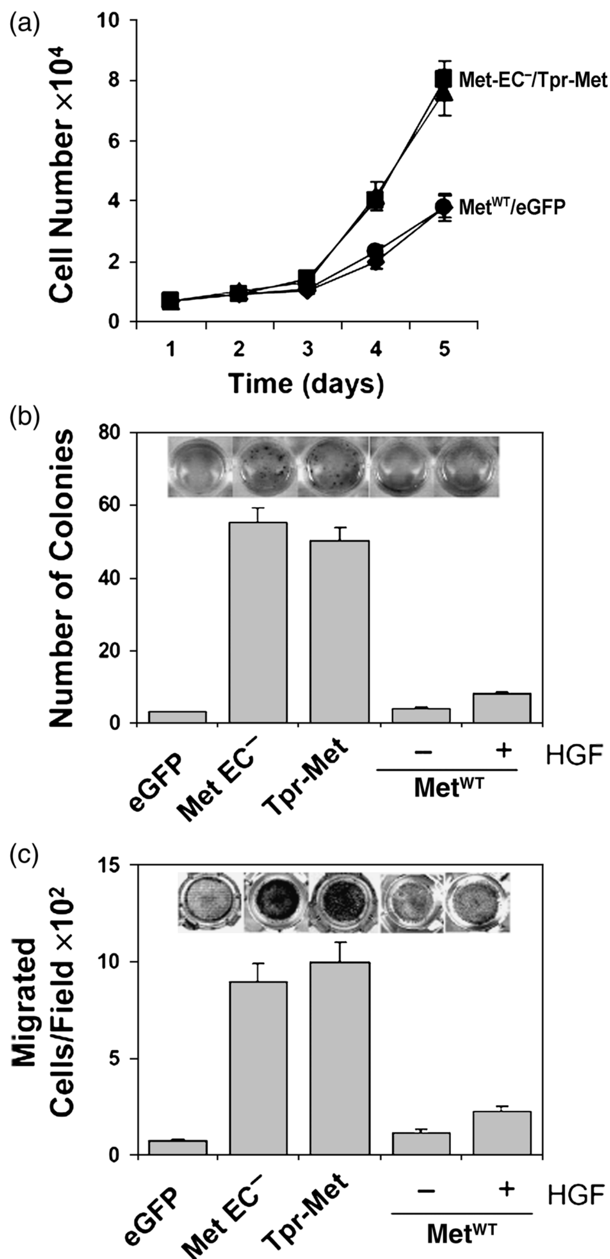

Fig. 2. Deletion of the hepatocyte growth factor receptor (Met) ectodomain enhances proliferation and confers transforming and invasive properties. (a) Proliferation: NIH-3T3 cells transduced with lentivirus (LV) for hepatocyte growth factor receptor devoid of the ectodomain (Met-EC$^-$), Tpr-Met, wild-type Met (Met^{WT}), or enhanced green fluorescent protein (eGFP) were plated at low density, then starved in low serum. Cell proliferation was evaluated at fixed times in 5% serum by a colorimetric assay. Colorimetric values were evaluated on the basis of a calibration curve. Reported numbers are means of quintuplicates (±SD). (b) Anchorage-independent growth: The same cells used in (a) (5×10^3 cells/4-cm^2 well) were seeded in soft agar (0.4% Noble agar in Dulbecco's modified Eagle's medium with 10% fetal calf serum [FCS]) and colonies were stained with MTT, counted, and photographed after 21 days. (c) Invasion: The ability of the same cells (10^5) as in (a) to pass through the 8-μm diameter pores of a transwell chamber filter coated with Matrigel was assayed 48 h after plating in 5% FCS. Cells attached to the lower side of the filter were fixed and stained with crystal violet. Numbers are from representative experiments; values represent the median cell number in 10 fields of two filters ±SD. These are representative experiments out of the three independent experiments carried out.

MAPK and PI3K to Akt, and it unleashes its transforming, invasive, and tumorigenic potential. These data have been obtained with exogenous expression of a truncated Met isoform in NIH-3T3 cells, consisting of its transmembrane and intracellular domains but devoid of the whole extracellular domain, in which are localized the two binding sites for HGF.[31,32] It is thus formally demonstrated that Met can be activated by deletion of the ectodomain, a ligand-independent mechanism already reported for other TRK.[3,4,33–37]

It has already been abundantly reported that the *MET* oncogene can be activated and can acquire oncogenic properties by other mechanisms that are either ligand independent, such as gene rearrangement, amplification and overexpression, and point mutation, or ligand dependent, such as autocrine or paracrine loops.[9,13,14] Previously, Met oncogenic activation upon deletion of the ectodomain was only indirectly suggested. Indeed, in some tumors a truncated Met isoform recognized only by antibodies directed against the cytoplasmic receptor tail and localized to the plasma membrane was detected and its expression was found to correlate with a severe outcome for the patients.[23,24] Recently, a short form of Ron (the MSP TKR belonging to the Met receptor family) lacking the ectodomain was found to be constitutively phosphorylated and to promote tumor progression.[25] This protein is synthesized from a shorter transcript due to the alternative use of two promoters.[8] In the case of Met, the truncated form expressed at the cell surface is generated by proteolytic cleavage, through a so-far-unidentified TIMP-3-sensitive transmembrane ADAM.[38]

Met undergoes basal ligand-independent shedding,[7,21] which can be upregulated by a series of different stimuli, including 12-*O*-tetradecanoylphorbol 13 acetate,[7] crosstalk with other receptors such as the activated epidermal growth factor receptor,[38] and specific Met agonists, such as HGF or monoclonal antibodies.[39] Under normal conditions this mechanism downregulates Met from the cell surface and thus temporally and spatially controls receptor-mediated biological responses and avoids ligand-dependent receptor overstimulation, potentially leading to cell transformation (see Jeffers *et al.*).[21] The data reported here, however, suggest that loss of the ectodomain results also in an opposite effect, that is, activation of the receptor, which, having oncogenic potential, triggers cell transformation and tumorigenesis. Indeed, this mechanism of receptor activation has already been reported for other TKR.[3,4,33–37]

Hepatocyte growth factor receptor shedding may be increased in tumors,[40] and this can be ascribed to the activity of extracellular proteases, which are often dysregulated in cancer. Two major Met fragments are thus generated. Although the 'decoy effect' of the soluble ectodomain fragment is still debated,[15,39,40] its increased levels were reported to correlate significantly with the malignant potential of different cell lines examined, and were independent of the steady-state receptor expression level.[40] The complementary Met fragment is normally not detected, unless cells are treated with lactacystin,[21] which seems to interfere with normal intracellular receptor traffic and degradation.[41] This fragment is tyrosine-phosphorylated and may undergo further regulated intramembrane proteolysis, releasing a fully soluble intracellular Met fragment,[21,39] possibly undergoing further degradation. This mechanism of control seems to be suppressed in malignant cells, as the presence of truncated Met-EC$^-$, detectable by immunohistochemistry on specimens of human breast carcinoma, correlates with a poorer prognosis.[23] Moreover, in a cell model of breast cancer progression, it was detected only in cells with a higher degree of malignancy.[40] Although these data support a role for Met-EC$^-$ in tumor progression, the experiments presented herein suggest that its expression can also trigger cell transformation and tumorigenesis from initial stages.

In similar experiments the transforming and oncogenic potentials of other forms of Met were established.[17,42,43] In particular, structure–function relationship studies have shown that a soluble cytoplasmic form of Met, encompassing the whole cytoplasmic

doi: 10.1111/j.1349-7006.2008.01079.x
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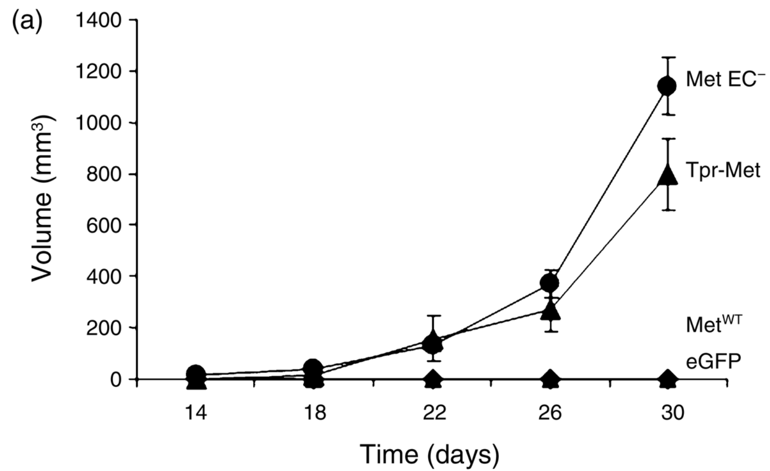

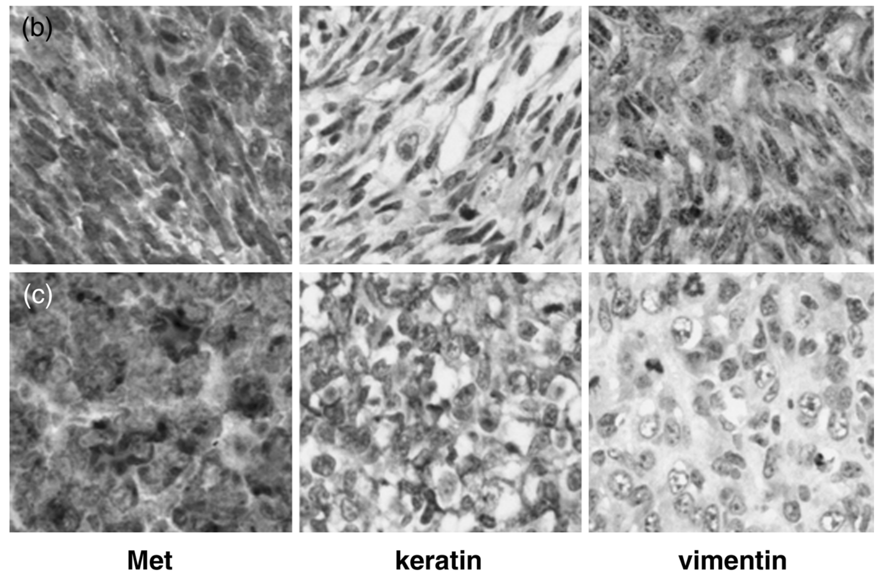

Fig. 3. Deletion of the hepatocyte growth factor receptor (Met) ectodomain confers tumorigenic properties. (a) NIH-3T3 cells transduced with lentivirus (LV) for hepatocyte growth factor receptor devoid of the ectodomain (Met-EC$^-$), Tpr-Met, wild-type Met (Met^{WT}), or enhanced green fluorescent protein (eGFP) (5×10^5 cells) were subcutaneously injected into the posterior flank of athymic Foxn1^{nu} mice. The tumor mass was measured constantly and animals were killed after 32 days. Tumors induced by injection of (b) NIH-3T3-Tpr-Met or (c) NIH-3T3-Met-EC$^-$ cells were excised, fixed in formalin, embedded in paraffin, sectioned at 5 µm, and stained with the anti-Met (DQ-13), anti-keratin, and anti-vimentin monoclonal antibodies, followed by the appropriate peroxidase-coupled secondary antibodies and reaction with 3,3′-diaminobenzidine (DAB). No DAB staining was detected when the primary antibodies were omitted (not shown). Original images were taken at ×450.

domain, is transforming and tumorigenic, although at significantly lower levels than Tpr-Met, used as a reference control,[17] possibly because it contains the juxtamembrane domain, which was shown to impair these activities.[44–49] Insertion of this soluble cytoplasmic form of Met into the plasma membrane by leaving the Met transmembrane domain in the construct (as is the case with Met-EC$^-$ described here) results in a transforming and oncogenic activity similar to the one triggered by Tpr-Met. Although this is in line with the observation that association of oncoproteins with the cell membrane enhances their oncogenicity (see beyond for Tpr-Met), it also shows that membrane association of Met neutralizes the negative effect of the juxtamembrane domain and a more potent oncogenic Met can be generated.

The expression of an activated Met receptor is associated with different tumor histological types, namely of epithelial and mesenchymal origin.[13,48] Here we have found that tumors induced by NIH-3T3-Met-EC$^-$ cells displayed a morphology typical of carcinomas with polygonal cells, in contrast to tumors induced by NIH-3T3-Tpr-Met cells, which, as reported also in other studies, was similar to sarcomas with spindle-shaped cells. Moreover, the expression of markers typical of the two histological types (epithelial- and carcinoma-specific keratins and mesenchyme- and sarcoma-specific vimentin) was consistent with morphologies observed in the two tumor types. Tumors with the morphology of sarcomas were also observed upon injection of NIH-3T3 cells expressing mutant, constitutively activated Met,[42] or coexpressing Met and HGF, resulting in an activating autocrine loop.[43] The different subcellular localization of the two transforming Met proteins, possibly directing the recruitment of different signal transducers, may be responsible for the different tumor phenotypes.[49,50] Because the two major transduction pathways, the PI3K to Akt and ras to MAPK pathways, were similarly activated in the two cell lines expressing Met-EC$^-$ and Tpr-Met, other more subtle differences may be involved. This contrasts with the differential activation of the PI3K to Akt pathway reported for membrane-localized Tpr-Met versus cytosolic Tpr-Met, the former leading to the activation of a hyaluronic acid–CD44 autocrine loop responsible for the myxoid phenotype of the tumors.[50]

In conclusion, exogenous expression of Met lacking the ectodomain confers transforming, invasive, and tumorigenic properties on the cells. Under normal quiescent conditions the ectodomain of intact Met would exert a negative impact on the enzymatic activity of the cytoplasmic kinase domain; cleavage of the ligand-binding receptor ectodomain, as well as the physiological ligand binding, would overcome this inhibition and thus lead to activation of the receptor and the downstream signaling pathways.

Acknowledgments

We thank Maria Flavia Di Renzo and Ricccardo Taulli for providing the NIH-3T3-Met^{WT} and NIH-3T3-Tpr-Met cells and for lentiviral vector preparation. We thank Maria Flavia Di Renzo, Lorenza Penengo, and Gianluca Baldanzi

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for helpful discussion. This work was supported by University of Piemonte Orientale, Novara, and Ricerca Finalizzata by Regione Piemonte. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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doi: 10.1111/j.1349-7006.2008.01079.x
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