Met, the Hepatocyte Growth Factor Receptor, Localizes to the Nucleus in Cells at Low Density

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Abstract

Some breast cancer cases in our previous immunohistochemical studies show Met expression in the nucleus. Given nuclear localization of other receptor tyrosine kinases, we proceeded to investigate Met. Nuclear Met is seen in numerous cell lines and in germinal regions of many tissues using four unique antibodies. Cell fractionation reveals a 60-kDa band recognized by COOH-terminal Met antibodies that is present independent of hepatocyte growth factor treatment. Green fluorescent protein (GFP) fusion proteins of the cytoplasmic domain of Met transfected into HEK293 cells are found in the nucleus whereas the full-length Met-GFP fusion is membranous. Further deletions of the Met-GFP fusions identify a region of the juxtamembrane domain required for nuclear translocation. In a CaCo2 cell line model for epithelial maturation, we find that Met is initially nuclear, and then becomes membranous, after confluence. This work suggests processing of Met. Nuclear Met is seen in numerous cell lines and its localization of other receptor tyrosine kinases, we proceeded to investigate Met. Nuclear Met is seen in numerous cell lines and in germinal regions of many tissues using four unique antibodies. Cell fractionation reveals a 60-kDa band recognized by COOH-terminal Met antibodies that is present independent of hepatocyte growth factor treatment. Green fluorescent protein (GFP) fusion proteins of the cytoplasmic domain of Met transfected into HEK293 cells are found in the nucleus whereas the full-length Met-GFP fusion is membranous. Further deletions of the Met-GFP fusions identify a region of the juxtamembrane domain required for nuclear translocation. In a CaCo2 cell line model for epithelial maturation, we find that Met is initially nuclear, and then becomes membranous, after confluence. This work suggests processing of the Met receptor, analogous to ErbB4, resulting in the release of the cytoplasmic domain and its translocation to the nucleus in cells at low density. (Cancer Res 2006; 66(16): 7976-82)

Introduction

Met (the c-Met gene product) is a receptor tyrosine kinase (RTK) for the hepatocyte growth factor (HGF) ligand that is expressed on the surface of epithelial and endothelial cells. The primary 150-kDa transcript is partially glycosylated to form the 170-kDa Met precursor that is then cleaved forming a heterodimer consisting of two subunits (45 and 150 kDa) joined by disulfide bonds. The smaller α subunit is entirely extracellular, whereas the larger β subunit traverses the plasma membrane and includes a juxtamembrane region responsible for negatively regulating Met, a tyrosine kinase domain with intrinsic kinase activity, and a COOH-terminal region that provides binding sites for target substrates (for review, see ref. 1). Binding of HGF to Met induces dimerization, which in turn triggers autophosphorylation of tyrosine residues in the activation loop of the kinase domain. Autophosphorylation then activates the intrinsic kinase activity of Met via the subsequent phosphorylation of two tyrosine residues in the COOH terminus, forming a multisubstrate docking site. Chimeric receptors with these residues can induce mitogenic, morphogenic, and motogenic responses similar to wild-type Met (reviewed in ref. 2). Activation of the docking site triggers signaling cascades, such as Gab1, Grb2, and phosphatidylinositol 3-kinase, leading to proliferation, scatter- ing, increased motility, invasion, and branching morphogenesis (reviewed in ref. 3). Met has also been shown to be activated in the absence of HGF ligand. Constitutive activation of Met can occur via mutations in the cytoplasmic domain and are associated with the genesis and progression of some human tumors (4).

A growing list of membrane proteins has been found to translocate to the nucleus. Members of the epidermal growth factor (EGF) and fibroblast growth factor family of RTKs have been shown to translocate to the nucleus as either intact receptors, or for ErbB4, as a truncated fragment, activating transcription of target genes (5, 6). ErbB4, a member of the EGF receptor family, plays an important role in mammary gland development and controls cell proliferation and differentiation. Ligand binding to ErbB4, such as Met, stimulates dimerization and autophosphorylation followed by substrate phosphorylation. ErbB4 undergoes cleavage of its intracellular domain via presenilin-dependent γ-secretase-like proteolysis, which then localizes to the nucleus and plays a role in regulating gene transcription (7, 8). Other membrane proteins that undergo similar cleavage events and subsequent nuclear localization include Notch, APP, CSF-1, E-cadherin, and CD44 (9–11). γ-Secretase cleavage of these proteins occurs in the transmembrane domain and is usually preceded by ectodomain shedding by a matrix metalloproteinase.

Our previous immunohistochemical study on ~600 cases of breast cancer showed that expression of the Met cytoplasmic domain and not the extracellular domain was correlated with poor patient outcome in lymph node–negative breast carcinomas (12). This difference is not easily explained, but one possibility is a processing event where a COOH-terminal fragment is present in the absence of the NH₂ terminus. Other RTKs are cleaved, and the resultant cleavage product translocates to the nucleus as for ErbB4 (7). Although we cannot yet prove cleavage, here we show a 60-kDa fragment of Met that localizes to the nucleus in a ligand-independent manner and is associated with lower density cell colonies.

Materials and Methods

Cell lines and treatments. HEK293, A431, NIH3T3, and HT-29 cell lines were obtained from American Type Culture Collection (ATCC, Manassas, VA) and cultured in DMEM with 10% fetal bovine serum (FBS). Mel1241 and Mel1335 cells were cultured in RPMI 1640 with 10% FBS. HMEC cells were obtained from ATCC and cultured in MEGM with 10% FBS. MCF-10A cells were obtained from ATCC and cultured in DMEM/F12 with 10% horse serum. CaCo2 cells were generously donated by the laboratory of Dr. Jon Morrow (Yale University School of Medicine, New Haven, CT). A431 cells were serum starved in DMEM containing 0.5% FBS for 18 hours and treated with 250 μmol/L ALLN (Calbiochem, La Jolla, CA) or 20 ng/mL HGF (R&D Systems, Minneapolis, MN). Treatment with ALLN and HGF combined was for the indicated time with a 30-minute pretreatment of ALLN. Primary antibodies to the COOH terminus of Met were monoclonal antibody 3D4 and polyclonal antibody CVD13 (Zymed Laboratories, Inc., San Francisco, CA) and polyclonal antibodies C12 and C28 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Other antibodies include lamin, E-cadherin, and extracellular signal-regulated kinase (ERK) 1 (BD Transduction Laboratories, San Diego, CA), α-tubulin (Zymed Laboratories), and pERK1/2 (Upstate, Lake Placid, NY).
Immunofluorescence and immunohistochemistry. Cells were grown on four-chamber slides (BD Biosciences, San Diego, CA) or coverslips, fixed in 4% paraformaldehyde, and permeabilized with 0.2% Triton X-100. Cells were blocked in 1% bovine serum albumin (BSA) for 1 hour. Primary antibodies at a dilution of 1:500 were added to cells and incubated for 1 hour. Cells were washed and incubated with goat anti-mouse or goat anti-rabbit Alexa 488 (Molecular Probes, Eugene, OR) secondary antibodies for 1 hour at room temperature. Cells were washed and mounted using Shandon Immu-mount medium (Thermo Electron, Waltham, MA) or Prolong Gold with 4',6-diamidino-2-phenylindole (DAPI; Molecular Probes). Cells were imaged on an Olympus (Center Valley, PA) BX41 microscope using a ×40 UPlanFl objective and equipped with a Sensys camera and IPLabs imaging software (Scanalytics, Inc., Fairfax, VA). Deconvolution images were taken with a ×60 PlanApo oil objective on a DeltaVision Nikon (Melville, NY) Eclipse T3200 microscope equipped with the Photometrics Series 300 camera and Softworx imaging software. All images were imported in to Adobe Photoshop 7.0.

The multitumor tissue array was constructed as described by Chung et al. (13). Immunoperoxidase staining was done as described previously in ref. 12.

Figure 1. Met localizes to the nucleus in normal and cancerous tissues and cell lines. A, immunohistochemistry of normal and cancerous tissue samples on a multitumor tissue microarray using the C28 antibody to the COOH terminus of Met. B, immunofluorescence of cell lines using four different antibodies to the cytoplasmic domain of Met shows varying levels of membranous, cytoplasmic, and nuclear expression. Immunofluorescence using an antibody to the extracellular domain of Met (DO24) shows cytoplasmic and membranous expression. Bottom, left, names of the antibodies used. Images were taken at ×40 (MCF-10A and HMEC images were taken at ×20).
Transfection. Constructs were made from the pMBI plasmid courtesy of Dr. X.Y. Fu (Indiana University School of Medicine, Indianapolis, IN). PCR primers were used to amplify Met sequences for insertion into the pEGFP-N1 vector (BD Biosciences). HEK293 cells were transiently transfected with 8 µg Maxi-prep DNA using the calcium phosphate method. Live cells were viewed on an Olympus Fluoview Scanning Laser Confocal microscope. The images were collected using an Olympus ×100 oil immersion lens.

Western blot analysis. Cells were harvested in radioimmunoprecipitation assay buffer (RIPA). Lysates were prepared using Bio-Rad (Hercules, CA) reagent, and total lysate (40 µg) was loaded onto 10% SDS-PAGE and transferred to Hybond enhanced chemiluminescence (ECL) nitrocellulose membrane (Amersham Biosciences, Piscataway, CA). Membranes were blocked in 1% BSA, TBS, and 1% Tween (BSA/TBS/Tween) for 1 hour. Primary antibodies at a 1:1,000 dilution were added to BSA/TBS/Tween and incubated for 1 hour at room temperature. Membranes were washed with TBS/Tween and then incubated with horseradish peroxidase–conjugated goat anti-mouse or goat anti-rabbit secondary antibody (The Jackson Laboratory, Bar Harbor, ME) at a dilution of 1:20,000 for 1 hour at room temperature. Bands were detected using ECL reagent (Amersham Biosciences) and exposed to film for 4 days.

Immunoprecipitation. Cells were grown to 60% confluence, treated with ALLN or DMSO as indicated above, and harvested in RIPA. Protein (1 mg) was incubated with antibody for 1 hour at 4°C followed by the addition of rec-Protein G-sepharose 4B beads (Zymed Laboratories) for 1 hour.

Cell fractionation. HEK293 cells were grown to confluence, A431 cells were grown to 60% confluence and treated with ALLN or DMSO as indicated above. Cells were harvested and washed in PBS, resuspended, and lysed in 0.2 mol/L sucrose buffer with 3 mmol/L CaCl₂, 2 mmol/L magnesium acetate, 0.1 mmol/L EDTA, 10 mmol/L Tris (pH 8.0), 1 mmol/L DTT, 0.5% NP40, and protease inhibitors centrifuged at 1,000 × g. The supernatant was used as the cytoplasmic fraction. The resuspended pellet was centrifuged on a 1.8 mol/L sucrose cushion with 5 mmol/L magnesium acetate, 0.1 mmol/L EDTA, 10 mmol/L Tris (pH 8.0), 1 mmol/L DTT, and protease inhibitors at 30,000 × g for 45 minutes. The resulting nuclear pellet was resuspended in glycerol buffer.

Northern blot analysis. Ninety percent confluent cells in T-75 flasks were lysed with 4 mol/L guanidine thiocyanate and 25 mmol/L sodium acetate. Lysates were then centrifuged on a 5.7 mol/L CsCl cushion in an ultracentrifuge at 30,000 rpm for 45 minutes. The resulting nuclear fraction was diluted to a final concentration of 2 µg/µL. The following probes were used: pMBI (1.5 KB), 1.2 KB, 0.9 KB, and 0.7 KB fragments which corresponded to the first, second, third, and fourth exons of the human Met gene, respectively. The probes were labeled with [α-32P]dCTP using the random primer labeling kit (Stratagene). The probe was boiled and added to the electrophoresis, gels were transferred, top down, to a nylon membrane (Invitrogen). The membrane was exposed to X-ray film for 4 days.

Results

Antibodies to the COOH terminus of Met localize to the nucleus. Immunohistochemistry, using an antipeptide polyclonal antibody to the COOH terminus of the Met receptor, shows Met in the cytoplasm and nucleus of normal and cancers tissues rather than the previously reported predominantly membrane staining (Fig. 1A; refs. 14, 15). Normal colon, skin, and testis show similar nuclear staining of Met in germinal tissue layers and aberrant expression throughout cancers tissue. The transition to Met-overexpressing cancers is most dramatically seen in lymphoma tissue where Met is completely absent in the normal lymph node tissue and localized almost exclusively to the nucleus in cancers tissue.

To confirm that the nuclear localization of Met expression was not an artifact seen only in formalin-fixed and paraffin-embedded tissue, a series of cell lines were stained with four different antibodies to the cytoplasmic domain. The antibodies selected were polyclonal antibodies C12, C28, and CVD13 to peptides very near or including the COOH terminus of Met, as well as 3D4, a monoclonal antibody to the tyrosine kinase domain. Mammary epithelial lines HMEC and MCF10-A, melanoma cell lines Mel1241 and Mel1335 (data not shown), and epidermoid carcinoma line A431 (Fig. 1B) all showed nuclear expression of Met to varying degrees depending on the antibody used. HEK293 and NIH3T3 cell lines express low levels of Met yet also showed nuclear staining equivalent to those known to express higher levels of Met. DO24, a monoclonal antibody to the extracellular domain of Met, shows cytoplasmic staining in all cell lines stained under the same culture conditions as showed nuclear expression (data not shown); however, HMEC, MCF10-A, HEK293, and A431 cell lines show membranous staining in dense regions of the cell colony. Images of NIH3T3 and Mel1241 cells stained with DO24 were overexposed to detect any staining of the extracellular domain of Met. The staining pattern seen in some cells under these conditions is perinuclear.

Verification of nuclear Met expression in cell lines. Because nuclear staining of Met was seen in cell lines previously considered low expressers or negative for Met, a Northern blot was done to confirm the presence of Met in these cell lines. Using a radioactively labeled probe representing 450 bp at the 3′-end of the translated sequence of Met, a 9.5-kb transcript was detected in all cell lines. The smaller 7.5-kb variant of Met was also seen in all cell lines, except NIH3T3 and Mel1335 (Fig. 2). No smaller isoforms were seen. Thus, although some lines (NIH3T3) have been used previously as negative controls for Met transfection (16), full-length message is observed for each cell line. Furthermore, this also suggests that the protein is not derived from a novel alternative splicing variant.

A 60-kDa Met fragment is found in the nuclear fraction. To determine if Met is present in the nucleus, cell fractionation was done to separate the nuclear fraction from the membranous and cytoplasmic fractions using standard sucrose gradient-based methods. The epidermoid carcinoma cell line A431 was used as a model. Separation of the cytoplasm from the nucleus resulted in a 60-kDa protein that is reactive with Met antibody localizing to the
nuclear fraction, whereas the 145-kDa Met receptor remained in the cytoplasmic fraction in both A431 (high Met expresser) and HEK293 (low Met expresser) cell lines (Fig. 3A). Tubulin is shown as a control for the cytoplasmic fraction, and lamin serves as a control for the nuclear fraction. Because the 60-kDa fragment has not been reported previously, we used a series of two polyclonal antibodies and one monoclonal antibody to prove that the band is actual Met and not a cross-reacting protein. Western blotting of A431 lysates from this line showed the expected band at 145 kDa and no band at 60 kDa. Immunofluorescence detected low levels of nuclear Met in A431 cells, suggesting that the nuclear fragment may need to be enriched in some manner to detect it on a Western blot; this is in contrary to subcellular fractionation results, as fractionation served to enrich the amount of nuclear protein seen on a Western blot. When A431 cells were serum starved and treated with ALLN, a proteasome inhibitor, a band that comigrates with 60-kDa markers is detected by three different antibodies to Met (Fig. 3B). The C12 antibody also detects bands at 120-kDa; these bands are cleavage products of the Met receptor (17) that are not detected by antibodies 3D4 and CVD13. To further prove specificity, we immunoprecipitated Met from total cell lysates of ALLN-treated and untreated A431 cells as well as untreated HEK293 and NIH3T3 cells. All cell lines show the cleaved Met precursor (190 kDa), full-length Met (145 kDa), and a band of 60 kDa (Fig. 3C) when immunoprecipitated with either a monoclonal or a polyclonal Met antibody. As seen in subcellular fractionation, the presence of the 60-kDa fragment in untreated cells is again due to the enrichment capability of the assay. Immunoprecipitation of NIH3T3 cells, which do not express Met at the membrane, does not pull down full-length Met when immunoprecipitated with the C12 antibody; however, the 3D4 antibody pulls down the uncleaved Met precursor. Additionally, the 3D4 antibody shows a band slightly higher than the 60-kDa fragment that also appeared faintly in the no antibody control lane. Together, these data support the presence of a 60-kDa fragment of Met in the nucleus.

**HGF, the Met ligand, does not effect expression of the 60-kDa fragment.** HGF is the ligand for Met, and its interaction with the extracellular domain of Met triggers dimerization and phosphorylation of the receptor and activation of several downstream signaling pathways, including the mitogen-activated protein kinase pathway (18). To assess the effect of HGF on the 60-kDa fragment, we treated two model cell lines with HGF over a 24-hour time course. Treatment with HGF did not induce the appearance of the 60-kDa fragment. Figure 4 shows that, although HGF is activating downstream signaling, as shown by phosphorylation of ERK1/2, the 60-kDa fragment is not induced. However, treatment with ALLN, which inhibits proteolysis, substantially increases the amount of the 60-kDa fragment in both cell lines in both the presence and the absence of HGF (Fig. 4).

**Met-green fluorescent protein constructs localize to the nucleus.** To verify that a region of the cytoplasmic domain is present in the nucleus, Met and NH2 terminally truncated forms of the cytoplasmic domain of Met were cloned into a green fluorescent protein (GFP) fusion vector so that GFP was located on the COOH terminus of the protein. We constructed a full-length Met protein fusion (Met), a fusion with the cytoplasmic domain truncated at the transmembrane/intracellular junction, amino acid K956 (Cyto), three constructs truncated after tyrosine residues (D972, R1004, and P1027) in the juxtamembrane region referred to as Jxm1, Jxm2, and Jxm3, the tyrosine kinase domain (Tkd1) beginning at I1084, and the tyrosine kinase domain truncated at L1157 (Tkd2). Each Met construct had a predicted molecular weight of 49, 47, 43, 41, 35, and 27 kDa, respectively; the GFP tag is

Figure 3. A 60-kDa fragment of Met localizes to the nucleus. A, cellular fractionation of HEK293 (lanes 1 and 2) and A431 cells (lanes 3 and 4). C, cytoplasmic fraction; N, nuclear fraction. The Met receptor (145 kDa) localizes to the cytoplasmic fraction, and a novel 60-kDa fragment recognized by the Met antibody localizes to the nuclear fraction. Purity of fractions was determined by the presence of tubulin (cytoplasmic marker) and lamin (nuclear marker). B, A431 total cell lysates treated with vehicle or 250 μmol/L ALLN show the 60-kDa fragment of Met after treatment with ALLN. Western blots were probed with three different antibodies to the COOH terminus of Met as labeled, C12, CVD13, and 3D4. C, immunoprecipitations of A431 cells, A431 cells treated with 250 μmol/L ALLN, HEK293 cells, and NIH3T3 cells (low expresser) all show a 60-kDa fragment recognized by antibodies to the COOH terminus of Met. Antibodies used to precipitate (IP) and Western blot (WB).

Figure 4. The nuclear fragment of Met is independent of HGF activation. A431 and CaCo2 cells were treated with 250 μmol/L ALLN and 20 ng/mL HGF or pretreated with ALLN for 30 minutes followed by ALLN/HGF treatment for 1, 6, or 24 hours. HGF treatment had no effect on the presence of the 60-kDa fragment when compared with the control in lane 1. Activation of Met by HGF was verified by phosphorylation of ERK. ALLN has function as both protease and phosphatase inhibitor, which may explain why pERK is present in all ALLN-treated lysates. Western blots using lysate from the same experiment were pieced together.
an additional 30 kDa. All of the constructs shown in a schematic in Fig. 5A were then transiently transfected into HEK293 cells. A Western blot of HEK293 cells transiently transfected with the cytoplasmic domain of Met confirmed the presence of the GFP-tagged Met construct (Fig. 5B). The full-length Met-GFP fusion localized to the plasma membrane of HEK293 cells as expected (Fig. 5C). Surprisingly, this construct has not been seen in the nucleus under any conditions. This may be due to the construction of an unnatural state of forced overexpression, as nuclear expression of Met is not readily seen in HT-29 cells, which overexpress Met (data not shown). As we have yet to determine the mechanism by which Met enters the nucleus, another possibility for the lack of nuclear Met-GFP is that the transfected cells were not properly stimulated to express nuclear Met-GFP. However, constructs of the cytoplasmic domain of Met localized to the nucleus of cells. Loss of the juxtamembrane region caused Met-GFP tyrosine kinase domain constructs to be partially excluded from the nucleus of cells, and loss of the NH2 terminus of the tyrosine kinase domain led to complete exclusion from the nucleus. (Fig. 5C). The same localization results were observed in transfections of MCF-10A cells (data not shown). Transient transfections of Met-GFP constructs confirm the ability of the cytoplasmic domain of Met to enter the nucleus. The region of the juxtamembrane domain preceding the tyrosine kinase domain (P1027-I1084) seems to be necessary for nuclear localization of the cytoplasmic domain.

**Localization of Met is dependent on cell density.** CaCo2 cells mature and differentiate as a function of time and density when grown in culture (19, 20). Preconfluent CaCo2 cells show nuclear and cytoplasmic expression of Met as seen in Fig. 6A. CaCo2 cells grown 3 days past confluence show a more distinct membranous as well as cytoplasmic expression of Met (Fig. 6B) using antibodies to the COOH terminus. Similar relocalization patterns were obtained with A431 cells (data not shown); however, it required A431 cells to be grown for a minimum of 10 days past confluence to detect regions of membranous Met expression. In addition, Met expression using DO24, an antibody to the extracellular domain of Met, was cytoplasmic (nonnuclear) at low density and membranous at high density in epithelial cells as discussed in Fig. 1B. This suggests that nuclear expression of Met may be detected only in cells in a state of rapid proliferation or in a less differentiated state, a hypothesis consistent with the expression patterns of Met in proliferative areas of normal tissues in Fig. 1A and in moderately or poorly differentiated cancers. Further work will be necessary to confirm this observation.

**Discussion**

Here, we show for the first time that a COOH-terminal fragment of the HGF receptor Met localizes to the nucleus in a ligand-independent manner. Using a series of monoclonal and polyclonal antibodies to the COOH terminus of Met, we show that Met expression is present not only at the membrane but also in the cytoplasm and nucleus in seven cell lines and predominantly in germinal regions in a range of tissues from normal organs. Nuclear translocation is reconstituted by transfection of GFP fusions with Met that migrate or do not migrate to the nucleus, dependent on the composition of the fusion. Cell lines expressing nuclear Met have either a mesenchymal phenotype or are on the leading edge of epithelial clusters. The maturation and increasing density of the CaCo2 cells provides the best model to illustrate this hypothesis.

All cell lines expressing Met in the nucleus show Met transcripts by Northern blot. Western blotting reveals a 60-kDa band recognized by antibodies to the COOH terminus of Met that localizes to the nucleus. The appearance of a smaller protein recognized by Met antibodies in the nucleus and the lack of a smaller transcript by Northern blot suggest that this fragment is derived from the full-length Met receptor by a processing event. The increase in amounts of the fragment in the presence of ALLN provides further evidence for a processing event. Although no nuclear localization sequence has been identified, serial deletion constructs of Met isolate a region of the juxtamembrane domain (P1027-I1084) that is required for nuclear localization of the cytoplasmic fragment of Met. This fragment is not an alternative splice as shown by Northern blotting and is most likely the product of a cleavage event, as the full-length Met-GFP fusion does not localize to the nucleus.

Studies have shown that a growing list of transmembrane receptors, including ErbB4, Notch, and APP, are cleaved within or near the transmembrane domain, a process referred to as regulated intramembrane proteolysis (RIP; ref. 21). This cleavage releases the cytoplasmic domain, which can translocate to the nucleus and regulate gene expression. RIP of membrane receptors, such as ErbB4 and Notch, is preceded by cleavage of the ectodomain (22, 23). It has been shown that Met undergoes ectodomain shedding by a TACE-related ADAM protease (24). This cleavage event leads to rapid ubiquitination of the cytoplasmic domain by c-Cbl, an E3 ubiquitin ligase (25), and for Notch, is followed by...
nuclear translocation of the cytoplasmic domain (26). We found that treatment with a proteolytic inhibitor, ALLN, substantially increased the levels of a 60-kDa COOH-terminal fragment of Met in A431 cells. This fragment localizes to the nucleus. Our results indicate that Met may also undergo RIP leading to nuclear localization of the cytoplasmic domain of Met. This process seems to be present at a basal level in all cell lines tested and independent of ligand binding.

The expression of nuclear Met in germinal tissue layers and at the periphery of some epithelial clusters suggests that nuclear Met may be associated with a mesenchymal or germinal phenotype. The reduction of nuclear expression of Met in maturing CaCo2 cells suggests that nuclear Met is linked to cell density and might also be a trait of less-differentiated cells. In addition, a recent study describing a yeast 2-hybrid screen identified nuclear proteins, such as SMC-1, a structural maintenance of chromosomes protein, as a novel interaction protein for the cytoplasmic domain of Met (27). The presence of Met in the nucleus indicates that it may play a role in enhancing signaling of the full-length Met receptor or may be indicative of a novel signaling pathway. Regardless, further studies on the function of Met in the nucleus will be important to fully understanding the Met signaling pathway. This is particularly important in light of the fact that several pharmaceutical companies are in various phases of testing Met-targeted therapies.

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References


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