Branching Tubulogenesis but Not Scatter of Madin-Darby Canine Kidney Cells Requires a Functional Grb2 Binding Site in the Met Receptor Tyrosine Kinase*

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Hepatocyte growth factor is a multifunctional cytokine that induces mitogenesis, motility, invasion, and branching tubulogenesis of several epithelial and endothelial cell lines in culture. The receptor for hepatocyte growth factor has been identified as the Met tyrosine kinase. To investigate the signaling pathways that are involved in these events, we have generated chimeric receptors containing the colony stimulating factor-1 receptor fused to the transmembrane and intracellular domains of the Met receptor. Madin-Darby canine kidney epithelial cells expressing the Met chimera dissociate scatter and form branching tubules in response to colony stimulating factor-1. From structure-function analyses, tyrosine residue 1356 within the carboxyl terminus of the Met receptor is critical for these events. The amino acid sequence downstream from tyrosine 1356 represents a consensus binding site for the Grb2 adaptor protein and forms a multisubstrate binding site for the P85 subunit of phosphatidylinositol 3-kinase, phospholipase Cγ, and the Shc adaptor protein. To distinguish which of these signaling pathways are required, we generated a mutant receptor that selectively fails to associate with the Grb2 adaptor protein. Cells expressing this mutant receptor scattered but were unable to form branching tubules, indicating that a Grb2 binding site in the Met receptor is critical for morphogenic responses.

Hepatocyte growth factor (HGF), also known as scatter factor (SF), is a multifunctional cytokine with activities on a wide variety of normal and neoplastic cells. HGF/SF is a prototype for a family of growth modulators that shares structural similarity with the serine protease plasminogen but lacks any proteolytic activity (1, 2). HGF/SF is primarily a mesenchymally derived factor (3) that stimulates mitogenesis, motility, and invasiveness of epithelial cells and induces branching tubulogenesis of kidney, breast, and lung epithelium grown in matrigel culture (4). In vivo, HGF/SF is a potent angiogenic factor (5) and is involved in organ regeneration (6) and tumorigenesis (7). A high affinity receptor for HGF/SF has been identified as the Met tyrosine kinase (8, 9). The Met receptor is a 190-kDa transmembrane protein, which is composed of an α subunit of 45-kDa disulfide linked to a β subunit of 145 kDa (10). The β subunit spans the plasma membrane, and its cytoplasmic portion contains a catalytic kinase domain as well as several potential sites of tyrosine phosphorylation (11, 12). Using heterologous chimeras containing the extracellular domain of the colony stimulating factor-1 receptor fused to the transmembrane and intracellular portion of the Met receptor, we and others have demonstrated that the Met receptor cytoplasmic domain is sufficient to mediate the pleiotropic biological responses to HGF/SF (13–15) and that these events require Met-dependent protein-tyrosine phosphorylation (13, 14).

Phosphorylated tyrosine residues in the non-catalytic cytoplasmic domain of receptor tyrosine kinases act as specific binding sites for substrates containing Src homology 2 (SH2) and phosphotyrosine binding (PTB) domains, which in turn transmit intracellular signals (reviewed in Ref. 16). Multiple substrates are activated and/or associated with the Met receptor following stimulation of cells with HGF/SF. These include Src, mitogen-activated protein kinase, phospholipase Cγ (PLCγ), phosphatidylinositol 3-kinase (PI 3-kinase), Ras, focal adhesion kinase, S6 kinase, and the adaptor proteins Grb2 and Shc (17–24). Upon stimulation with HGF/SF, the Met β subunit becomes highly phosphorylated on two tyrosine residues (tyrosine 1234 and 1235) within the kinase domain that are required for catalytic activity of the receptor (19, 25). In addition, tyrosine 1356 within the carboxyl terminus is phosphorylated (19) and is crucial for motility and branching tubule formation in Madin-Darby canine kidney (MDCK) cells (19, 26). The amino acid sequence downstream from tyrosine 1356 is VNV, which represents a consensus binding site for the Grb2 adaptor protein (27). In addition to binding Grb2, tyrosine 1356 forms a multisubstrate binding site for the P85 subunit of PI 3-kinase and PLCγ and, together with tyrosine 1349, is required for association and/or phosphorylation of the Shc adaptor protein (19, 20, 24, 28, 29). This implicates one or a combination of several signaling pathways originating at tyrosine 1356 in dissociation, motility, and morphogenesis of MDCK cells following stimulation with HGF/SF.

To distinguish which signaling pathways are required for cell motility and morphogenesis, we have taken advantage of the observation that the SH2 domain of Grb2 but not P85 or PLCγ has an absolute requirement for the Asn in the +2 position downstream of a phosphorylated tyrosine (27). We show in this...
paper that a mutant chimeric CSF-MET receptor containing an Asn to His substitution, two amino acids downstream from tyrosine 1356 (N1358H), fails to associate with Grb2 yet associates with PLCγ, the Shc adaptor protein, and the p85 subunit of PI 3-kinase. Cells expressing the N1358H mutant CSF-MET receptor scattered in response to rhCSF-1 but were unable to efficiently form branching tubules. Thus, a Grb2 binding site or pathways downstream from Grb2 are not essential for cell scatter but are critical for branching morphogenesis.

MATERIALS AND METHODS

Site-directed Mutagenesis

The construction of the CSF-MET chimera and the creation of the Y1356F and K1110A mutants have been previously described (13, 19). Creation of the Y1349F and Y1313F mutations was carried out as previously described (28, 30). The N1358H mutation was generated according to the Deng and Nickellod protocol (31), using the following oligonucleotide primer (substitutions are underlined): GTGAACGCTA CTAATGCTGCAACGTAAAATGT. To ensure that no additional mutations were introduced, all constructs were sequenced using the Sequenase 2.0 kit from Amersham.

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Cell Culture and DNA Transfections

COS-1 and MDCK cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Life Technologies, Inc.). Cell lines expressing either wild-type or mutant forms of CSF-MET were generated by retroviral infection of MDCK cells as described previously (32). Amphotropic retroviruses were produced following transient cotransfection of COS-1 cells with the pLXSN retrovirus vector containing the csf-met gene and the pSVY-ALMV plasmid, which encodes the retroviral packaging proteins (33). Supernatant from the COS-1 cells, which contained packaged virus, was used to infect MDCK cells. 24 h post-infection, MDCK cells were subcultured and selected in media containing 400 μg/ml G418, and 20 individual G418-resistant colonies were picked and expanded into cell lines for analysis.

Transient transfections were carried out by transfecting COS-1 cells (10⁶ cells/100-mm plate) with 6 μg of DNA encoding either the wt or mutant forms of the CSF-MET receptor using the DEAE-dextran method (32).

Immunoprecipitation and Western Blotting

Cells at 80–90% confluence were lysed in a lysis buffer containing 0.5% Triton X-100, 50 mM HEPES (pH 8.0), 150 mM NaCl, 10% glycerol, 0.5 mM phenylmethylsulfonylfluoride. Equal amounts of total protein were immunoprecipitated and immunoblotted as described previously (28). Immunoreactive bands were visualized by enhanced chemiluminescence (Amersham).

In Vitro Kinase Assays

COS-1 cells transfected with vectors encoding either the wt or mutant forms of the CSF-MET receptor were harvested in lysis buffer containing 0.5% Triton X-100, immunoprecipitated, and subjected to in vitro kinase as described previously (30).

In Vitro Association Assay

Using Cell Lysates—COS-1 cells transfected with vectors encoding either the wt or mutant forms of the CSF-MET receptor were harvested in 0.5% Triton X-100 lysis buffer as described above and subjected to in vitro association as described previously (28).

Using Fusion Proteins—Bacteria expressing the amino- and carboxy-terminal SH2 domains of PLCγ (nucleotides 547–752) fused to glutathione S-transferase (GST-N1-C-SH2 PLCγ), as well as the SH2 domain of Shc fused to GST (GST-SH2 Shc) were kindly provided by Dr. Tony Pawson, Mount Sinai Hospital, University of Toronto. Bacteria expressing the PTB domain of Shc fused to GST (GST-PTB Shc) was kindly provided by Dr. Michel Tremblay and Alain Charest, Dept. of Biochemistry, McGill University. Fusion proteins were produced by isopropyl-β-D-thiogalactopyranoside induction, and purified was carried out on glutathione-agarose beads (34). Transfected COS-1 cells were harvested as described above and incubated with approximately 0.5–1.0 μg of the GST fusion proteins for 2 h at 4 °C. Complexes were washed in 0.1% Triton X-100 lysis buffer and resuspended in Laemmli sample buffer. Proteins complexed with the GST fusion proteins were subjected to 8% SDS-PAGE and Western blotting as described above.

RESULTS

Generation and Characterization of Mutant CSF-MET Chimeras—The Met receptor cytoplasmic domain contains 16 tyrosine residues, 3 of which, including tyrosines 1349 and 1356 (Fig. 1A), are located in the carboxyl tail of the protein (37). We have previously shown that tyrosine 1356 (Tyr-1356) is the major site of autophosphorylation in the carboxyl terminus of the Met receptor (19) and is critical for efficient cell scatter and branching tubulogenesis of MDCK cells (19). Tyr-1356 and to a lesser extent Tyr-1349 form a multisubstrate binding site for the p85 subunit of PI 3-kinase, PLCγ (20), and the Shc adaptor protein (24), whereas only Tyr-1356 is required for the association of the Met receptor with the Grb2 adaptor protein (19, 20). To determine substrate associations important for signaling leading to cell scatter and branching tubulogenesis and to establish if Tyr-1349 plays a critical role, we have generated mutant CSF-MET chimera containing Tyr-Phe substitutions at Tyr-1349, a double mutation at Tyr-1349 and Tyr-1356, and at Tyr-1313, which has not been identified as a substrate binding site (38). Moreover, we have taken advantage of the fact that the Grb2 adaptor protein has an absolute requirement for an Asn in the +2 position downstream from a phosphorylated tyrosine residue (27) and have generated an additional mutant containing a substitution of the Asn for a His downstream of Tyr-1349 and Tyr-1356 (N1358H) (Fig. 1A). The p58 subunit of PI 3-kinase, Shc, and PLCγ have little specificity for this position (39); thus the N1358H mutant was predicted to lose only the ability to bind the Grb2 adaptor protein.

The synthesis and functional integrity of each mutant chimeric CSF-MET protein was examined following transient expression in COS-1 cells. CSF-MET proteins were immunoprecipitated with an antibody raised to a synthetic peptide in the extreme carboxyl terminus of the Met receptor (Ab144) (12). All the mutant CSF-MET proteins were synthesized and correctly processed into a mature CSF-MET chimeric protein of expected size (160 kDa) (data not shown). The catalytic activity of each mutant, as well as its ability to phosphorylate the exogenous substrate myelin basic protein, was examined by an in vitro kinase assay. A kinase-defective mutant, which substitutes the
lysine residue for an alanine (K1110A) in the catalytic phosphotransfer domain, showed no detectable catalytic activity (Fig. 1B, lane 2) (13), whereas the auto- and exogenous kinase activities of all mutants toward myelin basic protein were comparable to that of the wt chimera (Fig. 1B, lanes 3–7). Stable lines of MDCK cells expressing each mutant at variable levels were selected for further analyses; a representative sample is shown in Fig. 1C. The two bands identified by the Met antiserum represent the unprocessed (lower band) and mature (upper band) forms of the CSF-MET receptor (13).

The N1358H Mutant CSF-MET Receptor Fails to Bind the Grb2 Adaptor Protein yet Associates with PLC\_g\_ and the p85 Subunit of PI3-Kinase—To establish whether the N1358H mutant specifically eliminated the binding of Grb2 from the chimeric CSF-MET receptor as predicted, we performed in vitro association assays (Fig. 2). In vitro association assays have been used in studies of other receptor tyrosine kinases to identify protein-protein interactions that are difficult to detect utilizing metabolic labeling of tissue culture cells (40). Equal amounts of wt and mutant proteins were immunoprecipitated from COS-1 cells following transient transfections, collected on protein A-Sepharose, and subjected to an in vitro kinase reaction in the presence of 50 µM ATP. Lysates prepared from serum-starved MDCK cells were added to the phosphorylated CSF-MET receptor proteins, and the ability of Grb2 to associate with the CSF-MET receptor was detected by immunoblotting using a Grb2 antiserum. Grb2 associates with the wt CSF-MET chimera (19) (Fig. 2A, lane 2) as well as with the mutant Y1313F and Y1349F chimeric proteins (Fig. 2A, lanes 3 and 4). In contrast, the Y1356F (19) and Y1349/56F mutant chimeras were unable to associate with the Grb2 adaptor protein (Fig. 2A, lanes 5 and 7). More significantly, Grb2 was not co-immunoprecipitated by the N1358H mutant CSF-MET receptor (Fig. 2A, lane 6). The ability of the p85 adaptor subunit of the PI 3-kinase complex to associate with the CSF-MET receptor was assayed by in vitro association as described above. The p85 adaptor protein binds to mutant chimeric receptors Y1313F, Y1349F, and N1358H to similar levels as the wt CSF-MET receptor (Fig. 2A, lanes 2–4 and 6). As shown previously, the Y1349/56F and the Y1356F mutant receptors were unable to associate with the p85 subunit of PI3-kinase (Fig. 2A, lanes 5 and 7) (20).

The association of Grb2, p85, PLC\_g\_, and the Shc adaptor protein were also assayed by the ability of GST fusion proteins containing the SH2 domains from p85 (GST-N+C SH2 p85), PLC\_g\_ (GST-N+C SH2 PLC\_g\_), Grb2 (GST-SH2 Grb2), and Shc (GST-SH2 Shc) or the PTB domain of Shc (GST-PTB Shc) to associate with the wt CSF-MET and mutant chimeric proteins generated in COS-1 cells. With the exception of the Grb2 SH2 domain, the N1358H mutant chimera associated with all fusion proteins to wt levels (Fig. 2B, lanes 4 and 9), whereas the Y1356F and Y1349/56F mutant chimeras failed to associate.
with all proteins (Fig. 2B, lanes 7 and 8). The Y1349F mutant CSF-MET receptors were compromised in their ability to associate with both the GST-N+C SH2 PLCγ and GST-SH2 Shc fusion proteins (Fig. 2B, lane 6) yet associated with the GST-Grb2, GST-N+C SH2 p85, and GST-Shc PTB domain fusion protein to wt levels (Fig. 2B, lane 6). These data collectively demonstrate that the N1358H CSF-MET mutant receptor selectively eliminates the binding of the Grb2 adaptor protein to the receptor while retaining the ability to associate with the p85 subunit of PI 3-kinase, PLCγ, and with Shc through both its SH2 and PTB domains.

MDCK Cells Expressing the N1358H Mutant CSF-MET Chimaera Scatter in Response to rhCSF-1 but Fail to Form Branching Tubules—To investigate if a functional Grb2 binding site is required for the biological activity of the CSF-MET chimaera, the ability of MDCK cells expressing this mutant to scatter and form branching tubes in response to rhCSF-1 was assessed. In the presence of 5 units/ml rhCSF-1, MDCK cell lines expressing the wt CSF-MET, Y1349F, or Y1313F mutants were able to dissociate from small colonies of 10–20 cells and scatter (Fig. 3; data not shown), whereas both the Y1349/56F mutant and, as shown previously, the Y1356F mutant (19) failed to scatter. Thus, the association of the Grb2 adaptor protein with the CSF-MET receptor is not essential for the activation of pathways required for cell dissociation and scatter.

The ability of MDCK cells expressing the N1358H, Y1349F, and Y1313F mutants to invade a collagen matrix and form branching tubules was examined. MDCK cells expressing wt or all mutant CSF-MET receptors formed three-dimensional cysts when suspended in collagen (Fig. 4) and formed branching tubules when stimulated with 5 units of rhHGF/SF (Fig. 4; data not shown). In contrast, upon stimulation with 5 units/ml rhCSF-1, MDCK cell lines expressing the Y1349F at high (Y1349F-7) or low (Y1349F-14) (data not shown) levels or cells expressing Y1313F mutant receptors efficiently formed tubules (data not shown), whereas six independent MDCK cell lines expressing the N1358H mutant receptor at high levels were unable to form branching tubes (including N1358H-1, -17, -22; Fig. 4; data not shown). Following stimulation with rhCSF-1, five out of six MDCK cell lines expressing the N1358H mutant maintained a cyst-like structure that appeared morphologically distinct from the untreated controls, whereas one cell line (N1358H-1) formed, on occasion, single tubular structures that failed to branch (Fig. 4).

To investigate these structures, thin layer sections of collagen blocks containing cysts or tubules of both the wt and N1358H CSF-MET expressing MDCK cells were examined (Fig. 5A). Characteristically, MDCK cells and cells expressing CSF-MET mutants formed cysts that are composed of a single layer of epithelia, surrounding a hollow interior (36) (Fig. 5A, a and b). As shown by others (36), the epithelial layer is polarized with the basolateral surface to the outside of the cyst and the apical surface facing the lumen of the cyst (Fig. 5B, a). Cysts derived from MDCK cells expressing the wt and mutant receptors, when stimulated with rhHGF/SF, formed branching tubules and maintained a single layer of polarized epithelial cells (36) (Fig. 5, A (c and d) and B (b)). Similarly, MDCK cells expressing wt, Y1349F, or Y1313F chimeric CSF-MET receptor formed branching tubules of polarized epithelia in response to 5 units/ml rhCSF-1 (Fig. 5, A (e) and B (a); data not shown). In contrast, MDCK cells expressing the N1358H mutant CSF-MET chimaera formed dense cysts comprised of a mass of epithelial cells in response to rhCSF-1 (N1358H-1, Fig. 5, A (f) and B (c)), suggesting that cells dissociate in response to rhCSF-1 but are unable to organize into a tubular structure of polarized epithelium.

**DISCUSSION**

HGF/SF is a multifunctional cytokine that stimulates mitogenesis, motility, and branching tubulogenesis of epithelial and endothelial cells in culture. Although a role for many signaling pathways has been established for cell mitogenesis, little is known about signaling pathways involved in cell motility and morphogenesis. The Met receptor tyrosine kinase mediates the pleiotropic responses to HGF/SF and from a structure-function
analyses, tyrosine residue 1356 within the carboxyl terminus of the Met receptor is critical for these events (19, 26). Tyrosine 1356 is a binding site for the Grb2 adaptor protein (19, 20) and, together with Tyr-1349, is reported to form a multi-substrate binding site, required for full activation of and/or association with several substrates including PLC\(_g\), the p85 subunit of PI 3-kinase, and the Shc adaptor protein (20, 24).

In an attempt to elucidate the role of signaling pathways in cell motility and tubulogenesis, we generated the Y1349F, Y1349/1356F, and N1358H mutant receptors (Fig. 1). The Y1349F mutant retains the ability to associate with all substrates, although association with PLC\(_g\) and the SH2 domain of Shc is reduced (Fig. 2). However, when expressed in MDCK cells, the Y1349F mutant stimulates cell motility and branching tubulogenesis to wt levels (Figs. 3 and 4), whereas the Y1349/1356F mutant receptor, which retains wt levels of in vitro kinase activity (Fig. 1) fails to associate with known substrates (Fig. 2) and is biologically inert (data not shown). These data support those of Weidner et al. (26) using heterologous Trk-Met chimeras and supports our previous data that tyrosine 1356 is the most highly phosphorylated residue in the Met receptor carboxyl terminus and provides the major binding site for substrates (19, 30).

In contrast, the N1358H mutant failed to selectively associate with the Grb2 adaptor protein yet associated with the p85 subunit of PI 3-kinase, PLC\(_g\), and the Shc adaptor protein to wt levels (Fig. 2), confirming the specificity of this mutation. Interestingly, MDCK cells expressing the N1358H mutant chimera scattered in response to rhCSF-1 (Fig. 3) but failed to form branching tubules (Fig. 4). Thus, a functional Grb2 binding site and association of the CSF-MET chimera with the Grb2 adaptor protein is not essential for MDCK cell dissociation or motility but is critical for a morphogenic response.

Genetic and biochemical studies have established a role for Grb2 as an adaptor protein that couples activated receptor tyrosine kinases to the Ras guanine nucleotide exchange factor, mSos, promoting activation of Ras (41, 42). Moreover, activation of Ras or Ras-dependent pathways involving Rac and Rho are required for HGF/SF-induced cell scatter (43–45). Although the N1358H mutant chimera cannot activate the Ras pathway through direct coupling with Grb2, it still retains the ability to bind the adaptor protein Shc (Fig. 2). Tyrosine phosphorylation of the Shc adaptor protein at tyrosine 317 (YVNV) forms a consensus binding site for the Grb2 adaptor protein-Sos complex (46, 47), and, where studied, this association promotes activation of Ras (48, 49). Shc is tyrosine phosphorylated following stimulation of cells with HGF/SF (24) and in cells expressing the Met oncoprotein (28). Furthermore, overexpression of Shc in MDCK cells enhances cell motility in response to HGF/SF (24), supporting a critical role for Shc in this response. The ability of the N1358H receptor to bind Shc in the absence of direct Grb2 association further supports a role for Shc in cell dissociation and motility.

Shc contains both an SH2 domain and a PTB domain, which...
PTB domain and SH2 domain may be required for high affinity interactions with the Met receptor. Although activation of Ras or downstream pathways are essential for cell dissociation and scatter of MDCK cells in response to HGF/SF, the expression of an activated Ras or Rac alone is not sufficient to mediate a fully motile response (43, 45), and additional events are required. Of these, activation of PI 3-kinase (23, 55) but not PLCγ (23) is required for dissociation of cells in response to HGF/SF. The observation that the N1358H mutant associates with the p85 adaptor of PI 3-kinase and Shc to wt levels (Fig. 2) is consistent with the involvement of these substrates in regulating pathways involved in dissociation and motility of MDCK cells in response to HGF/SF.

MDCK cells expressing the N1358H CSF-MET receptor fail to form branching tubules, in response to CSF-1, when suspended in a collagen matrix (Fig. 4). These cells form cysts containing a single layer of polarized epithelia in a similar manner to cells expressing the wt receptors (Fig. 5A, a and b), thus their ability to polarize is not compromised by expression of the N1358H chimera. However, in response to rhCSF-1 they fail to form branching tubules and most frequently form a solid ball of epithelia (Fig. 5A, f). This is consistent with their ability to dissociate in monolayer culture but demonstrates that an intact Grb2 binding site is required for MDCK cells to reorganize or maintain a polarized structure and promote branching tubulogenesis. This suggests that branching tubulogenesis either requires pathways downstream from Grb2 or, alternatively, that the binding of an as yet unknown substrate requires an asparagine at position 1358.

Branching tubulogenesis depends upon cell-cell interactions and cell-substrate interactions mediated at least in part by E-cadherin and integrins and requires a complex regulation of cell division, degradation, and deposition of extracellular matrix (reviewed in Ref. 56). A role for the Met receptor in stimulating cell mitogenesis and degradation of the extracellular matrix has been reported (57–59); however, the pathways regulating these events have not been delineated. Ras activation is associated with a mitogenic response following stimulation of many receptor tyrosine kinases (reviewed in Ref. 60). Thus, although sufficient for cell dissociation, the inability of the N1358H mutant to couple directly with Grb2 may reduce the threshold of Ras activation below that required for a mitogenic response. However, activation of mitogen-activated protein kinase, a downstream target of Ras involved in a mitogenic response, is comparable following stimulation of cells expressing wt or N1358H receptors (data not shown). In addition to binding Sos and activating Ras, the Grb2 SH3 domains target Grb2 to the cytoskeleton (61) and can also couple to other signaling proteins. Thus, Grb2 interactions distinct from those that activate the Ras pathway may play a critical role in the morphogenic response, and experiments are underway to investigate this.

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REFERENCES
1. Lokker, N. A., Mark, M. R., Luis, E. A., Bennett, G. L., Robbins, K. A., Baker, J. B., and Godowski, P. J. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 11574–11578
2. Hartmann, G., Naldini, L., Weidner, K. M., Sachs, M., Vigna, E., Comoglio, P. M., and Birchmeier, W. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 11574–11578
3. Stoker, M., Gherardi, E., and Gray, J. (1987) Nature 327, 239–242
4. Brinkman, V., Foroutan, H., Sachs, M., Weidner, K. M., and Birchmeier, W. (1995) Cell Biol. 131, 1575–1586
5. Rosen, E. M., Grant, D. S., Kleinman, H. K., Goldberg, I. D., Bhargava, M. M., Nickoloff, B. J., Kinsella, J. L., and Polverini, P. (1993) Symp. Soc. Exp. Biol. 47, 227–234

Fig. 5. Thin layer microscopy of branching tubules suspended in collagen. A, MDCK cells expressing wild-type CSF-MET-18 and N1358H-1 were cultured in collagen as described in Fig. 4 and were unstimulated (a, b) or stimulated with 5 units/ml of either rhHGF/SF (c, d) or rhCSF-1 (e, f). Following fixation in 2% glutaraldehyde (in 0.1 M sodium cacodylate buffer (pH 7.4)) and embedding in Epon 812, thin sections were stained with uranyl acetate and Reynold’s lead and examined by electron microscopy. All pictures are at a magnification of 800×. B, thin sections of MDCK cells expressing wt CSF-MET-18 stimulated with 5 units/ml of rhCSF-1 (a) or N1358H-1 CSF-MET stimulated with either 5 units/ml of rhHGF/SF (b) or rhCSF-1 (c) from A were further analyzed by electron microscopy at a magnification of 3,668×.
