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Invasiveness and metastasis of NIH 3T3 cells induced by Met-hepatocyte growth factor/scatter factor autocrine stimulation

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ABSTRACT **The *met* protooncogene product, Met, is the tyrosine kinase growth factor receptor for hepatocyte growth factor/scatter factor (HGF/SF). NIH 3T3 cells express HGF/SF endogenously and become tumorigenic in nude mice via an autocrine mechanism when murine Met is expressed ectopically (Met^{mu} cells) or when human Met and human HGF/SF are coexpressed (HMH cells). Here, we show that Met^{mu} and HMH cells are invasive *in vitro* and display enhanced protease activity necessary for the invasive phenotype. In experimental and spontaneous metastasis assays, Met^{mu} or HMH cells metastasize to the lung, but lower numbers of subcutaneously injected Met^{mu} and HMH cells produced invasive tumors in the heart, diaphragm, salivary gland, and retroperitoneum. It has been reported elsewhere that Met expression increased with tumor passage in athymic nude mice, and these tumor explants show enhanced activity in the metastasis assays. Autocrine-mediated Met-HGF/SF signal transduction in NIH 3T3 mesenchymal cells may provide an important system for understanding the biological process of metastasis.**

The *met* protooncogene, a member of the tyrosine kinase receptor family, is widely expressed in adult and embryonic tissues as well as in primary cells and cell lines (1–4). The ligand for Met is hepatocyte growth factor/scatter factor (HGF/SF) (5, 6). HGF, a mitogen for hepatocytes as well as a wide variety of epithelial and endothelial cells (7), is identical to scatter factor (SF), an activity that disperses epithelial cells *in vitro* and promotes cell movement (8). HGF/SF has been shown to promote chemotaxis, chemokinesis, and invasiveness *in vitro* (9, 10); to induce blood vessel formation and contribute to tumor angiogenesis (11, 12); and to stimulate endothelial cell migration and capillary-like tube formation (13) *in vitro*. We have recently shown that Met-HGF/SF mediates mesenchymal to epithelial transition (14).

These pleiotropic effects of HGF/SF could contribute to tumor invasion and metastasis. While tumorigenic and metastatic cells are highly mitogenic, metastatic cells additionally exhibit increased attachment to extracellular matrix, enhanced proteolysis of basement membranes, increased motility, and the ability to colonize target organs (15). Several oncogenes including *src*, *ras* (16), and *neu* (17) confer increased tumorigenic and metastatic properties to NIH 3T3 cells in nude mice.

met is frequently amplified and overexpressed in spontaneous NIH 3T3 cell transformants (18), and overexpression of *met* in NIH 3T3 cells directly leads to cell transformation and tumorigenicity (1). We have shown that NIH 3T3 cells express murine HGF/SF (HGF/SF^{mu}) endogenously and that, with ectopic murine Met expression, these cells become tumorigenic by an autocrine mechanism (19, 20). We have recently reported that Met autocrine activation also may play a role in human soft tissue sarcomas (3). While HGF/SF^{mu} does not efficiently activate human Met (Met^{hu}) to transform NIH 3T3 cells, coexpression of Met^{hu} with the human ligand (HGF/SF^{hu}) efficiently transforms these cells, rendering them tumorigenic in nude mice (19, 20). Here, we show that autocrine Met activation in NIH 3T3 cells enhances cell motility, collagenase activity, and invasiveness *in vitro* as well as experimental and spontaneous metastasis activity *in vivo*.

MATERIALS AND METHODS

Cell Lines and Immunoprecipitation. NIH 3T3 cells transformed with the oncogenic form of *met* (ref. 2; Tpr-Met cells), with murine *met* (Met^{mu} cells), or by the coexpression of human *met* and human *HGF/SF* (HMH cells) (19, 20) have been described. Met^{mu} and HMH cells are either parental cells or primary or secondary tumor explants (19, 20). NIH 3T3 cells transformed by *ras* (gift of Donald Blair, NCI-FCRDC) or *src* (21) were used as controls. A rare tumorigenic clone of Met^{hu}-transfected NIH 3T3 cells, MT cells, are transformed by a nonautocrine mechanism and were used as controls for the autocrine-transformed Met^{mu} and HMH cells (14). All cells were maintained as described (19, 20). C28 anti-peptide antibody was raised in rabbits by immunization with the C-terminal 28 amino acids of Met^{hu} (2). The SP260 anti-peptide antibody is a rabbit antiserum directed against the C-terminal 21 amino acids of Met^{mu} (5). A3.1.2 is a monoclonal antibody against human recombinant HGF (IgG, subclass G2a; a gift from T. Nakamura, Osaka). Immunoprecipitation of Met and HGF/SF was carried out as described (19, 20).

Experimental and Spontaneous Metastasis. All test cells were washed twice and resuspended in 0.1 ml of serum-free medium (DMEM; GIBCO) prior to injection. For experimental metastasis assays, the cells were injected intravenously into the tail vein of weanling athymic (BALB/c *nu/nu*), NFS, BALB/c or triple-deficient (NIH *bg-nu-Xid*, TDM) (Harlan–Sprague–Dawley) mice. For spontaneous metastasis assays, cells were injected subcutaneously into the back of athymic mice. Tumor formation was monitored twice weekly. Fast-growing tumors at the site of inoculation (15–20 mm in size) were surgically removed. Spontaneous metastasis assays were also performed by injecting cells into the mammary fat pad (22). For each assay, major organs (lung, liver, spleen, kidney, stomach, colon, brain, and skin) were examined as described below for the development of metastases. Animals were sacrificed when they appeared distressed; those remaining after 12 weeks were sacrificed and examined for tumors.

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Abbreviations: HGF/SF, hepatocyte growth factor/scatter factor; HGF/SF^{mu}, murine HGF/SF; HGF/SF^{hu}, human HGF/SF; Met^{hu}, human Met; Met^{mu}, murine Met; Neo^{r}, neomycin resistant.

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Organs of test mice were immersion fixed in formalin and embedded in paraffin. A 5-μm section was mounted on superfrost slides, stained with hematoxylin/eosin and evaluated by light microscopy for identification of metastases.

***In Vitro* Invasiveness Assay.** Cell invasiveness assays were performed *in vitro* as described by Albini *et al.* (23) using 10-well chemotaxis chambers (NeuroProbe). Lower and upper wells were separated by Matrigel-coated (1 μg/mm^2; Collaborative Research) Nucleopore filters (8-μm pore size). HGF/SF, when used, was diluted in 0.4 ml of DMEM containing bovine serum albumin at 1 mg/ml and placed into the lower well. Cells (10^5) in DMEM/BSA were seeded into the upper wells; chambers were incubated overnight at 37°C. Filters were fixed and stained, and invading cells were counted using a light microscope at ×40 magnification.

Collagenase Assay. Type I and type IV collagenase activities were measured by the method of Nakajima *et al.* (24). Aliquots of [^{3}H]proline-labeled type I or type IV collagen (NEN) diluted with cold rat tail type I or type IV collagen (Sigma) (8000 cpm per 6 μg in 0.5 M acetic acid) were placed in 96-well plates (Costar) and dried overnight. Transfected cells (2×10^4) were added and incubated overnight. Cell supernatants were mixed with 10% (wt/vol) trichloroacetic acid/0.5% tannic acid; ^{3}H radioactivity in the supernatant was measured by scintillation counting. Activity was determined by comparison to a known amount of bacterial collagenase (10 units/ml) added to control wells. Each experiment was performed in duplicate; the averaged data are presented as a percentage of the control.

RESULTS

***In Vitro* Invasiveness Assay.** HGF/SF has been shown to induce invasive activity *in vitro* (9, 10, 25, 26). Histopathological examination of tumors generated by Met^{mu} and HMH cells showed that the tumors invaded adjacent tissue (data not shown). To determine if *met*-transformed NIH 3T3 cells displayed invasive activity *in vitro*, we tested Met^{mu} cells and HMH cells in Boyden chamber basement membrane Matrigel assays. We also tested nontransformed NIH 3T3, Tpr-Met, and MT cells. Met^{mu}, HMH, and Tpr-Met cells migrated spontaneously through the Matrigel-coated filter in the absence of exogenous HGF/SF in the lower chamber, while the MT and NIH 3T3 cells did not (Table 1). When HGF/SF^{hu} was placed in the lower well, the MT cells showed dramatic migration into the membrane, but migration was much less when the mouse ligand was used. Marked increase in cell migration was also observed with Met^{mu} cells when either HGF/SF^{mu} or HGF/SF^{hu} was present (Table 1). These results support our earlier conclusion that HGF/SF^{mu} has low affinity for Met^{hu}, whereas HGF/SF^{hu} activates both Met^{mu} and Met^{hu} (19). We conclude that NIH 3T3 cells expressing Met become invasive either spontaneously through autocrine stimulation, or in a chemotaxic response, when HGF/SF is placed in the lower well. There was no significant increase in invasiveness of HMH cells in the presence of HGF/SF^{hu}, but we have shown that these cells secrete high levels of HGF/SF^{hu} (19, 20). Met^{mu} cells, in contrast, express an excess of receptor over the endogenous ligand (20) and, therefore, respond to HGF/SF (Table 1). The spontaneous migration activity of Tpr-Met cells suggests that certain signal transduction pathways are shared by the activated oncogene and the autocrine-activated receptor. Thus, both autocrine and paracrine Met signaling in mesenchymal cells can promote *in vitro* invasiveness. Activation of the Met receptor may induce the motility necessary for this invasion, but the cells also must synthesize the proteases necessary for penetrating the Matrigel.

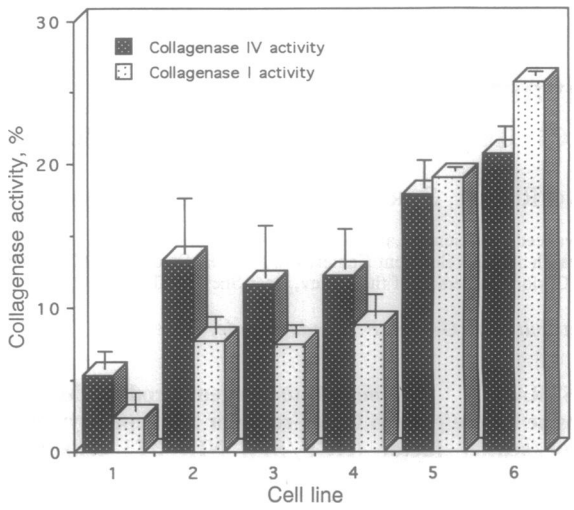

FIG. 1. Type I and type IV collagenase activity secreted by transfected NIH 3T3 cells. Collagenase activities were measured as described in *Materials and Methods*. Bars: 1, NIH 3T3 cells; 2, Met^{mu} cells; 3, Tpr-Met cells; 4, parental HMH cells; 5, primary HMH tumor explant cells; 6, secondary HMH tumor explant cells.

Collagenase Secretion. The ability to proteolytically degrade basement membrane collagen has been correlated with metastatic potential (15, 24). We compared the collagenase activity of parental NIH 3T3, HMH, Met^{mu}, MT, and Tpr-Met cells. These analyses showed that enhanced type I and IV collagenase activities were present in HMH, Met^{mu}, and Tpr-Met cells (Fig. 1). Comparable results were obtained using denatured collagen (data not shown). The collagenase levels were also high in the MT tumor cells (data not shown), even though their invasive activity was stimulated only by exogenous HGF/SF^{hu} (Table 1). These data imply that increased collagenase secretion may be required for invasiveness but, by itself, is not sufficient to induce the invasive phenotype. Furthermore, while the parental HMH cells expressed high levels of collagenase compared to control NIH 3T3 cells, primary and secondary HMH tumor cell explants expressed higher levels (Fig. 1), which correlated with a significant increase in spontaneous *in vitro* invasiveness (Table 1).

Table 1. *In vitro* motility invasiveness assay

	HGF/SF^{hu}, units/ml				HGF/SF^{mu}, units/ml		
Cells	0	5	30	200	5	30	200
Neo^r	−	−	−	−	−	−	−
MT	−	+++++	++++	++	+	++	−
Met^{mu}	+/−	+++	++	+	+	++++	+
HMH	+/−	+/−	+	ND	−	−	ND
1° HMH explant	++	+	+	ND	+	+	ND
Tpr-Met	++	ND	+	ND	ND	ND	ND

+/−, 5–10 cells per field; ++, ≈150 cells per field; up to +++++, ≈650 cells per field; ND, not determined; Neo^r, neomycin resistant; 1°, primary.

Table 2. Met-mediated experimental lung metastasis

Cells	Athymic	BALB/c	NFS	TDM
Neo^r	2/11 (10)	0/3	0/2	0/2
Tpr-Met	4/4 (3)	2/3 (3)	2/3 (7)	2/2 (2)
Met^{mu}	6/6 (6–7)	0/6	0/6	2/3 (8)
HMH	2/6 (7)	0/3	0/3	1/3 (8)
HMH 1° explant	2/3 (3)			
HMH 2° explant	4/5 (2–3)			
MT	1/6 (7)			

The values given are the (number of mice with a metastasis)/(total number of mice). The numbers in parentheses are the latency in weeks. 1°, primary; 2°, secondary.

Experimental Metastasis. Met-HGF/SF autocrine signaling in NIH 3T3 cells stimulates invasive migration *in vitro* and protease production (Fig. 1 and Table 1). To determine whether these activities paralleled metastatic activity *in vivo*, we tested whether HMH, Met^{mu}, MT, and Tpr-Met cells would colonize the lung of nude mice. Histopathological analyses revealed that parental Met^{mu} cells efficiently induced lung metastases (Table 2). Parental HMH cells were poorly active in this assay, but primary and secondary tumor cell explants, which express much higher levels of Met^{hu} and HGF/SF^{hu} (19, 20), produced lung tumors within 3 weeks. The explanted lung tumor cells generated by HMH primary tumor cells exhibited increased levels of Met^{hu} and HGF/SF^{hu} (Fig. 2 *A* and *B*, lanes 3–6), similar to the enhanced levels of expression found in secondary tumor explants in nude mouse tumor assays (20). Met was also abundantly expressed in lung tumor cell explants generated by parental Met^{mu} cells (Fig. 2*C*, lanes 1 and 2). Interestingly, two of the animals injected with control Neo^r NIH 3T3 cells developed lung tumors that expressed high levels of endogenous Met^{mu} (Fig. 2*C*, lanes 4 and 5). Amplification of endogenous Met occurs frequently in spontaneous NIH 3T3 cell transformants (18), and Met^{hu} is also amplified in human sarcomas (3). Here, we show that endogenous Met^{mu} amplification has occurred spontaneously to induce experimental metastasis. The Tpr-Met cells were also very efficient at populating the lung, whereas MT cells, which are highly tumorigenic in nude mice (data not shown), did not efficiently colonize the lung (Table 2).

We also injected HMH, Met^{mu}, and Tpr-Met cells into the tail vein of immune competent BALB/c and NFS mice, as well as into triple-immunodeficient mice (Table 2). As observed with nude mice, HMH, Met^{mu}, and Tpr-Met cells colonized the lung of triple-immunodeficient animals, but only the Tpr-Met cells established tumors in the lung of the immune competent animals (Table 2). It is not clear how the *tpr-met* oncogene can induce tumors that evade recognition in immune intact animals.

Spontaneous Metastasis. We tested the various *met*-transformed NIH 3T3 cells for spontaneous metastasis activity in nude mice. We observed multifocal lung metastases with Tpr-Met cells, but only the HMH and Met^{mu} primary tumor cell explants (Table 3 and Fig. 3 *A* and *B*) showed greatly enhanced metastatic activity. Interestingly, at low cell inoculation densities, the Met^{mu} cells also metastasized to the parotid and sublingual salivary gland and obliterated the entire submandibular gland (Fig. 3*D*). The Met^{mu} cells also metastasized to the retroperitoneum (Fig. 3*F*), while HMH cells could colonize and invade both the heart (Fig. 3*C*) and the diaphragm (Fig. 3*E*). The MT cells did not produce metastases in subcutaneous assays. NIH 3T3 cells transformed by activated *ras* or *src* oncogenes are known to generate efficient lung metastasis (18) and served as a positive control (Table 3).

We also tested HMH, Met^{mu}, and Tpr-Met cells for metastatic activity after inoculating cells into the mammary fat pad of nude mice. All three cell types induced spontaneous lung metastases (Table 3) but again, the primary and tertiary athymic nude mouse tumor explants were more active in this assay.

DISCUSSION

Tumor invasion and metastasis are complex, multistep processes that are associated with only a small percentage of primary tumor cells. For tumor cells to metastasize, they must acquire the ability to alternately penetrate and exit through the walls of primary and target organs, as well as the vascular and/or lymphatic circulatory systems, and undergo the cell proliferation and angiogenesis required for colonization (15). Gene products that regulate the processes of cell adhesion, activation and secretion of proteases, cell motility, cell growth, and angiogenesis could contribute to the metastatic potential. Many of these phenotypes have been associated with Met-HGF/SF-mediated signal transduction, suggesting that Met and HGF/SF could play a role in metastasis.

Stoker (25) first proposed that HGF/SF may be involved in tumor invasion and metastasis, since the ligand elicits scattering and motility in epithelial cells *in vitro*. HGF/SF and other cell motility factors are postulated to be involved in inflammatory reactions, tissue repair, and interactions in the immune system (8). Here, we show that Tpr-Met cells grow

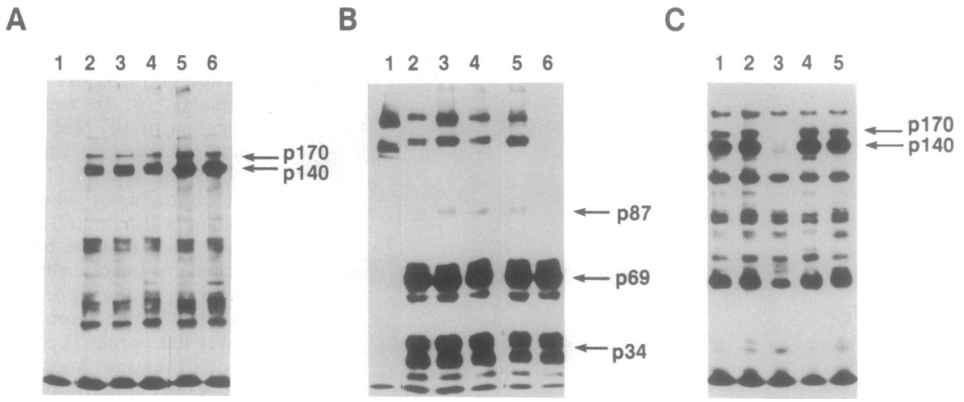

FIG. 2. Met and HGF/SF expression in cell explants of lung metastases. Cell cultures were metabolically labeled with [^{35}S]methionine and [^{35}S]cysteine (ICN) for 6 h, and cell lysates were immunoprecipitated with C28 anti-peptide antibody for Met^{hu} (*A*) or SP260 anti-peptide antibody for Met^{mu} (*C*). Concentrated conditioned medium was immunoprecipitated with anti-HGF monoclonal antibody A3.1.2 (*B*). (*A* and *B*) Lane 1, NIH 3T3 cells; lane 2, secondary HMH tumor cells; lanes 3–6, explanted lung metastasis from a primary HMH tumor explant. (*C*) Lanes 1 and 2, explanted lung metastasis cells from Met^{mu} cells; lane 3, NIH 3T3 cells; lanes 4 and 5, explanted lung metastases cells from Neo^r control cells.

Table 3. Met-mediated spontaneous metastasis in athymic mice

Cells	Metastasis/ total*	Organs	Cells injected $\times 10^{-5}$	Latency, weeks
		Subcutaneous injection		
Neo^r	0/4	—	10	—
MT	0/11	—	10	—
Tpr-Met	2/3	Lung	10	6
Met^{mu}	1/9	Lung	10	12
	1/3	Lung	3	10
	0/3	—	1	—
Met^{mu} 1° explant	2/6	Lung	10	5
	3/3	Lung	3	6–7
	2/3	Lung/SG/RP	1	6
HMH	0/8	—	10	—
	0/3	—	3	—
	0/2	—	1	—
HMH 1° explant	2/7	Lung	10	6–9
	1/2	Lung	3	9
	2/3	Lung/DP/heart	1	5–8
ras	2/3	Lung	10	4–5
src	1/3	Lung	10	2.5
		Mammary fat pad injection		
Neo^r	0/5	—	4	—
Tpr-Met	3/4	Lung	4	3–4
Met^{mu}	1/5	Lung	4	6
Met^{mu} 1° explant	1/5	Lung	4	3–4
HMH	0/5	—	4	—
HMH 1° explant	2/4	Lung	4	1–3
HMH 3° explant	3/4	Lung	4	3

1°, primary; 3°, tertiary; SG, salivary gland; RP, retroperitoneum; DP, diaphragm.
*(Number of mice with a metastasis)/(total number of mice).

unabated in heterologous immune intact mice, escaping immune surveillance. Moreover, HGF/SF induces blood vessel formation and contributes to tumor angiogenesis *in vivo* (15, 16) and stimulates endothelial cell migration and capillary-like tube formations *in vitro* (13). We have observed abundant vascular space and extensive microvascular vessel formation in Met tumors (data not shown). The inappropriate or ectopic expression of Met in NIH 3T3 cells leads to tumorigenesis through an autocrine mechanism (19, 20). In these studies, we show that the same autocrine interaction in NIH 3T3 cells

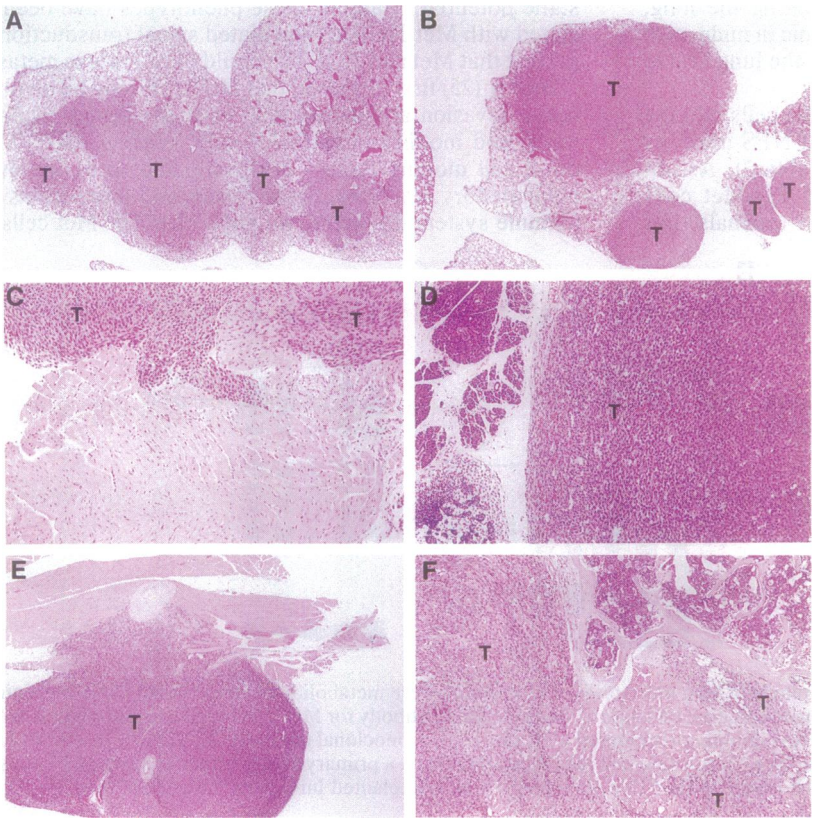

FIG. 3. Histopathology of metastatic tumors. (*A*, *C*, and *E*) Tumors from injection of 10^5 HMH primary tumor cell explants. (*B*, *D*, and *F*) Tumors from injection of 10^5 Met^{mu} primary cell explants. (*A* and *B*) Lung with multifocal metastatic tumors (T). (*C*) Heart with metastatic tumor (T) invading heart base and myocardium. (*D*) Submandibular salivary gland that has been completely replaced by tumor cells (T). The parotid gland is in the upper left corner, and submandibular lymph node is in the lower left corner. Epithelioid areas evident in the large tumor mass are similar to those previously observed in Met-mediated mesenchymal to epithelial conversion (14). (*E*) Diaphragm with metastatic tumor (T) on pleural surface and invading muscle. (*F*) Retroperitoneal tumor (T) extending through muscle and fascial planes and along a vertebral body (upper right).

confers the phenotypes of invasiveness *in vitro* and experimental and spontaneous metastasis *in vivo*. Abundant Met expression was found in human sarcoma cell lines and tumors (3), raising the possibility that similar mechanisms may function in these human malignancies.

HGF/SF serves as a paracrine effector of mesenchymal–epithelial interactions (8, 27). When epithelial cells scatter *in vitro*, they resemble fibroblasts in culture (25), and there are examples of cells and tissues that could display this phenotype. Thus, HGF/SF has been detected in various carcinoma cell lines (28, 29), and epithelial carcinoma cell lines expressing both Met and HGF/SF grow as single cells but grow as colonies when HGF/SF signaling is blocked (30). Further, we recently demonstrated that autocrine Met-HGF/SF expression can mediate mesenchymal to epithelial cell transition (14). These observations suggest the possibility that alternating between high and low levels of Met and/or HGF/SF expression could play a role in the selection for phenotypes of migration and colonization necessary for metastasis.

Met-HGF/SF autocrine expression in NIH 3T3 cells increases not only cell motility but also type I and type IV collagenase activities, two enzymes that are essential for invasiveness. Similarly, HGF/SF stimulates bovine brain endothelial cell motility and increases the secretion of plasminogen activator activity (13). Our studies suggest that motility and protease production can be independently regulated, since the MT cells that overexpress only Met^{hu} produce elevated levels of collagenase but are not invasive *in vitro* in the absence of HGF/SF (Table 1).

We have shown that with increasing passage of HMH tumors, there is a concomitant increase in the levels of HGF/SF and Met protein expression (19, 20). Here we show that the level of Met and HGF/SF expression directly correlates with invasiveness activity *in vitro* (Table 1), collagenase activity (Fig. 2), and enhanced experimental (Table 2) and spontaneous (Table 3) metastases. Further, the rare metastatic lung tumors obtained with control NIH 3T3 cells (Table 2) overexpress Met^{mu}, showing that endogenous Met can participate in the development of the metastatic phenotype. However, we cannot exclude the possibility that other genetic events that are responsible for or contribute to these phenotypes occur with tumor passage.

What is the mechanism of Met-induced metastasis? The Met signal transduction pathway has been linked with *src* and *ras* activation (31, 32), and, perhaps, it is through these oncogenes that Met functions to confer a metastatic phenotype (16). Further, an engineered secreted form of basic fibroblast growth factor (33) and an activated human c-*erb B2/neu* oncogene (17) have been shown to increase invasiveness *in vitro*, implying that other ligands and receptors have this activity. To our knowledge, Met-HGF/SF is the first example of an unmodified receptor–ligand pair mediating a metastatic phenotype, implying that the abnormal process of metastasis can proceed through a conventional signal transduction pathway but in an inappropriate cell background.

met was first isolated as a transforming gene, *tpr-met*, from MNNG-treated HOS cells, and its trivial name was derived from *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (34), but from our present studies, metastasis is also appropriate.

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1. Iyer, A., Kmiecik, T. E., Park, M., Daar, I., Blair, D., Dunn, K. J., Sutrave, P., Ihle, J. N., Bodescot, M. & Vande Woude, G. F. (1990) *Cell Growth Differ.* **1**, 87–95.
2. Gonzatti-Haces, M., Seth, A., Park, M., Copeland, T., Oroszlan, S. & Vande Woude, G. F. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 21–25.
3. Rong, S., Jeffers, M., Resau, J., Tsarfaty, I., Oskarsson, M. & Vande Woude, G. F. (1993) *Cancer Res.* **53**, 5355–5360.
4. Sonnenberg, E., Weidner, K. M. & Birchmeier, C. (1993) in *Hepatocyte Growth Factor-Scatter Factor (HGF/SF) and the c-Met Receptor*, eds. Goldberg, I. D. & Rosen, E. M. (Birkhaeuser, Basel), pp. 381–394.
5. Bottaro, D. P., Rubin, J. S., Faletto, D., Chan, A. M.-L., Kmiecik, T. E., Vande Woude, G. F. & Aaronson, S. A. (1991) *Science* **152**, 802–804.
6. Naldini, L., Vigna, E., Narsimhan, R. P., Gandino, G., Zarnegar, R., Michalopoulos, G. K. & Comoglio, P. M. (1991) *Oncogene* **6**, 501–504.
7. Nakamura, T. (1992) *Prog. Growth Factor Res.* **3**, 67–86.
8. Gherardi, E. & Stoker, M. (1991) *Cancer Cells* **3**, 227–232.
9. Rosen, E. M., Knesel, J. & Goldberg, I. D. (1991) *Cell Growth Differ.* **2**, 603–607.
10. Weidner, K. M., Hartmann, G., Naldini, L., Comoglio, P. M., Sachs, M., Fonatsch, C., Rieder, H. & Birchmeier, W. (1993) in *Hepatocyte Growth Factor-Scatter Factor (HGF/SF) and c-Met Receptor*, eds. Goldberg, I. D. & Rosen, E. M. (Birkhaeuser, Basel), pp. 311–328.
11. Grant, D. S., Kleinman, H. K., Goldberg, I. D., Bhargava, M. M., Nickoloff, B. J., Kinsella, J. L., Polverini, P. & Rosen, E. M. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 1937–1947.
12. Bussolino, F., DiRenzo, M. F., Ziche, M., Bacchietto, E., Olivero, M., Naldini, L., Gaudino, G., Tamagnone, L., Coffer, A. & Comoglio, P. M. (1992) *J. Cell Biol.* **119**, 628–641.
13. Rosen, E. M., Grant, O., Kleinman, H., Jaken, S., Donovan, M. A., Setter, E., Luckett, P. M. & Carley, W. (1991) in *Cell Motility Factors*, eds. Goldberg, I. D. & Rosen, E. M. (Birkhaeuser, Basel), pp. 76–88.
14. Tsarfaty, I., Rong, S., Resau, J. H., Rulong, S., Pinto da Silva, P. & Vande Woude, G. F. (1994) *Science* **263**, 98–101.
15. Liotta, L. A. (1992) *Sci. Am.* **266**, 54–63.
16. Greenberg, A. H., Egan, S. E. & Wright, J. A. (1989) *Invasion Metastasis* **9**, 360–378.
17. Yu, D. & Hung, M. (1991) *Oncogene* **6**, 1991–1996.
18. Cooper, C. S., Tempest, P. R., Beckman, P. M., Heldin, C.-H. & Breakers, P. (1986) *EMBO J.* **5**, 2623–2628.
19. Rong, S., Bodescot, M., Blair, D., Nakamura, T., Mizuno, K., Park, M., Chan, A., Aaronson, S. & Vande Woude, G. F. (1992) *Mol. Cell. Biol.* **12**, 5152–5158.
20. Rong, S., Oskarsson, M., Faletto, D. L., Tsarfaty, I., Resau, J., Nakamura, T., Rosen, E., Hopkins, R. & Vande Woude, G. F. (1993) *Cell Growth Differ.* **4**, 563–569.
21. Kmiecik, T. E., Johnson, P. J. & Shalloway, D. (1988) *Mol. Cell Biol.* **8**, 4541–4546.
22. Leone, A., Flatow, V., VanHoutte, K. & Steeg, P. S. (1993) *Oncogene* **8**, 2325–2333.
23. Albini, A., Iwamoto, Y., Kleinman, H. K., Martin, G. R., Aaronson, S. A., Kozlowski, J. M. & McEwan, R. N. (1989) *Cancer Res.* **47**, 3239–3245.
24. Nakajima, M., Welch, D. R., Belloni, P. N. & Nicolson, G. L. (1987) *Cancer Res.* **47**, 4869–4876.
25. Stoker, M. (1989) *J. Cell. Physiol.* **139**, 565–569.
26. Giordano, S., Zhen, Z., Medico, E., Gaudino, G., Galimi, F. & Comoglio, P. M. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 649–653.
27. Birchmeier, C., Sonnenberg, E., Weidner, K. M. & Birchmeier, W. (1993) *BioEssays* **15**, 1–6.
28. Yoshinaga, Y., Fukita, S., Gotoh, M., Nakamura, T., Kikuchi, M. & Hiroashi, S. (1992) *Jpn. J. Cancer Res.* **83**, 1257–1261.
29. Tsao, M.-S., Zhu, H., Giaid, A., Viallet, I., Nakamura, T. & Park, M. (1993) *Cell Growth Differ.* **4**, 571–579.
30. Adams, J. C., Fulong, R. A. & Watt, F. (1991) *J. Cell Sci.* **96**, 385–394.
31. Faletto, D. L., Kaplan, D. R., Halverson, D. O., Rosen, E. R. & Vande Woude, G. F. (1993) in *Hepatocyte Growth Factor-Scatter Factor (HGF/SF) and the c-Met Receptor*, eds. Goldberg, I. D. & Rosen, E. R. (Birkhaeuser, Cambridge, MA), pp. 107–130.
32. Graziani, A., Gramaglia, D., dalla Zonca, P. & Comoglio, P. (1993) *J. Biol. Chem.* **268**, 9165–9168.
33. Yayon, A. & Klagsbrun, M. (1990) *Cancer Metastasis Rev.* **9**, 191–202.
34. Cooper, C. S., Park, M., Blair, D. G., Tainsky, M. A., Huebner, K., Croce, C. M. & Vande Woude, G. F. (1984) *Nature (London)* **311**, 29–33.