Invasive Properties of Murine Squamous Carcinoma Cells: Secretion of Matrix-degrading Cathepsins Is Attributable to a Deficiency in the Mannose 6-Phosphate/Insulin-like Growth Factor II Receptor

Kim Lorenzo, Phuong Ton, Jason L. Clark, Sogué Coulibaly, and Lukas Mach

Department of Biochemistry, University of Western Australia, Nedlands WA 6907, Australia [K. L., P. T., J. L. C., L. M.], and Zentrum für Angewandte Genetik, Universität für Bodenkultur Wien, A-1190 Vienna, Austria [S. C., L. M.]

ABSTRACT

Penetration of basement membrane layers is a hallmark feature of metastatic tumor cells. The invasive propensity of murine SCC-VII squamous carcinoma cells is in part attributable to the extracellular action of the lysosomal cysteine protease cathepsin B. Although most noncancerous cells store this enzyme in the lysosomes, we found that SCC-VII cells release a large fraction (42%) of their newly synthesized procathepsin B into the culture medium. Procathepsins D and L, the precursors of other major lysosomal proteinases, are also secreted in significant amounts (24 and 75%, respectively). In contrast, normal murine 3T3-L1 fibroblasts exocytose only minor amounts of their newly synthesized procathepsins B (10%), D (<1%), and L (16%). Western blotting analysis revealed that SCC-VII cells are deficient in the 300 kDa mannose 6-phosphate/insulin-like growth factor-II receptor (M6P/IGF2R), a tumor suppressor with a central role in the intracellular transport of lysosomal enzymes. Consistent with the absence of M6P/IGF2R, SCC-VII cells lack dense lysosomes, with the bulk of intracellular acid hydrolases residing in late endosomes/prespools. On the other hand, the synthesis of the M6P recognition marker on lysosomal enzymes is not impaired in SCC-VII cells, because [33P]Pi is incorporated into the carbohydrate moieties of procathepsins B, D, and L. Furthermore, 69% of the phosphorylated N-linked oligosaccharides synthesized by SCC-VII cells carry phosphomonoester groups and as such constitute high-affinity ligands for M6P receptors. SCC-VII cells express the 46 kDa cation-dependent M6P receptor (MPR46), but intracellular retention of procathepsins B, D, and L is not affected by ammonium chloride and chloroquine, agents known to perturb the M6P receptor system. Our results indicate that failure to express the 300 kDa M6P/IGF2R may enhance the metastatic capacity of tumor cells by inducing the secretion of procathepsins B, D, and L.

INTRODUCTION

Malignant cancer cells display a distinct capacity to degrade extracellular matrix components, a feature indispensable for penetration of basement membranes and subsequent spreading to distant sites. Various proteinases have been implicated in tumor invasion and metastasis, including the lysosomal enzymes cathepsins B, D, and L (1–3). In most noncancerous cells, the subcellular location of these cathepsins is restricted to lysosomes. However, the proteinases are frequently redistributed in tumor cells to peripheral vesicles, promoting exocytosis of the enzymes (4). In addition, tumor cells secrete precursors of cathepsins B, D, and L into the pericellular fluid, because of leakage in the biosynthetic transport of the latent proenzymes to lysosomes. For cathepsin L, secretion was attributed to the intrinsic low affinity of the proteinase to its sorting receptors (5). Once released, self-activation of the individual proenzymes may occur (6).

Intracellular trafficking of cathepsins to lysosomes depends on the presence of M6P in the carbohydrate moieties of the proteinases. These residues mediate binding to specific M6P receptors (7). Mutant cells with either impaired synthesis of the M6P recognition marker or a M6P receptor deficiency fail to retain their newly synthesized lysosomal hydrolases (8). Two distinct M6P-binding proteins occur in mammalian cells, the 300 kDa M6P/IGF2R and the 46 kDa cation-dependent MPR46. Besides representing the main targeting receptor for lysosomal enzymes, M6P/IGF2R binds IGF-II and TGF-β precursor (9). M6P/IGF2R participates in the degradation of IGF-II, a potent growth stimulant and mitogen that is often overproduced in tumors (9, 10). Furthermore, the receptor facilitates activation of latent TGF-β, a growth-suppressing cytokine (11). Because of this dual role in the control of cellular growth, M6P/IGF2R is considered a tumor suppressor (10). Indeed, the M6P/IGF2R gene is frequently mutated in liver, breast, and gastrointestinal cancers (12–14). Recently, direct evidence for a growth-suppressive role of M6P/IGF2R in tumors has been provided (15). However, the impact of M6P/IGF2R on tumor invasion and metastasis has not yet been investigated.

We have shown previously that the invasive properties of murine SCC-VII squamous carcinoma cells are in part attributable to the extracellular action of the major lysosomal cysteine proteinase cathepsin B (16). In this report, we demonstrate that SCC-VII cells are deficient in M6P/IGF2R, causing hypersecretion of cathepsin B and other lysosomal proteinases. Our results indicate that loss of M6P/IGF2R may promote the invasiveness of malignant tumor cells.

MATERIALS AND METHODS

Reagents. Trans-[35S]label metabolic labeling reagent (>1000 Ci/mmol) was obtained from ICN Pharmaceuticals (Costa Mesa, CA). [3H]Mannose (10–20 Ci/mmol), [33P]Pi (>2500 Ci/mmol), UDP-[3H]galactose (5–20 Ci/mmol), unlabeled and [3H]-methylated molecular mass standards, Amplify fluorographic reagent, Percoll, protein A-Sepharose 4B, Sephadex G-75, and Enhanced Chemiluminescence Western blotting reagents were obtained from Amersham Pharmacia Biotech (Rainham, United Kingdom). Endoglycosidase H and peptide N-glycosidase F were from Roche Diagnostics Mannheim, Germany). Calf intestinal alkaline phosphatase, chloroquine, HRP (type VI-A), 4-nitrophenyl-β-D-galactoside, 4-nitrophenylphosphosphate, 3,3′,5,5′-tetramethylbenzidine, diethyl(2-hydroxypropyl)aminoethyl-Sephadex, leupeptin, E-64, sodium β-glycerophosphate, BSA (fraction V), and chicken egg ovalbumin (grade V) were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of reagent grade.

Antibodies. The production and characterization of a rabbit antiserum against purified human liver cathepsin B has been reported previously (17). The antiserum has been shown to cross-react with all forms of the murine gene is frequently mutated in Endoglycosidase H and peptide N-glycosidase F were from Roche Diagnostics Mannheim, Germany). Calf intestinal alkaline phosphatase, chloroquine, HRP (type VI-A), 4-nitrophenyl-β-D-galactoside, 4-nitrophenylphosphosphate, 3,3′,5,5′-tetramethylbenzidine, diethyl(2-hydroxypropyl)aminoethyl-Sephadex, leupeptin, E-64, sodium β-glycerophosphate, BSA (fraction V), and chicken egg ovalbumin (grade V) were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of reagent grade.

Antibodies. The production and characterization of a rabbit antiserum against purified human liver cathepsin B has been reported previously (17). The antiserum has been shown to cross-react with all forms of the murine gene is frequently mutated in

Received 11/2/99; accepted 5/30/00.

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.

1 This work was supported in part by Australian Research Council Grants 04/15/412/170, 04/15/412/284, and 04/15/412/343 (to L. M.).

2 These authors contributed equally to this study.

3 Present address: Department of Animal Housing, Baxter AG, A-2304 Orth, Austria.

4 To whom requests for reprints should be addressed, at Zentrum für Angewandte Genetik, Universität für Bodenkultur Wien, Muthgasse 18, A-1190 Vienna, Austria. Phone: 43-1-36006-6360; Fax: 43-1-36006-6392; E-mail: l.mach@edv2.boku.ac.at.

5 The abbreviations used are: M6P, mannose 6-phosphate; IGF-II, insulin-like growth factor II; IGF2R, IGF-II receptor; HRP, horseradish peroxidase; MPR46, 46-kDa cation-dependent M6P receptor; TGF, transforming growth factor; αPAR, urokinase-type plasminogen activator receptor.
Dr. Braulke also provided an anti-peptide antisera against the COOH terminus of murine MPR46, with the kind permission of Dr. Annette Hille-Rehfeld from the same department. Antiserum against mouse cathepsins D and L were donated by Dr. John S. Mort (Shriners Hospital for Crippled Children, Montreal, Quebec, Canada) and Dr. Ann H. Erickson (University of North Carolina, Durham, NC), respectively.

**Cell Culture.** Murine SCC-VII squamous carcinoma cells were propagated in Minimal Essential Medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 µg/ml penicillin, and 100 µg/ml streptomycin at 37°C as described (16). Human HepG2 hepatoma cells and normal human GM5522 skin fibroblasts were obtained from the American Type Culture Collection (Rockville, MD) and the Human Genetic Mutant Cell Repository (Camden, NJ), respectively. Both cell lines were cultured as outlined above. Murine 3T3-L1 fibroblasts, supplied by Dr. David James (University of Queensland, Brisbane, Queensland, Australia), were maintained in DMEM supplemented with 10% bovine calf serum, 2 mM glutamine, 100 µg/ml penicillin, and 100 µg/ml streptomycin at 37°C. Murine NIH 3T3 fibroblasts were obtained from the American Type Culture Collection and propagated in DMEM supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 µg/ml penicillin, and 100 µg/ml streptomycin at 37°C. All tissue culture reagents were purchased from Life Technologies, Inc. (Gaithersburg, MD).

**Metabolic Labeling and Immunoprecipitation.** Confluent cell monolayers were metabolically labeled for 1 h with [35S]methionine (100 µCi/ml) or [3H]PPi, (250 µCi/ml) and were subsequently chased for 4 h as described (17). When added, NH4Cl (10 mM) and chloroquine (30 µM) were present throughout the entire experimental procedure. Immunoprecipitation of antigens from labeled cell and medium extracts, followed by SDS-PAGE and fluorography, was performed as reported (17, 18). To determine the amount of label incorporated into individual polypeptides, the corresponding gel areas were excised and solubilized in 30% H2O2/0.25% NH4OH prior to quantification by liquid scintillation counting (17). The raw data were corrected for the number of methionine residues present in each polypeptide. The complete sequences of mouse procathesins B, D, and L are available from the National Center of Biotechnology Information.6

**Endocytosis Experiments.** Confluent layers of 3T3-L1 and SCC-VII cells were incubated with NH4Cl-induced secretions of [35S]methionine-labeled HepG2 cells (3 x 106 cpm/ml) for 20 h at 37°C. Internalization of HepG2 procathesins B and D by the respective recipient cells was determined by immunoprecipitation, followed by SDS-PAGE and fluorography as reported (18).

**Subcellular Fractionation.** HepG2- and SCC-VII cells were incubated in complete culture medium containing 2 mg/ml HRP for 5 min at 37°C. The cells were then washed prior to a 10-min chase in HRP-free medium to label endosomes. Postnuclear supernatants were obtained and fractionated by Percoll density gradient centrifugation as described (19). The activity of lysosomal β-N-acetyhexosaminidase was determined spectrophotometrically with 4-nitrophenyl-β-D-glucoside, and peptide hydrolyses to these compartments. Furthermore, NH4Cl and chloroquine inhibited procathepsin B processing in 3T3-L1 cells, which also requires an acidic environment (Fig. 1A).

**RESULTS**

**Bio synthesis of Cathepsin B in SCC-VII Cells and 3T3-L1 Fibroblasts.** To follow the biosynthesis of cathepsin B, SCC-VII cells and control mouse 3T3-L1 fibroblasts were metabolically labeled with [35S]methionine. Upon immunoprecipitation with an antisera to the purified enzyme, a protein of 42 kDa was detected in both cell types, which corresponds to procathepsin B, the latent precursor of the proteinase (not shown). When 3T3-L1 cells were chased, this precursor was found to be rapidly converted into mature single-chain cathepsin B (32 kDa), indicating delivery of the enzyme to lysosomes. In contrast to human cells (17, 18), the two-chain form of the proteinase was not produced, even after prolonged chase periods (not shown). Under normal conditions, only a small fraction (10%) of the newly synthesized proenzyme was secreted into the culture medium. Procathepsin B release was significantly stimulated by the presence of 10 mM NH4Cl (22%), albeit weaker than in human GM5522 fibroblasts (>60%; not shown). Similar results were obtained with 30 µM chloroquine (not shown). Both lysosomotropic bases are known to raise the pH in the lumen of endosomes and lysosomes, which perturbs M6P receptor-mediated trafficking of acid hydrolyses to these compartments. Furthermore, NH4Cl and chloroquine inhibited procathepsin B processing in 3T3-L1 cells, which also requires an acidic environment (Fig. 1A).

In contrast to 3T3-L1 fibroblasts, SCC-VII cells released a substantial fraction (42%) of newly produced procathepsin B into the culture medium. The fraction (58%) retained inside the cells was processed to

---

the mature enzyme (Fig. 1A). The heterogeneity of mature SCC-VII cathepsin B (32–36 kDa) was found to be attributable to differences in glycosylation, because removal of N-linked oligosaccharides by treatment with peptide N-glycosidase F yielded a discrete 31 kDa band (Fig. 1B). Interestingly, procathepsin B secretion by SCC-VII cells was not significantly enhanced by NH4 Cl (44%). Similar results were obtained with chloroquine (not shown). However, procathepsin B maturation was incomplete (86%) when SCC-VII cells were treated with either base, indicating that endosomal/lysosomal alkalinization had indeed occurred. Therefore, the residual retention of cathepsin B by SCC-VII cells is apparently mediated by a pH-insensitive mechanism that differs from the classic M6P receptor pathway.

**Biosynthesis of Cathepsin L in SCC-VII Cells and 3T3-L1 Fibroblasts.** The most prominent mammalian lysosomal cysteine proteinase besides cathepsin B is cathepsin L. In 3T3-L1 cells, only 16% of newly synthesized procathepsin L (37 kDa) was secreted into the culture medium, with the remainder being intracellularly retained. Unlike cathepsin B, the retained procathepsin L was processed in these cells via a single-chain intermediate (29 kDa) into the mature double-chain enzyme, as indicated by the appearance of its heavy chain (20 kDa). NH4Cl substantially stimulated procathepsin L secretion (62%). As for cathepsin B, residual intracellular procathepsin L did not undergo proteolytic maturation in the presence of the base (Fig. 2A).

In contrast, SCC-VII cells secreted 75% of their newly made procathepsin L into the culture medium. Any proenzyme retained by the cells was rapidly processed via the single-chain intermediate (29 kDa) into mature two-chain cathepsin L. The presence of NH4Cl did not significantly enhance procathepsin L release (78%). However, NH4Cl did inhibit the formation of the mature forms of the proteinase to 69%, which confirms that NH4Cl is effective in elevating the pH of lysosomes in SCC-VII cells (Fig. 2A).

Our results demonstrate that SCC-VII cells and 3T3-L1 fibroblasts...
sort procathepsin L less efficiently than procathepsin B to lysosomes. This discrepancy may be attributable to the intrinsic low affinity of procathepsin L for M6P receptors (5) or indicate the involvement of distinct M6P-independent sorting receptors, with preference for cathepsin B over cathepsin L.

**Biosynthesis of Cathepsin D in SCC-VII Cells and 3T3-L1 Fibroblasts.** Cathepsin D, the major aspartic proteinase in mammalian lysosomes, was found to be initially synthesized by SCC-VII cells and 3T3-L1 fibroblasts as a latent 46 kDa precursor. 3T3-L1 cells released <1% of newly made procathepsin D into the medium. All proenzymes retained by the cells were converted to mature single-chain cathepsin D (44 kDa). Treatment with NH4Cl induced secretion of the latent proenzyme to some extent (21%), but most cathepsin D remained inside the cells even in the presence of the lysosomotropic agent. In contrast to cathepsins B and L, proteolytic maturation of intracellular procathepsin D in these cells was not significantly affected by NH4Cl (Fig. 2B).

SCC-VII cells secreted substantial amounts (24%) of their newly synthesized procathepsin D. Any intracellularly retained proenzyme was processed into the mature single-chain form of the proteinase. Procathepsin D secretion was only slightly stimulated by NH4Cl (33%). As in 3T3-L1 cells, NH4Cl did not inhibit intracellular procathepsin D processing (Fig. 2B).

Our results indicate that SCC-VII cells and 3T3-L1 fibroblasts preferentially retain cathepsin D as compared with cathepsins B or L. This finding is consistent with an alternative, M6P-independent lysosomal targeting mechanism for cathepsin D, as it has been reported for human breast cancer cells (23).

**Phosphorylation of Lyosomal Enzymes in SCC-VII Cells.** Because SCC-VII cells hypersecret, in addition to cathepsins B, D, and L, also the lysosomal marker β-N-acetylhexosaminidase (not shown), we investigated whether the cells are capable of forming M6P residues on lysosomal enzymes. Secreted procathepsin B (42 kDa) was immunoprecipitated and then treated with endoglucosaminidase H to test for the presence of high-mannose-type, phosphorylated N-linked oligosaccharides (5). Treatment with endoglucosaminidase H resulted in the appearance of two novel polypeptides of 40 and 38 kDa reflecting the loss of one and two N-glycans, respectively. Complete deglycosylation of procathepsin B with peptide N-glycosidase F reduced the apparent molecular mass of the protein to 36 kDa, consistent with the removal of three N-linked oligosaccharide side chains. Similar results were obtained for procathepsin B synthesized in the presence of NH4Cl. These data indicate that one or two of the three carbohydrate side chains of procathepsin B contain M6P residues (Fig. 3A).

To directly establish phosphorylation of lysosomal enzymes, SCC-VII cells and 3T3-L1 fibroblasts were metabolically labeled with [35S]methionine in the absence (−) or continuous presence (+) of NH4Cl as described in the legend of Fig. 1. Procathepsin B was immunoprecipitated from the culture media and treated with (+) or without (−) 2 milliunits of endoglucosaminidase H (Endo H) or 0.2 unit of peptide N-glycosidase F (PNGase) for 16 h at 37°C prior to analysis by SDS-PAGE and fluorography as in Fig. 1. ProcB(3), fully glycosylated procathepsin B carrying three N-linked oligosaccharide side chains; ProcB(0), completely deglycosylated procathepsin B. B. 3T3-L1 fibroblasts (1) and SCC-VII cells (2) were metabolically labeled for 1 h with 100 μCi/ml [35S]methionine (33P) or 250 μCi/ml [35S]methionine (35P) and subsequently chased for 4 h in the continuous presence of NH4Cl as outlined in “Materials and Methods.” Procathepsins D and L were immunoprecipitated from the culture media and analyzed by SDS-PAGE and fluorography as in Fig. 1. C. Procathepsin L secreted by 35P- and 33P-labeled SCC-VII cells as described above was immunoprecipitated and treated with (+) or without (−) 0.2 unit of peptide N-glycosidase F (PNGase) or 10 units of alkaline phosphatase (AlkPhos) for 16 h at 37°C prior to analysis by SDS-PAGE and fluorography, as outlined in the legend of Fig. 1.
line phosphatase treatment (which cleaves exclusively linkages involving phosphomonoester groups). These results demonstrate that 69% of the phosphorylated N-linked oligosaccharides synthesized by SCC-VII cells contain phosphomonoester groups that serve as high-affinity ligands for M6P receptors (7). This is in good agreement with data reported for human fibroblasts (22). Thus, it appears that phosphorylation of lysosomal enzymes is fully functional in SCC-VII cells.

Expression of M6P Receptors in SCC-VII Cells. Mammalian cells usually synthesize both known M6P receptors, M6P/IGF2R and MPR46. We have investigated the expression of both receptors in SCC-VII cells and 3T3-L1 fibroblasts. Upon immunoblotting with antibodies against MPR46, a diffuse 45-kDa protein was detected in membrane extracts of SCC-VII cells. A slightly smaller immunoreactive polypeptide (43 kDa) was present in 3T3-L1 membranes. No reaction was observed with the corresponding soluble protein fractions (Fig. 4A). It has been established that differences in glycosylation may account for cell type-specific molecular forms of MPR46 (24).

When extracts of 3T3-L1 fibroblasts labeled with $^{35}$Smethionine were immunoprecipitated with antibodies against M6P/IGF2R, a polypeptide of $>250$ kDa was detected. However, this protein was not present in extracts of SCC-VII cells. No cross-reactive polypeptides were detectable in the culture medium of either cell line, ruling out the possibility that shedding of cell surface-resident M6P/IGF2R was responsible for the absence of this protein in SCC-VII cell lysates (Fig. 4B).

Although both M6P receptors contribute to intracellular sorting of lysosomal enzymes, receptor-mediated uptake of exogenous M6P-containing ligands is exclusively mediated by M6P/IGF2R (7). We have shown previously that procathepsin B synthesized by human hepatoma HepG2 cells is efficiently internalized by autologous recipient cells and human fibroblasts in a M6P/IGF2R-dependent manner (18). As a functional test for the presence of M6P/IGF2R, NH$_4$Cl-induced secretions of HepG2 cells labeled with $[^35]$S)methionine were offered to unlabeled SCC-VII cells and 3T3-L1 fibroblasts for receptor-mediated endocytosis. Although procathepsin B was internalized by 3T3-L1 cells in a M6P-inhibitable manner, no uptake was detectable in SCC-VII cells. Identical results were obtained for procathepsin D (data not shown). We conclude that SCC-VII cells lack any endogenous synthesis of M6P/IGF2R, which induces hypersecretion of newly synthesized lysosomal enzymes as well as impairs their recapture via receptor-mediated endocytosis.

The Formation of Mature Lysosomes Is Impaired in SCC-VII Cells. Recent studies have shown that M6P/IGF2R plays a key role in the biogenesis of lysosomes (8). To assess the status of lysosomes in SCC-VII cells, postnuclear organelles were separated by density-gradient centrifugation. When the distribution of cathepsin B in the gradient fractions was determined, a marked peak of enzyme activity was observed at a buoyant density of 1.06 g/ml (Fig. 5A). The same distribution was found for the lysosomal marker $\beta$-N-acetylgalactosaminidase and acid phosphatase, a lysosomal enzyme transported to the bulk of the cathepsin B (and $\beta$-N-acetylgalactosaminidase) activity of control HepG2 cells was located in compartments with a density of $>1.08$ g/ml, as typical for mature lysosomes (Fig. 5B). However, the subcellular distribution of lysosomal enzymes in SCC-VII cells overlaps significantly with the locations of the Golgi marker galactosyltransferase and the endocytic tracer HRP (Fig. 5A), a feature reminiscent of late endosomal/prelysosomal compartments. In fact, mutant human fibroblasts with a defect in the formation of dense lysosomes exhibited the same sedimentation pattern as observed for SCC-VII cells (19).

In normal cells, lysosomes are usually located in the perinuclear region (8, 19). Such a pattern was observed when control NIH 3T3 fibroblasts were immunostained with anti-cathepsin D antibodies (Fig. 6A). In contrast, immunocytochemical detection of cathepsin D in SCC-VII cells revealed numerous vesicles distributed throughout the cytoplasm (Fig. 6B). A similar subcellular distribution of lysosomal markers was observed in M6P/IGF2R-deficient murine fibroblasts, where the labeled structures were found to resemble late endocytic compartments (8). Thus, SCC-VII “lysosomes” display at least some characteristics of late endosomes/prelysosomes, indicating impaired formation of mature lysosomes in these cells as a direct consequence of the absence of M6P/IGF2R.

DISCUSSION

Our results demonstrate that SCC-VII cells secrete lysosomal proteins attributable to the absence of functional M6P/IGF2R. Similarly, lack of M6P/IGF2R synthesis has been observed for the highly deviated Morris 7777 hepatoma, a malignant rat tumor (24). Indeed, Morris 7777 hepatoma cells display enhanced secretion of procathepsin D as compared with normal rat hepatocytes (25). Interestingly,
transformation of murine BALB/3T3 fibroblasts with Moloney murine sarcoma virus triggers exocytosis of procathepsins B and L, concomitant with the loss of M6P/IGF2R activity (26). M6P/IGF2R deficiency has been also detected in leukemia cells, which secrete large quantities of lysosomal hydrolases (27). Hence, the release of matrix-degrading cathepsins as a consequence of M6P/IGF2R absence may represent a common feature of many tumor cells.

M6P/IGF2R plays a pivotal role in the formation of mature lysosomes (7, 8). SCC-VII cells fail to synthesize the receptor and thus lack a functional set of these organelles. Consequently, these cells store lysosomal enzymes in compartments with properties reminiscent of late endosomes/prelysosomes. Similar results were obtained for other M6P/IGF2R-negative tumor cell lines, such as murine J774 myeloid leukemia cells (28) and Morris 7777 hepatoma cells (29). Interestingly, cathepsin B was relocated to endosomal vesicles in human MCF-10A breast epithelial cells upon transfection with an oncogenic version of the c-Ha-ras gene (30). Furthermore, transformation of mouse NIH 3T3 fibroblasts with Kirsten murine sarcoma virus leads to redistribution of cathepsin L and other lysosomal enzymes to endosomes/prelysosomes (31). Because both latter cell types are not deficient in M6P/IGF2R,7 impaired formation of lysosomes may also occur in M6P/IGF2R-positive tumor cells.

Despite the lack of M6P/IGF2R, SCC-VII cells retain a large proportion of their newly synthesized lysosomal proteinases. Although the other known mammalian M6P receptor, MPR46 (8), is present in SCC-VII cells, the insensitivity of the intracellular transport of cathepsins B, D, and L to lysosomal alkalinization suggests that M6P-independent sorting of lysosomal enzymes may take place in these cells. Similarly, M6P-independent targeting mechanisms have been proposed for procathepsins D and L in normal and transformed mouse NIH 3T3 fibroblasts (32) and for procathepsin D in human breast cancer cells (23). Interestingly, procathepsin D transiently associates during its biosynthesis with prosaposin (33), a protein delivered to lysosomes via interaction with the low-density lipoprotein receptor-related protein (34). Thus, interaction of procathepsin D with endogenous prosaposin may account for its preferential retention in SCC-VII cells as compared with cathepsins B and L.

It has been reported recently that M6P/IGF2R interacts with uPAR through a domain distinct from its M6P- and IGF-II binding sites (35). In the absence of M6P/IGF2R, uPAR is not efficiently internalized, effectively increasing the number of surface binding sites for plasminogen activators, which may cause enhanced focal proteolysis. Because SCC-VII cells produce plasminogen activators (16), deficient uPAR internalization could further add to the proteolytic load in the pericellular environment of the cells. Hence, M6P/IGF2R deficiency may enhance the invasiveness of tumor cells by at least two means, elevated secretion of matrix-degrading cathepsins and increased amounts of cell-surface plasminogen activators. Interestingly, uPAR may also promote tumor cell invasion in a protease-independent manner through stimulation of integrin-mediated cell migration (36).

---

7 P. Ton and L. Mach, unpublished data.
The M6P/IGF2R gene is often inactivated in human and animal tumors. Rodents appear particularly susceptible because M6P/IGF2R displays monooalcal expression in mice and rats, with the paternal allele being repressed. In contrast, this genomic imprinting of M6P/IGF2R is a polymorphic trait in humans, with both alleles being transcribed in most humans. Thus, inactivation of the M6P/IGF2R locus generally requires two genetic events in humans but only one in mice (10). Interestingly, loss of one M6P/IGF2R allele is a common feature of human hematopoietic and breast cancers (12, 13). M6P/IGF2R contains several microsatellite sequences in its coding region, and deletions within the remaining M6P/IGF2R allele arise frequently as a consequence of microsatellite instability (14). These mutations generally cause frameshifts that result in premature termination of translation, giving rise to the synthesis of truncated, soluble receptors (12). However, M6P/IGF2R-related polypeptides were not detected in the culture supernatants of SCC-VII cells, thus indicating that the molecular defect in the M6P/IGF2R gene of these cells is probably not directly related to microsatellite instability.

M6P/IGF2R exerts at least two growth-suppressive functions: (a) the receptor accounts for the internalization and degradation of IGF-II, a powerful growth-stimulating factor; and (b) M6P/IGF2R binds latent TGF-β1 and stimulates activation of the growth-suppressive cytokine by recruitment of plasminogen and uPAR-bound plasminogen activators (37). In M6P/IGF2R-mutant gastrointestinal tumors, IGF-II degradation and TGF-β1 activation are significantly reduced (38). Recently, transfection of wild-type M6P/IGF2R cDNA into M6P/IGF2R-mutant human SW48 colorectal carcinoma cells was shown to suppress growth and induce apoptosis (15). Because our results indicate that M6P/IGF2R-negative tumor cells display an increased potential to degrade extracellular matrix components, inactivation of the M6P/IGF2R gene may support growth as well as metastasis of malignant cancers.

ACKNOWLEDGMENTS

We express our gratitude to Drs. Ann H. Erickson, Annette Hille-Rehfell, Thomas Braulke, David James, and John S. Mort for providing antibodies and cell lines. We also thank Melinda Abas and Prof. Josef Gloessl for critical reading of the manuscript.

REFERENCES

4. Rozhin, J., Sameni, M., Ziegler, G., and Sloane, B. F. Pericellular pH affects reading of the manuscript.
Invasive Properties of Murine Squamous Carcinoma Cells: Secretion of Matrix-degrading Cathepsins Is Attributable to a Deficiency in the Mannose 6-Phosphate/Insulin-like Growth Factor II Receptor

Kim Lorenzo, Phuong Ton, Jason L. Clark, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/60/15/4070

Cited articles
This article cites 36 articles, 19 of which you can access for free at:
http://cancerres.aacrjournals.org/content/60/15/4070.full#ref-list-1

Citing articles
This article has been cited by 9 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/60/15/4070.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.