Matrix Metalloproteinase-9 Is Upregulated in Nucleophosmin-Anaplastic Lymphoma Kinase–Positive Anaplastic Lymphomas and Activated at the Cell Surface by the Chaperone Heat Shock Protein 90 to Promote Cell Invasion

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Abstract

Many anaplastic large cell lymphomas (ALCL) express the chimeric oncogene NPM-ALK, which drives malignant transformation and invasion. In this study, we show that NPM-ALK expression increases matrix metalloproteinase-9 (MMP-9) expression. Accordingly, we found that 100% of a large panel of ALK(+) ALCL biopsies examined were also MMP-9(+), in contrast to only 36.3% of ALK(-) tumors. Mechanistic studies revealed that Rac1 drove MMP-9 secretion. The MMP inhibitor GM6001 and MMP-9 blocking antibodies abolished the invasiveness of NPM-ALK(+) cells. Interestingly, the hyaluronan receptor CD44 acted as a docking surface for MMP-9 and the chaperone heat shock protein 90 on the cell surface, where MMP-9 was cleaved and activated. Membrane-associated MMP-9 was localized to invadopodia, which display a strong gelatinase activity. Taken together, our observations strengthen the concept that chaperones have a major extracellular role in the regulation of protein activation status, and reveal new factors that are crucial for spreading and invasion of ALK(+) ALCL. They also point out new factors crucial for ALK(+) ALCL. Cancer Res; 70(17); 6978–87. ©2010 AACR.

Introduction

Anaplastic large cell lymphomas (ALCL) are non–Hodgkin lymphomas (NHL) with a high grade of malignancy. The most frequent rearrangement, a t(2;5)(p23;q35) reciprocal translocation, fuses the NH2-terminal portion of nucleophosmin 1 (NPM) to the COOH-terminal portion of ALK that contains the catalytic domain to produce the NPM-ALK fusion protein (1). Oligomerization of NPM-ALK through the NPM domain results in constitutive hyperactivation of tyrosine kinase activity responsible for transformation (2). NPM-ALK is highly phosphorylated on tyrosine and recruits a “signalsome” of more than 40 proteins, thereby triggering activation of transduction pathways largely involved in proliferative and antiapoptotic responses (3–5).

The majority of ALK(+) ALCL are diagnosed at advanced stages (III and IV), displaying systemic disease with generalized lymphadenopathy and extranodal metastasis, especially in the skin and in soft tissues such as the liver, lung, or spleen (6). Similar observations were made in NPM-ALK(+) transgenic mice, confirming that ALK(+) malignancies are very invasive neoplasms (2, 7–9). In agreement with these observations, NPM-ALK(+) cells display a distorted morphology with enhanced motility and invasiveness (8, 9). We and others have implicated regulators of cytoskeletal dynamics in NPM-ALK–dependent invasion, such as the Rho GTPases Rac1 and Cdc42, p130Cas, and pp60c-src (7, 8, 10). GTPases drive metastasis by disrupting polarity and intercellular contacts and increasing cell motility. They also promote remodeling of the extracellular matrix (ECM) through regulation of matrix metalloproteinases (MMP), thereby helping tumor cells to cross tissue boundaries (11). MMPs belong to a family of zinc-dependent endopeptidases. They play a central role in many physiologic and pathologic conditions including wound healing, embryogenesis, angiogenesis, arthritis, and cancer (12). Besides metastasis, they also promote cancer cell growth by releasing bioactive molecules from the ECM, such as chemokines, growth factor precursors, proangiogenic factors, and other proteases (13). MMP-2 and MMP-9 (gelatinases A and B) are particularly important in cancer progression because of their action on type IV collagen and basal membrane components (14). MMP-9 expression and secretion is especially tightly regulated. It is secreted as an inactive proform (zymogen) of 92 kDa and activated in the pericellular space by proteolytic cleavage, leading to the active 85-kDa enzyme. Association of MMP-9 to cell surface transmembrane proteins such as integrins, membrane-associated collagen, and variants of CD44 induces its activation by mechanisms that remain elusive (14–17). Experiments performed in mice...
show that elevated levels of MMP-9 are deleterious in cancers (18). In breast cancer and hematologic malignancies such as acute myeloid leukemia (AML), B-cell chronic lymphocytic leukemia (B-CLL), and NHL, high levels of MMP-9, either in the tumor or serum of patients, correlate with a poor clinical outcome (19–21).

Herein, we show that MMP-9 expression increases in NPM-ALK(+) cells. Interestingly, the majority of MMP-9 are complexed with CD44 and the chaperone heat shock protein 90 (Hsp90), responsible for MMP-9 activation. MMP-9 localizes in invadopodia, where the physical barrier of the ECM gets digested. Moreover, disruption of the CD44/MMP-9/Hsp90 complex abolishes invasion of NPM-ALK(+) cells. Thus, we have identified a new cell surface complex in ALCL cells that is likely responsible for lymphoma cell dissemination.

Materials and Methods

Patients and cell lines

Peripheral lymph nodes biopsies were obtained from patients diagnosed for ALCL at the Department of Pathology of Toulouse University Medical Center (France), after informed consent. The institutional review board of the Purpan’s Hospital approved the study. Control or NPM-ALK-expressing NIH-3T3 fibroblasts were maintained in DMEM (Invitrogen) supplemented with 10% FCS, 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.5 mg/mL gentamicin. The ALCL cell lines Cost, Karpas 299, SU-DHL-1 [harboring the t(2;5)(p23q35) translocation and expressing NPM-ALK], and Fepd, NPM-ALK(−), were cultured in Iscove’s modified Dulbecco’s medium (Invitrogen) supplemented with 15% FCS. NPM-ALK-expressing NIH-3T3 cells (9) and Cost ALCL cell lines (22) were obtained from G. Delsol (INSERM U563, Toulouse, France). Fepd, Karpas 299, and SU-DHL-1 ALCL cell lines were obtained from DSMZ (German Collection of Microorganisms and Cell Culture). Cells were authenticated by checking their morphology and the expression of NPM-ALK. Cells lines were checked twice a month for contamination.

Reagents and antibodies

GM6001, NSC23766, geldanamycin, and WHI-154 were from Calbiochem. Aprotinin and leupeptin were from Euromedex. FITC-coupled gelatin was from Invitrogen. All other chemicals were from Sigma-Aldrich. The following antibodies were used in this study: ALK [ALK1 (DakoCytomation) and Sp8 (Abcam)], Rac1 (23A8, Upstate Biotechnology), α-tubulin (B-5-1-2, Sigma-Aldrich), vinculin (hVIN-1, Sigma-Aldrich), cortactin (4F11, Upstate Biotechnology), phospho-ALK-Y1604 (recognizes Y664 on NPM-ALK; Cell Signaling Technologies), MMP-9 (mAb13415, mAb3309, and Ab13458; Chemicon), Hsp90α/β (F-8, Santa Cruz Biotechnologies), CD44 (A020, Calbiochem), and control IgG1 (MOPC-21, BioLegend). Horse-radish peroxidase–conjugated secondary antibodies were from Promega. AlexaFluor-labeled secondary antibodies were from Invitrogen.

MMP-9, CD44, and Hsp90 in ALCL Invasiveness

Immunohistochemistry using tissue microarrays

Formalin-fixed, paraffin-embedded tissues of ALCL collected from 38 patients [28 cases were ALK(+) j were used to prepare a tissue microarray (TMA). For immunohistochemical stainings, sections were incubated with the antibodies to ALK (Sp8) or MMP-9 (mAb3309). Development was described previously (23). Cases were considered positive when more than 10% of neoplastic cells were stained for MMP-9.

Cell lysis, immunoprecipitation, and immunoblotting

Protein extraction was already described (8). Secretion of MMP-9 in the culture media was evaluated after 24 hours of serum deprivation. Briefly, 500 μL of a 2-mL culture supernatant from 8 × 10^5 ALCL cells or 3 × 10^6 NIH-3T3 were concentrated and analyzed by SDS-PAGE and immunoblotting. For cell fractionation, cells were scrapped in detergent-free lysis buffer [50 mmol/L Tris-base (pH 8.0), 150 mmol/L NaCl, 5 mmol/L EGTA, 1 mmol/L phenylmethylsulfonyl fluoride, 50 mmol/L NaF, 1 mmol/L Na_3VO_4, 10 μg/mL leupeptin, and 2 μg/mL aprotinin]. Homogenates were centrifuged for 30 minutes at 100,000 × g and the pellet was resuspended in lysis buffer supplemented with 1% Triton X-100 and 0.5% sodium deoxycholate to obtain the membrane fractions. For immunoprecipitations, clarified homogenates were incubated overnight at 4°C with anti–MMP-9 (mAb13415) or control IgG antibodies and protein G-Sepharose beads. Eluted proteins were analyzed by SDS-PAGE and immunoblotting. Semiquantification of immunoreactive bands was done with GeneTools 6.03 software (SynGene).

siRNA-mediated knockdown

ON-TARGETplus SMARTpool siRNA (50 nmol/mL) targeted to CD44 (L-041132-01) or nontargeting (D-001810-10) as a control from Dharmacon siGenome was transfected into Cost ALCL cells using Lipofectamine RNAiMax (Invitrogen) according to the manufacturer’s instructions. Cells were cultured for 48 hours before being assayed.

Cell invasion assays

For transwell experiments, 5 × 10^4 NIH-3T3 cells were processed as described (8). For MMPs inhibition, cells were preincubated with 10 μmol/L GM6001 and antibodies directed against MMP-9 (mAb13415) or control IgG at 10 μg/mL.

The Matrigel invasion assay measures the ability of ALCL to invade a dense Matrigel matrix (BD Biosciences) that mimics the basement membranes. Overnight serum-starved ALCL cells (2 × 10^5) were labeled for 90 minutes with 1 μmol/L CellTracker Orange CMTMR (Invitrogen). After washes, cells were seeded in LabTek chambers (Nunc) and overlaid with 5 mg/mL Matrigel +/- 10 μmol/L GM6001. After 90 minutes at 37°C, medium containing 15% FCS and 1 μg/mL stromal-derived factor 1α (SDF1α; Pepro Tech) was added. After 24 hours, cells were fixed in 3.7% formaldehyde, 0.05% Triton X-100 containing PBS and labeled with AlexaFluor488-phalloidin and analyzed by confocal microscopy (LSM510, Carl Zeiss) using a 63× objective. Optical z sections were taken every 2.5 μm starting from the base and extending 100 μm into the Matrigel. To quantify invasion, CMTMR
fluorescent cells in the sections above 20 μm were added and then divided by the sum of cells in all the z sections. Data represent the analysis of ≥200 cells per condition. Three-dimensional imaging and cell quantification were realized using the Imaris 6.4.2 software (Bitplane AG).

**Immunofluorescence microscopy**

Cells were fixed with 3.7% formaldehyde, permeabilized when required with 0.2% Triton X-100, saturated with 5% FCS containing PBS, and labeled with the indicated primary antibodies and AlexaFluor-labeled secondary antibodies at room temperature. Confocal images were captured with a LSM710 (Carl Zeiss) using a 63× objective. Profiling of fluorescence intensity was done with the MetaMorph 7.5.0.0 software (Molecular Devices) and fluorescence intensity was quantified with the ImageJ 1.42q software (NIH).

**ECM degradation assays**

Cells were seeded on FITC-gelatin–coated coverslips as described previously (24). Briefly, a thin layer of FITC-gelatin was deposited on the coverslips and cross-linked with 0.5% glutaraldehyde for 15 minutes at room temperature. After a PBS wash, coverslips were kept in serum-free medium at 37°C until used. For colocalization experiments, cells were fixed, stained, and analyzed as described earlier using the confocal Zeiss LSM710 microscope. To quantify ECM degradation, we determined the percentage of cells that presented areas of degradation of the FITC-gelatin relative to the total number of cells in each condition.

**Statistical analysis**

The mean ± SE was calculated for each data point. Data are representative of three to five independent experiments. Differences between groups were analyzed by Student’s t test. In all cases, P < 0.001 (*).

**Results**

**NPM-ALK(+) lymph node biopsies express MMP-9**

ALCL are invasive lymphomas. Thus far, there is no clear consensus about the levels of MMPs in NHL (19, 25). We therefore sought to analyze the expression of MMP-9 by immunohistochemistry on a TMA prepared with 38 biopsies of ALCL. Twenty-eight cases expressed ALK chimera and corresponded to common types or small-cell variants according to the WHO classification (26). Although 36.3% of the ALK(−) samples were positive for MMP-9, 100% of the ALK(+) biopsies were strongly labeled with anti–MMP-9 antibodies, suggesting that the oncogene might have altered MMP-9 expression (Fig. 1A). Anaplastic cells produced MMP-9, which could be detected in the cytosol (Fig. 1B). Accordingly, strong MMP-9 expression was observed in NPM-ALK(+) ALCL cell lines when compared with the ALK(−) Fepd cells (Fig. 2A). Exogenous expression of NPM-ALK in NIH-3T3 fibroblasts also led to a robust increase in MMP-9 (Supplementary Fig. S1A), whereas treatment of ALCL cells with the ALK inhibitor WHI-154 resulted in a dramatic drop in metalloproteinase (Fig. 2B).

MMP-9 was detected both in culture supernatant and in cell lysates (Fig. 2A). Several articles described that MMP-9 localizes at the cell surface (15, 16, 27). Confocal microscopy showed a specific MMP-9 cell surface staining when cells were not permeabilized (Supplementary Fig. S1B; Fig. 3B). At high magnification, MMP-9 appeared vesicular in permeabilized cells and as patches in nonpermeabilized cells, suggesting that it was locally restrained on the membrane on secretion (Supplementary Fig. S2).

**Active extracellular MMP-9 is located in invadopodia**

To analyze the in situ proteolytic activity associated with the plasma membrane of NPM-ALK(+) cells, fluorescent matrix degradation assays were carried out. Cells were plated onto coverslips coated with FITC-gelatin. ECM degradation was visualized as black areas devoid of fluorescence, which can be measured and quantified (24). After 48 hours, about 25% of NPM-ALK(+) cells formed clustered black dots, which disappeared on treatment with the MMP inhibitor GM6001 (Fig. 2C and D). Confocal sections followed by orthogonal plotting of the images (z-projections) showed that the
digested zones contained MMP-9 (Fig. 2D), indicating that the protease might be involved in degradation. These structures displayed specific features of invadopodia, podosome-type adhesions with strong proteolytic activity specific of highly invasive tumoral cells. They are located on the ventral side of cells and active protrusions. By immunofluorescence, invadopodia display patch signals enriched in proteins regulating cytoskeleton and membrane remodeling, cell-ECM adhesion, and matrix degradation (28). Accordingly, the MMP-9–containing ventral protrusions of NPM-ALK(+) cells contained F-actin, cortactin, and vinculin, clearly identifying them as functional invadopodia (Fig. 2D). These data suggest

Figure 2. NPM-ALK increases the expression of MMP-9, which localizes within invadopodia. A, MMP-9 is highly expressed in NPM-ALK(+) cells. Lysates or culture media of serum-deprived ALCL cells were immunoblotted with anti–MMP-9 (Ab13458) or anti-ALK antibodies. Tubulin was used as a loading control. B, ALK inhibition decreases the expression of MMP-9. Serum-starved ALCL cells were treated with 15 μmol/L WHI-154 and immunoblotted with the indicated antibodies. C, NPM-ALK(+) cells display a strong surface-associated gelatinase activity. NPM-ALK(+) NIH-3T3 cells were cultured on FITC-gelatin–coated coverslips for 48 h with DMSO (control) or 10 μmol/L GM6001. Degradation areas were visualized as dark holes in the FITC-gelatin matrix. The percentage of cells that presented at least one area of gelatin degradation was determined. D, NPM-ALK(+) NIH-3T3 cells cultured as above were stained with phalloidin, anti-cortactin, anti–MMP-9, or anti-vinculin antibodies. Top images are xy sections of the ventral cell surface and bottom images are xz sections taken by confocal microscopy. Horizontal bar, 10 μm; vertical bar, 5 μm.
that MMP-9 is a major component of invadopodia in anaplastic lymphomas.

**Rac drives membrane localization of MMP-9**

We previously showed that Rac1 was crucial for NPM-ALK–dependent invasion (8). Rho GTPases (Rac and RhoA) can modulate the remodeling of ECM by regulating the levels and secretion of MMPs (11). We used the small-molecule Rac inhibitor NSC23766 to check whether it was involved in MMP-9 regulation (29). Overnight preincubation with NSC23766 did not affect the level of MMP-9 in total cell lysates, whereas membrane-associated MMP-9 was dramatically decreased in ALCL cells (Fig. 3A) and NPM-ALK(+) NIH-3T3 fibroblasts (Fig. 3A–C). Concomitant with the decrease in MMP-9 at the membrane, we observed a drop in gelatinase activity when Rac was inhibited, suggesting that Rac-driven MMP secretion is of major importance for ECM degradation by ALCL cells and, therefore, for invadopodia function (Fig. 3D).

**MMP-9 physically interacts with CD44 and the Hsp90 chaperone**

Interestingly, it has been described that association of MMP-9 with receptors such as CD44 or integrins favored...
ECM proteolysis at the leading edge of invasive tumoral cells (16, 17, 27). Immunoprecipitations of MMP-9 from lysates obtained from NPM-ALK(+) ALCL Cost and Karpas 299 and transfected NIH-3T3 showed that CD44 associated with the 85-kDa cleaved MMP-9 (Fig. 4A). Confocal microscopy confirmed that CD44 and MMP-9 colocalized on the plasma membrane (Fig. 4B). In fibroblasts, NPM-ALK induces a typical spindle shape (Supplementary Fig. S1B; Figs. 3B and 4B; ref. 7). Image analysis showed enrichment of the MMP-9/CD44 complexes at the extremity of the elongated dynamic cellular processes specific to NPM-ALK–expressing fibroblasts (Fig. 4B and C). Interestingly, some cells also showed a strong membrane colocalization at the ventral side in a zone close to the nucleus that often corresponds to the location of invadopodia (28).

Because only mature MMP-9 associated with CD44, we suspected that another protein regulating MMP-9 proteolysis was present in the complex. MMPs are secreted as proforms that get activated by proteolytic cleavage outside the cell. It was described that secreted Hsp90 chaperone promoted extracellular maturation of MMP-2 (30). Hsp90 is important in ALK(+) anaplastic lymphomas because it regulates the stability of the
oncogene (31). Immunoprecipitation showed that Hsp90 was indeed part of the MMP-9/CD44 complex (Fig. 4A). Confocal microscopy confirmed the colocalization of MMP-9, CD44, and Hsp90 on the cell surface (Fig. 4B and C). Interestingly, Hsp90, which is one of the most abundant ubiquitous proteins located in the cytosol, was present on the plasma membrane only at sites that contained MMP-9, reinforcing the idea that they function together to promote a common response.

**CD44 anchors MMP-9 and Hsp90 at the membrane**

We then pushed further the study of the MMP-9/CD44/Hsp90 complex. Hsp90 can be secreted by tumoral cells (fibrosarcomas and breast cancer cells) and associate with the plasma membrane, but the mechanism of its recruitment is not understood. In the case of MMP-2, it was proposed that the gelatinase itself could be the anchor (30). In our case, depletion of CD44 by siRNA resulted in a drop in membrane association for both MMP-9 and Hsp90, which were released into the culture media (Fig. 5A and B). Again, confocal microscopy confirmed that cells expressing very low to indetectable levels of CD44 showed a decrease in MMP-9 and Hsp90 surface association, suggesting that CD44 acted as a docking protein (Fig. 5C and D).

**Hsp90 is required for pro-MMP-9 cleavage and activation**

We then used the drug geldanamycin, which is a specific inhibitor of Hsp90 (32). Treatment of ALCL cells with geldanamycin induced the disappearance of the 85-kDa active MMP-9 in total cell lysates (Fig. 6A). It has been shown that geldanamycin leads to a rapid proteosomal degradation of NPM-ALK. Indeed, we found that geldanamycin induced a decrease in NPM-ALK (Fig. 6A). To make sure that the effect of geldanamycin on the maturation of MMP-9 was coming...
from inhibition of Hsp90 and not from a drop in ALK, we treated the cells with the ALK inhibitor WHI-154 to abolish ALK activity without affecting protein levels. The inhibition of ALK activity had no effect on the maturation of MMP-9 because both the 92-kDa and the 85-kDa bands were detected in WHI-154–treated cells (Fig. 6A; Supplementary Fig. S3). Quantification showed that geldanamycin inhibition of Hsp90 resulted in a very strong blockade of MMP-9 maturation (90% drop in the 85-kDa form), suggesting that the presence of active Hsp90 on the cell surface is a prerequisite to MMP-9 activation (Supplementary Fig. S3B).

**CD44 and MMP-9 regulate NPM-ALK(+) cell invasiveness**

We focused on functional aspects of the CD44/MMP-9/Hsp90 complex by assessing the invasiveness of NPM-ALK(+) ALCL. NPM-ALK(+) cells display high invasive properties (8, 9). To mimic their environment, we overlaid the cultures with three-dimensional Matrigel and checked their ability to move toward a gradient of 15% FCS and 1 μmol/L SDF1α. After 24 hours, the proportion of cells invading over 20 μm was determined by taking optical sections by confocal microscopy. Cost, Karpas 299, and SU-DHL-1 cells were found as high as 100 μm above the bottom of the wells, with an average of 30% of the cells being in sections superior to 20 μm. Treatment of cells with the MMP inhibitor GM6001 resulted in a blockade of invasion because less than 5% of cells moved above 20 μm (Fig. 6B; Supplementary Fig. S4). Interestingly, xz projections of migrated cells showed a strong polarization of actin motile structures toward the gradient, whereas GM6001 treatment totally abolished the formation of those structures, and as a result, cells remained in the <20-μm zone of the gel (Supplementary Fig. S5). To assess more precisely the role of the MMP-9/CD44 complex, we tested the invasiveness of NPM-ALK(+) NIH-3T3 in Matrigel-coated Boyden filters.
chambers. Cells were seeded in the top chamber in 2% FCS-supplemented medium and allowed to migrate through the Matrigel layer toward a gradient of 10% FCS. Again, cells treated with GM6001, specific blocking anti-MMP-9 antibodies, or CD44-targeting siRNA showed a 90% drop of their invasive capabilities in comparison with untreated NPM-ALK cells (Fig. 6C). These data clearly showed that active CD44-associated MMP-9 is of crucial importance for NPM-ALK(+) cell invasiveness and highlight the importance of the MMP-9/CD44/Hsp90 complex for spreading of ALK(+) neoplasms.

Discussion

We uncovered an important mechanism that drives ALK (+) ALCL invasiveness by deciphering the functional relationship between the receptor CD44, the Hsp90 chaperone, and the metalloproteinase MMP-9 at invadopodia (model in Supplementary Fig. S6). Cancer metastasis is a complex process that involves degradation of the basement membrane as well as penetration and adaptation to the microenvironment of the targeted organ. These steps rely largely on MMPs, which are recognized as major factors of cancer spreading (14, 33).

There was a controversy about the levels and origin of MMP-2/MMP-9 in ALCL biopsies (19, 25). By assessing protein and not mRNA levels as it has been done previously, we showed that NPM-ALK(+) anaplastic cells were expressing MMP-9, suggesting that MMP-9 was produced by the oncogenic cells. Moreover, we could not detect any MMP-2 in ALCL cells or tumors. It is known that activated T-lymphocytes express increased amounts of MMP-9. Therefore, its overexpression in anaplastic lymphomas could just be a remnant of lymphocyte response (34). However, the majority of ALK(-) ALCL do not express high levels of MMPs even though they are in an activated state because of tumoral transformation. It is therefore unlikely that the increase in MMP-9 only results from cellular activation.

Because MMP-14 has been described as a marker of invadopodia in many solid tumors, we checked for its presence in MMPs, which are recognized as major factors of cancer spreading (14, 33).

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The implication of CD44 in tumor growth and dissemination has been shown in various cancers. In hematologic cancers, its role was reported in B-CLL and AML (17, 36). Clinical studies in NHL showed that high levels of CD44 were associated with a bad prognosis for invasive large-cell lymphomas (37, 38). Binding of MMP-9 and its activator (Hsp90) to CD44 provides an explanation for the proinvasive functions of CD44 because maturation of MMP-9 at the plasma membrane will allow directed remodeling of the ECM.

Interestingly, soluble MMP-9 recovered from culture medium or from the serum of patients is often found to be inactive because of association with inhibitors from the tissue inhibitors of metalloproteinases (TIMP) family, whereas cell-associated MMP-9 displays strong gelatinase activity (39). Because CD44 is TIMP-free, we can hypothesize that it could act by recruiting MMP activators and dissociating the TIMP-MMP complexes (14, 39).

Both CD44 and MMP-9 were found within podosomes or invadopodia in various cell types, including B-CLL (35). We and others have already reported that Rac1 and Cdc42 drove invasion and cytoskeleton rearrangements in NPM-ALK(+) cells (8, 40). Among other means, GTPases regulate motility by linking the cytoskeleton to the membrane via the ERM proteins (Ezrin, Moesin, Radixin) and CD44. They also promote MMP production by modulating their transcription and secretion through acto-myosin motors (41, 42). This work highlights a new aspect of the role of Rac in dissemination of aggressive lymphomas by showing that it is required to secrete MMP-9 and form functional invadopodia. MMP-9 appeared as spots on the cell surface, suggesting that it is captured by CD44 as soon as it is secreted and concentrated at dynamic areas requiring proteolytic activity.

An important concept that arose from this study is that the Hsp90 chaperone can be recruited at the plasma membrane by the same docking protein as the MMP. Hsp chaperones guide the proper folding, activation, transport, and turnover of key regulators of cell biology. It is not surprising that their functions are subverted during oncogenesis, hence their important role in cancer (43). Recently, the demonstration that secreted Hsp90 induced MMP-2 activation showed that it had functions outside the cell (30, 44). Our data suggest that extracellular Hsp90 is crucial for MMP-9 activation and ALCL invasion. The regulation of metalloproteinase activity at motile membrane sites could be a general feature of extracellular chaperones. Future work will determine whether maturation of MMPs by extracellular Hsp is a general phenomenon. This is the first description of how anaplastic lymphoma invasiveness is regulated in relationship with the microenvironment, opening new avenues of research that need to be explored.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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