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Large Scale Matrix Degradation by Stromal Cells Independent of Invadopodia

Hong Cao
Mayo Clinic

Robbin Eppinga
Dordt College, robbin.eppinga@dordt.edu

Eugene W. Krueger
Mayo Clinic

Jing Chen
Mayo Clinic

Mark A. McNiven
Mayo Clinic

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Abstract

Invadopodia are actin-rich structures at the base of many neoplastic cells that sequester matrix metalloproteases that act to degrade the surrounding stroma to facilitate the invasive process. Conventional invadopodia are dependent upon Src kinase and the large GTPase dynamin 2 (Dyn 2). Whether invadopodia are the only mechanism by which cells degrade matrix is unclear. We have observed that cells of mesenchymal origin degrade matrix in an unique way different from tumor cells. The HYPOTHESIS of this study is that fibroblasts, and other cells of mesenchymal origin, degrade matrix by a mechanism distinct from that of epithelial-based tumor cells. The CONCLUSION is that stromal cells degrade matrix by a novel mechanism distinct from traditional invadopodia.

Keywords

invadopodia, tumor cells, stromal cells, cancer

Disciplines

Cancer Biology | Cell and Developmental Biology

Comments

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Large Scale Matrix Degradation by Stromal Cells Independent of Invadopodia

Hong Cao, Robbin Eppinga, Eugene W. Krueger, Jing Chen, and Mark A. McNiven

Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, Minnesota USA

Biology Department, Dordt College, Sioux Center, Iowa USA

Background and Goals

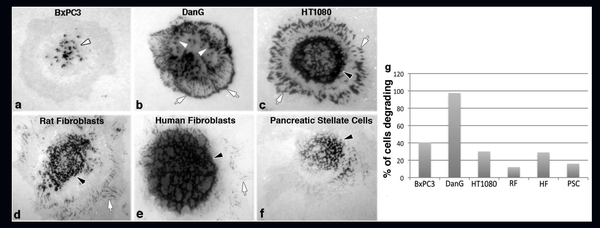
Invadopodia are actin-rich structures at the base of many neoplastic cells that sequester matrix metalloproteases that act to degrade the surrounding stroma to facilitate the invasive process. Conventional invadopodia are dependent upon Src kinase and the large GTPase dynamin 2 (Dyn 2). Whether invadopodia are the only mechanism by which cells degrade matrix is unclear. We have observed that cells of mesenchymal origin degrade matrix in an unique way different from tumor cells.

The HYPOTHESIS of this study is that fibroblasts, and other cells of mesenchymal origin, degrade matrix by a mechanism distinct from that of epithelial-based tumor cells.

The GOALS of this study were to define the mechanisms supporting this novel form of matrix remodeling by:

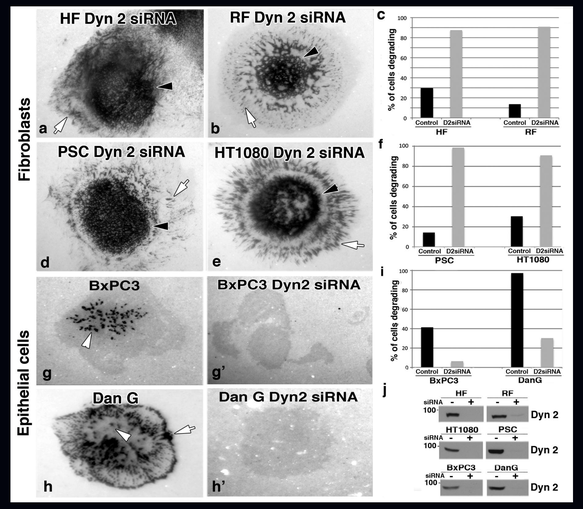
- Defining the requirements of Dyn 2 and Src kinase in matrix degradation by fibroblasts and tumors of epithelial origin.
- Testing the role of Dyn 2 depletion on MT1-MMP accumulation by cells both internally and at the plasma membrane.

Figure 1. Tumor Cells and Stromal Cells Exhibit Distinct Patterns of Matrix Degradation



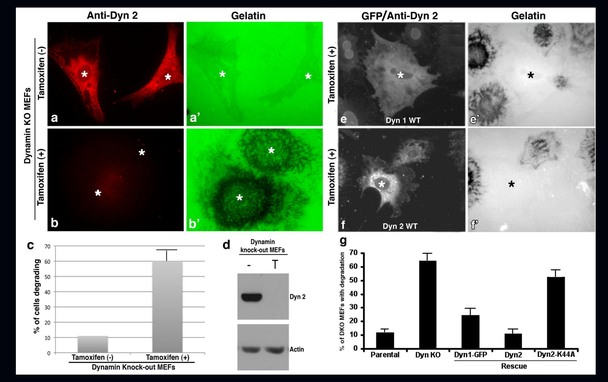
A variety of different tumor and mesenchymal cell types plated on fluorescent gelatin and cultured for 8h prior to fixation. (a,b) The pancreatic tumor cells BxPC3 and DanG show largely invadopodial-like (white arrowheads) and focal adhesion-like (arrows) degradation patterns. (c) The fibrosarcoma cell line HT1080 exhibits focal adhesion-like degradations and a unique pattern of centrally positioned degradation structure in a labyrinth or reticular-like display. (d-f) Mesenchymal cells exhibit a unique pattern of matrix degradation, whether they be rat fibroblasts, human fibroblasts, or pancreatic stellate cells. (g) Graph depicting the percentage of each cell type that degrades matrix after 8 hours.

Figure 2. Reduction of Dynamin 2 (Dyn 2) Induces a Differential Response in Matrix Degradation Between Fibroblasts and Epithelial Cells



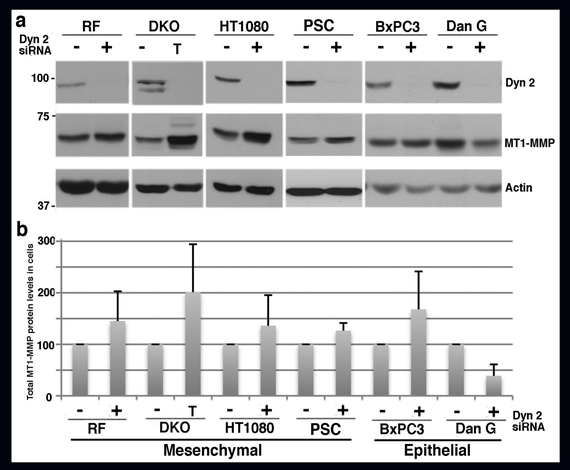
Assessing the effects of Dyn2 knockdown on matrix degradation in six different fibroblast and epithelial cell types. Cells were treated with siRNA to reduce Dyn2 levels over 72 h, then trypsinized, resuspended, and plated on fluorescent gelatin-coated coverslips for six hours in the presence of the protease inhibitor BB94. Cells were then rinsed and allowed to degrade for 8 hrs prior to fixation. (a-e) A variety of mesenchymal fibroblastic cells that normally exhibit modest amounts of degradation show increased matrix remodeling by 4-5-fold upon reduction of Dyn 2. While some focal adhesion-like degradation (white arrows) is observed, most of it is a reticular-like degradative pattern at the center of the cell. In contrast, pancreatic epithelial tumor cells that spontaneously degrade via invadopodia are markedly inhibited in their degradative capacity by Dyn2 knockdown (g-i). (j) Western blot analysis of cell homogenates showing near complete elimination of Dyn2 expression in the six cell types examined.

Figure 3. Tamoxifen-Induced Knockout of Dyn 2 in MEFs Induces a Substantial Increase of Matrix Degradation Compared to Controls



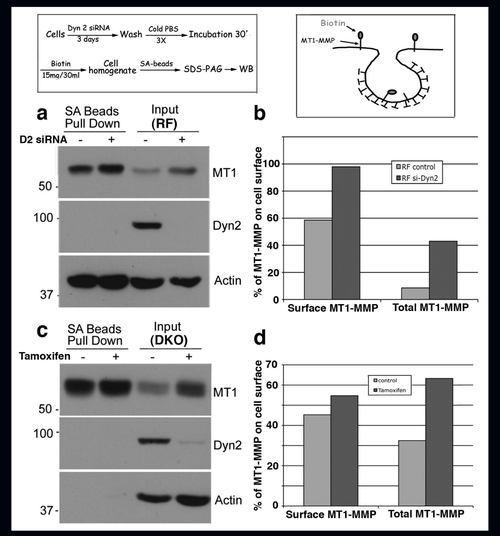
To examine another fibroblast cell line from a Dyn 2 genetic knockout model (kindly provided by Ferguson and De Camilli, Yale), matrix degradation was analyzed in MEFs from these knockout mice in the presence and absence of tamoxifen. (a,b) Immunostaining of control or tamoxifen-treated cells (4-5 days) stained for Dyn 2, or revealing matrix degradation on a fluorescent gelatin substrate. Control cells show substantial amounts of endogenous cytoplasmic Dyn 2 but exhibit almost no matrix degradation. In contrast, cells in which Dyn 2 has been reduced exhibit a 6-7-fold increase in matrix degradation with a characteristic reticular network pattern. (d) Western blot analysis of cells +/- tamoxifen treatment blotted for Dyn 2. (e-g) Re-expression of either Dyn 1 or Dyn 2 in tamoxifen-induced Dyn 2 knockout MEFs results in a reversal of the degradation phenotype (reduced degradation). Fluorescence images showing re-expression of either of the neuronal-specific Dyn 1 or ubiquitously expressed Dyn 2 with corresponding degradation patterns on fluorescent gelatin. Note that the surrounding cells in which dynamin is not expressed degrade large amounts of matrix in comparison to the rescued cells (*) that degrade no matrix at all. (g) Quantitation of the effect of dynamin knockout on matrix degradation by MEFs. Note the substantial increase in the tamoxifen-treated Dyn KO cells, which is markedly reduced by expression of wild type Dyn 2 and Dyn 1-GFP. In contrast, the Dyn 2K44A mutant-expressing cells continue to degrade large amounts of matrix.

Figure 4. A Reduction of Dyn 2 Causes Accumulation of MT1-MMP



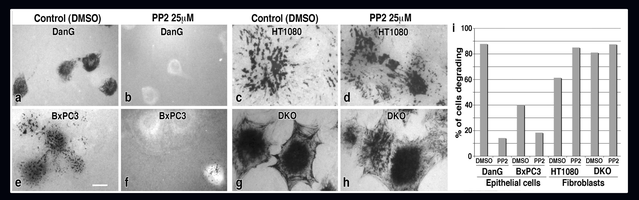
Western blot analysis of six different mesenchymal and epithelial cell lines in which endogenous Dyn 2 levels have been experimentally reduced, followed by SDS-PAGE and Western blot analysis with antibodies to MT1-MMP. (b) Quantitation of three distinct experiments shows a modest accumulation of MT1-MMP protein upon Dyn 2 knockdown. While levels were increased by nearly 50% in the RF, DKO MEFS, and BxPC3 cells, there was only a modest incease observed in the PSC cells and an actual loss of protease in the DanG cells.

Figure 5. Knockdown of Dyn 2 Induces a Modest Retention of MT1-MMP on the Cell Surface



(a,c) Western blot analysis of cell homogenates of either rat fibroblasts treated with siRNA to knockdown dynamin, or MEFs treated with tamoxifen to induce the Dyn 2 knockout. Following reduction of Dyn 2 levels, cells were incubated in biotin to label all cell surface proteins, then homogenized followed by a pull-down with streptavidin beads, then SDS-PAGE and Western blot (see cartoon for experimental sequence). (b,d) Graphs represent quantitation of three different experiments showing both total, and surface populations of MT1-MMP from cells both before and after Dyn2 knockdown. As shown before in Fig. 4, total MT1 levels were increased by 2 fold, although changes in the levels of MT1 residing at the cell surface remain only modestly increased (20-40%) following Dyn2 knockdown. This minor increase suggests that the massive increase in matrix degradation by the mesenchymal cells following Dyn2 reduction may not be dependent upon an accumulation of active protease at the cell surface.

Figure 6. Matrix Degradation by Epithelial Cells and Fibroblasts Exhibits Distinct Requirements for Src Kinase



Invadopodia function is dependent upon Src kinase. To determine if the distinct matrix degradation processes observed in the different cell types shared a requirement for Src kinase, we treated cells with the Src inhibitor PP2. Cells were plated on gelatin in the presence of the MMP inhibitor BB94 for 5 hours during plating to prevent degradation during attachment. Subsequently cells were washed free of BB94 and incubated in 25μM PP2 for 13 hours. (a, e) The pancreatic cancer lines DanG and BxPC3 both show robust degradation under control conditions, but are almost completely inhibited when treated with 25μM PP2 (b, f). By contrast, the fibrosarcoma cell line HT108 (c, d) and the Dyn2 knockout fibroblast cell line DKO (g, h) both continue to degrade large amounts of matrix even under these high concentrations of inhibitor. (i) A graph depicting the percentage of cells that degrade matrix upon Src inhibition. Note the graphic contrast in drug sensitivity between the epithelial tumor cells and the fibroblasts/fibrosarcoma cell lines.

Summary

In this study we have found that:

- **Fibroblasts and cells of mesenchymal origin degrade matrix but appear to do so by a mechanism distinct from epithelial-based tumor cells.**
- **Matrix degradation in fibroblasts appears to be mediated by focal adhesion sites or at a reticular structure at the cell base. Epithelial tumor cells degrade by both focal adhesions and traditional invadopodia.**
- **Matrix degradation by invadopodia in epithelial tumor cells is markedly reduced by Dyn2 knockdown or inhibition of Src kinase while degradation by fibroblasts is increased substantially by Dyn 2 depletion and is Src independent.**
- **Reduction in Dyn2 levels results in a 2 fold increase of MT1-MMP with a modest proportion of this protease remaining at the cell surface.**

Conclusions

Stromal cells degrade matrix by a novel mechanism distinct from traditional invadopodia.