Differential Effects of Protease Inhibitors in 2-Dimensional and 3-Dimensional Cell Invasion Assays

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Abstract

Tumor cell invasion through an extracellular matrix has long been established as a key component in the metastasis of cancer. Previous studies have shown that inhibitors of proteases, such as matrix metalloproteinases (MMPs), urokinase, and elastase, are effective in reducing tumor cell lines in 2-D assays, that employ matrix coated porous transwell inserts. We have recently developed a 3-D invasion assay in a 96-well plate format where cells were seeded into a layer of basement membrane extract (BME) in an amniotic fusion around a centrally placed silicone stopper within the plate wells. Adherent cells were then allowed to invade BME coated wells 48 hours, after which the stoppers were removed and the wells were incubated in an incubator for an additional 48 hours. The wells were then stained with rhodamine and imaged using a Zeiss Axioskop microscope (2x magnification). The images, captured with an automated image analysis system, were used to evaluate the invasion potential of the cell lines, as well as to evaluate the effect of chosen inhibitors on cell invasion. Results: The use of this assay system is particularly relevant for drug discovery efforts as these results substantiate the lack of efficacy of protease inhibitors observed in 2-D assays. The results from this assay also indicate that all cell lines tested were able to invade BME coated wells in the absence of inhibitors. However, inhibitors of cathepsin B and MMPs were able to significantly reduce invasion in 3-D assays. As previously reported, these results are consistent with known protease characteristics observed at invadopodia sites.

Introduction

Proteases have been implicated in many important physiological processes, including development, wound healing, and inflammation. In cancer, proteases play a key role in the ability of tumor cells to invade surrounding tissues, a process known as tumor cell invasion. In 2-D assays, tumor cell invasion is often assessed using a Transwell™ membrane based assay, which allows for the study of cell migration in response to external stimuli. However, this assay is limited in its ability to accurately reflect the complex, three-dimensional (3-D) microenvironment of the tumor, as it lacks the extracellular matrix (ECM) and basement membrane (BM) components that are present in vivo. In contrast, 3-D invasion assays, such as the one described in this study, are able to account for these factors and provide a more accurate representation of tumor cell invasion in vivo.

Materials and Methods


Results: The use of this assay system is particularly relevant for drug discovery efforts as these results substantiate the lack of efficacy of protease inhibitors observed in 2-D assays. The results from this assay also indicate that all cell lines tested were able to invade BME coated wells in the absence of inhibitors. However, inhibitors of cathepsin B and MMPs were able to significantly reduce invasion in 3-D assays. As previously reported, these results are consistent with known protease characteristics observed at invadopodia sites.

Conclusions

1. HT-1080 cells moving within the 3-D ECM express several classes of proteases within interconnected structures, such as Cathepsins and MMPs, as evidenced by enzymatic activity assays and immunocytochemistry.
2. Serum stimulation enhances invasion through 3-D collagen matrices.
3. The serum growth factor mimics the effects of invasion in vivo.
4. Inhibitors, including those for serine and cysteine proteases, and MMPs, have little effect alone or in combination on cell invasion into 3-D ECM matrix.

References