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Limited Processing of Pro-Matrix Metalloprotease-2 (Gelatinase A) Overexpressed by Transfection in PC-3 Human Prostate Tumor Cells: Association With Restricted Cell Surface Localization of Membrane-Type Matrix Metalloproteinase-1

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The expression and activation of matrix metalloproteinases (MMPs) by tumor cells is correlated with progression to invasive and metastatic status. The purpose of this study was to examine the role of increased MMP-2 (gelatinase A) expression in prostate cancer progression utilizing human prostate PC-3 cancer cells that overexpress MMP-2 using gene transfection. PC-3 cells were transfected with pCR-3 vector only and pCR-3 MMP-2 plasmids employing the LipofectAMINE method, and stable transfectants were selected with G418. The expression of MMP-2, tissue inhibitor of metalloproteinase-2 (TIMP-2), and membrane-type MMP 1 (MT1-MMP) in PC-3 parental and transfected cells under serum-free conditions was determined by zymography, immunoblotting, immunofluorescent microscopy, Northern blotting, and/or reverse transcriptase-polymerase chain reaction (RT-PCR). MMP-2 transfected cells produced primarily the proenzyme form of MMP-2; the parental and vector control transfected PC-3 cells did not express any MMP-2 that was detectable by the methods we employed. Treatment of PC-3 MMP-2 transfected cells with Concanavalin A (Con A), in contrast to HT-1080 cells, processed only a small amount of the secreted 72-kd proenzyme to a 62-kd intermediate and a cell-associated 59-kd active form. The low level of secreted pro-MMP-2 processing induced by Con A was inhibited by serine protease inhibitors and was unaffected by cyclic adenosine monophosphate (cAMP). Immunoblotting showed that these cells produced abundant TIMP-2 and lower amounts of MT1-MMP in comparison with Con A–responding HT-1080 cells. HT-1080 cells respond to Con A by translocating MT1-MMP from intracellular localization sites to the plasma membrane, an effect not observed in PC-3 cells. The molecular basis for the low level of processing of pro-MMP-2 by PC-3 cells may be due to an overabundance of TIMP-2 and/or a low level of cell surface active MT1-MMP.

Key words: Tissue inhibitor of metalloproteinase-2, prostate cancer, Concanavalin A, reverse transcriptase-polymerase chain reaction