Role of RECK in invasiveness of soft tissue sarcoma cell lines
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Introduction: Soft tissue sarcomas (STS) are malignant tumors of the soft tissue that are histologically diverse but exhibit similar aggressive features. Matrix metalloproteinases (MMPs) are a family of enzymes capable of degrading extracellular matrix and thus endow the tumor cell with the invasive capacity. RECK (reversion inducing cysteine rich protein with Kazal motifs), a novel MMPs inhibitor, is known to down-regulate MMPs and suppress the invasive potential in many types of tumors. We have previously shown in osteosarcoma that RECK suppresses tumor invasiveness by decreasing MMPs activation (1). However, the role of RECK in STS has never been studied. The purpose of this study was to investigate the role of RECK in tumor invasiveness in STS.

Materials and Methods: SW 684 (fibrosarcoma, HTB-91), SW 872 (liposarcoma, HTB-92), SW 982 (synovial sarcoma, HTB-93), A 204 (rhabdomyosarcoma, HTB-82) STS cell lines were used in the experiments (American Type Culture Collection, Rockville, Maryland). Cells were cultured as previously described (2). RECK gene was constructed as previously described (1) and transfected into the 4 STS cell lines. Reverse transcriptase-PCR was used to analyze the expression of RECK and MMP-14 (MT1-MMP) before and after gene transfection. Activity level and expression of MMPs was assessed by measuring gelatinolytic activities of pro-MMP-2, MMP-2, pro-MMP-9, and MMP-9. The in vitro invasive properties of STS cells were evaluated in the transwell chamber invasion assay (Becton Matrigel Invasion Chamber; Becton Dickinson, Bedford, MA).

Results: The level of RECK mRNA expression was increased in all of the STS cell lines after gene transfection (Fig 1). To assess the role of RECK in MMPs expression, gelatin zymography was performed performed in RECK-transfected cells. The activation of pro-MMP-2 was decreased in RECK-transfected cell lines. The expression of MMP-14, as assessed by RT-PCR, was also decreased in RECK-transfected cell lines (Fig 2). To investigate the role of RECK in the invasiveness of STS cells, the invasive capacities of SW 892 cells, before and after transfection, were assessed using transwell chamber assay. The invasive ability of SW 892 cells decreased significantly after RECK transfection (Fig 3).

Discussion: Our findings suggest that RECK suppresses tumor invasiveness by decreasing MMPs activation in STS. These results may raise the possibility that RECK is a promising target for treating STS.


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Figure 1. Expression of RECK mRNA before and after gene transfection. Representative result of RT-PCR of RECK and actin is presented. (Con: cells without transfection, Vec: cells transfected with an empty vector, RECK: cells transfected with RECK-containing vector)

Figure 2. Effects of RECK transfection in MMPs activation in STS cell lines. Representative result of gelatin zymography (upper row) and RT-PCR (lower row) is presented. Positions of the major gelatinolytic bands are shown on left.

Figure 3. Effects of RECK transfection on the invasive capacity of STS cell lines. (A) Representative transwell chamber assay of RECK-transfected SW 892 cells. Note the decreased number of cells in the RECK-transfected group. (Vec: cells transfected with an empty vector, RECK: cells transfected with RECK-containing vector) (B) Comparison of the number of cells that migrated in Matrigel chamber after RECK transfection. Columns represent the mean of cell numbers counted in 3 randomly selected fields on light microscope (200x). Error bars indicate standard deviation.