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Study the Role of CD93 in cell migration

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Abstract

Background and Purpose: CD93 (C1qRp) is a cell surface glycoprotein predominantly expressed on myeloid lineage cells, microglia cells, endothelial cells and early stem cell precursors. CD93 was considered to be involved in the C1q-mediated enhancement of phagocytosis. However, other reports suggested that CD93 was not required for enhancing phagocytosis in vitro. The extracellular domain of CD93 contains a carbohydrate-recognition domain, which is known as a bind site for carbohydrates from ECM and to mediate cell adhesion and locomotion. Thus the specific aim of this study is to investigate the role of CD93 on cell migration. **Methods:** We established green fluorescent protein-tagged CD93 or cytoplasmic domain–deleted CD93 (CD93(ΔC)) transfectants in A2058 melanoma. Cell migration was measured by in vitro wound healing assay. Cells were grown to confluence in 6-well plates and wounded with a micropipette tip. After different time period, the cells migrated into the wound zone and the wound closure area were photographed and analyzed. Invasion assays were performed by using Boyden chamber. Cells were seeded to the upper chambers, which were separated by the Matrigel-coated 8 μm pore size polycarbonate membrane from the lower chamber. The cells that invaded through Matrigel were counted. For immunofluorescent staining, cells were fixed by formaldehyde and incubated with primary antibody followed by fluorescent-labeded secondary antibody. The activities of MMP in the conditioned medium were determined by gelatin zymography assay. The expression of MMP-2/9 mRNA was analyzed by real-time PCR. To study small GTPases activity, Rac1-GTP pull-down assay was performed. **Results:** Using in vitro wound healing assay and Boyden chamber invasion assay, we demonstrated that the migration and invasion of A2058-CD93 cells and A2058-ΔC CD93 cells were increased than that of control A2058-GFP cells. We also found that A2058-CD93 cells migrated directionally while A2058-GFP cells migrated haphazardly. The results suggest that CD93 can mediate the directional cell migration. In addition, we showed that The MMP-2/9 activities in the conditioned medium from A2058-CD93 cells were elevated that that from A2058-GFP cells. The real-time PCR experiment also demonstrated that the expressions of MMP-2/9 mRNA in A2058-CD93 cells were increased than that in A2058-GFP cells. Moreover, we demonstrated that the Rac-1 activity of A2058-CD93 cells was increased that of control cells. These results suggested that the effect CD93 on mediating cell migration and invasion might be through the Rac-1 pathway and the induction of MMP-2/9 activities. **Conclusions:** We demonstrated that the expression of CD93 could enhance cell migration and invasion, and the effects of CD93 might be explained at least partly through the induction of the MMP2/9 and Rac-1 activities.