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Study the function of CD93 on cell adhesion
CD93 在細胞貼附的功能研究

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Abstract

Background and Purpose: CD93 (C1qRp) is a transmembrane glycoprotein that is expressed on endothelial cells, neutrophils, myeloid lineage cells and haematopoietic stem cells. The structure of CD93 is similar with thrombomodulin which was reported to play an important role in mediating cell-cell adhesion through its lectin-like domain. The specific aim of this study is to investigate the role of CD93 on cell adhesion. **Methods:** We established green fluorescent protein-tagged CD93 or cytoplasmic domain–deleted CD93 (CD93(ΔC)) transfectants in A2058 melanoma. Confocal microscopy was used to examine the subcellular distribution of CD93 proteins in A2058 cells and cell morphology. The cell-cell adhesion was performed by cell monolayer permeability assay and Ca^{2+}-switch assay. The spreading area of A2058-GFP, A2058-CD93 and A2058-CD93ΔC was measured after cells were seeding for various time periods. The interaction of CD93 and ezrin was determined by immunofluorescent staining and co-immunoprecipitation assay. **Results:** Fluorescent microscopy demonstrated that CD93-GFP distributed in the plasma membrane and particularly concentrated at cell-cell junction. CD93-expressed cells grew as closed cluster colonies, while CD93(ΔC)-expressed cells grew loosely. The permeability of the monolayer of CD93 cell cultures was significantly reduced compare with the CD93(ΔC) and A2058-GFP control cells. The cell-cell adhesion in A2058-CD93 cells was Ca^{2+}-dependent and was inhibited by monoclonal antibody against CD93. These results suggested that the expression of CD93 could enhance cell-cell adhesion in a Ca^{2+}-dependent manner. The effect of CD93-mediated cell adhesion was also abolished by the addition of mannose, suggesting that the carbohydrate-recognition domain of CD93 might be participated in CD93-mediated cell-cell adhesion. Determination of the spreading area of single cells indicated that the membrane extension of A2058-CD93 cells was larger than that of A2058-GFP cells. In addition, the adhesion of A2058-CD93 was more tightly than that of A2058-GFP cells. In order to elucidate whether the effect of CD93 on cell adhesion is mediated by anchoring to actin cytoskeleton, we investigate the interaction of CD93 and actin-binding protein ERM family, ezrin. We demonstrated that CD93 colocalized with ezrin at cell-cell junction on A2058-CD93 cells. We also showed that CD93 interacted with ezrin directly by co-immunoprecipitation assay. Thus, CD93 protein might connect to actin cytoskeleton by ezrin and regulated cell adhesion. Furthermore, we demonstrated that CD93- and CD93(ΔC)-expressed cells possessed higher proliferation rate than A2058-GFP. The CD93 distribution and its interaction with ezrin were also confirmed on human umbilical vein endothelial cells. **Conclusions:** These results suggested that the expression of CD93 could enhance cell adhesion, which might be through the interaction of CD93 to F-actin by ezrin.