RalGAP, the Inactivator of Ral Small GTPase, Suppresses the Invasion and Metastasis of Prostate Cancer

Introduction and Objective: Recently Ral small GTPase, RalA and RalB, has been attracting increasing attention, because this protein is involved in tumorigenesis and cancer progression in several cancers. In prostate cancer, it is reported that Ral activation promotes the bone metastasis of prostate cancer cells. RalGAPs, which are protein complexes consisting of a catalytic α1 or α2 subunit and a common β subunit, inactivate Ral. Therefore we hypothesized that RalGAPs suppress tumor progression of prostate cancer.
Materials and Methods: Three human prostate cancer cell lines were used: PC3, DU145 and LNCaP. The expression of mRNA and protein were examined by quantitative RT-PCR and Western blotting, respectively. Ral activation level was evaluated by GST-sec5 pulldown assay. BD Matrigel Invasion chamber were used for invasion assay. The extent of metastasis was evaluated by bioluminescence imaging. All mice were maintained under the guidelines of the Animal Research Committee in the Kyoto University. Tissues of prostate cancer were obtained surgically under the informed consent of all patients.
Results: We first evaluated the expression of RalGAPs in prostate cancer cell lines. Among the three subunits of RalGAPs (α1, α2 and β), α2 expression was remarkably downregulated in PC3 and DU145 in which activation levels of RalA and RalB were higher than in LNCaP. Then we established subclones of PC3 cells that overexpressed α2 or mutant α2 (N1742K) lacking enzyme activity. Ral activation assay showed that the active form of RalA and RalB was reduced by the forced expression of α2, but not by N1742K. To evaluate the function of α2, we performed wound healing assay and invasion assay in the PC3 subclones. Expression of α2, but not of N1742K, suppressed cell migration and invasion of PC3 cells. We then carried out bone metastasis assay by injecting the PC3 cells into left ventricle. Expression of α2, but not of N1742K, suppressed bone metastasis formation of the PC3 cells. We further examined the expression of α2 in human prostate cancer tissues by immunohistochemistry. The staining intensity of α2 were significantly decreased in prostate cancer tissues than in normal ductal tissues ($p<0.001$, chi-square test).
Conclusions: These results suggest that RalGAPα2 suppresses the progression of prostate cancer.