Expression of Dominant Negative Form of Ets-1 Suppresses Fibronectin-stimulated Cell Adhesion and Migration Through Down-regulation of Integrin a5 Expression in U251 Glioma Cell Line.

D. Kita*, T. Takino, M. Nakada*, H. Miyamori, J. Yamashita* and H. Sato (*Dept. Neurosurgery, Med. Sch., Kanazawa Univ.)

Ets transcription factors are associated with tumor malignancy. We previously reported that the stable transfection of the dominant negative form of Ets-1 (Ets-DN) in the glioma cell line U251 induced down-regulation of urokinase-type plasminogen activator (uPA) mRNA expression and invasiveness (M. Nakada, et al., J Neuropathol Exp Neurol., 58: 329-34, 1999). Here we analyzed effects of Ets-DN-expression on cell adhesion, migration and phosphorylation of focal adhesion kinase (FAK) (1). U251 cells expressing Ets-DN (U251-DN) showed reduced cell adhesion, spreading and extension of actin stress fibers on dishes coated with fibronectin but not on dishes coated with collagen. Migration of U251-DN cells was found to be significantly inhibited compared to that of parental cells when examined by wound-induced migration assay on fibronectin-coated dishes. Phosphorylation levels of FAK in U251-DN cells were also attenuated on dishes coated with fibronectin. Reduced expression level of integrin5 subunit in U251-DN cells was demonstrated by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis. Furthermore, down-regulation of transcription from the integrin $\alpha 5$ promoter by expression of Ets-DN was shown by luciferase reporter assay. Semi-quantitative RT-PCR of surgical samples of brain tumors revealed that the expression level of Ets-1 mRNA correlated with that of integrin $\alpha 5$ mRNA in glioma. The experimental metastatic ability of U251-DN cells examined in chick embryo was considerably lower than that of parental cells. These results suggest that Ets-1 contributes to glioma malignancy by up-regulating expression of the integrin $\alpha 5$ subunit, which composes integrin $\alpha 5 \beta 1$, mediates intracellular signaling and the subsequent acceleration of the invasive process including cell adhesion and migration.

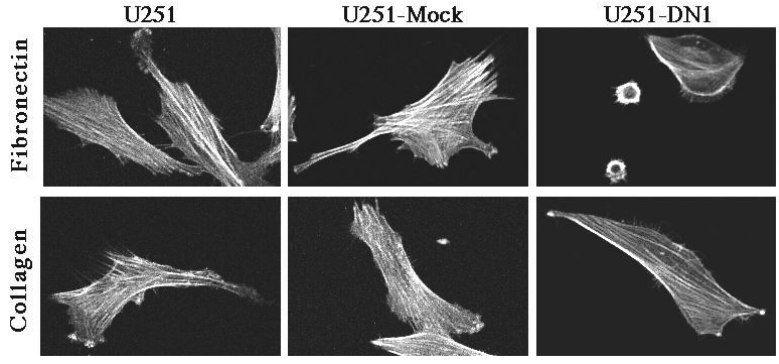

Figure 1. Cell adhesion and cytoskeleton formation. U251, U251-Mock or U251-DN cells were plated onto dishes coated with fibronectin (upper panels) or collagen (bottom panels) and incubated for 2 h at 37°C, and then stained with rhodamine-labeled phalloidin.

Reference: Kita, D., Takino, T., Nakada, M., Takahashi, T., Yamashita, J., and Sato, H. (2001) Expression of dominant negative form of Ets-1 suppresses fibronectin-stimulated cell adhesion and migration through down-regulation of integrin $\alpha 5$ expression in U251 glioma cell line. Cancer Res., 61, 7985-7991.