Introduction: The coordination of the steps involved in the invasion of normal tissue by tumor cells requires the regulation of a number of complex cytoplasmic processes: cell adhesion to the extracellular matrix (ECM), the matrix destruction, and cell migration. Among the cell adhesion molecules, the integrins is known to play a significant role in cell adhesion processes. The integrins are a family of type I transmembrane proteins composed of a large extracellular domain and a short cytoplasmic domain. Upon binding to extracellular ligands, integrins cluster on the plane of the plasma membrane and promote the assembly of molecular complexes containing both cytoskeletal and signaling elements. Although the integrin signaling pathways have not been completely characterized, many involve tyrosine kinase phosphorylation of neighboring cell and cytoplasmic domain proteins. The study of ECM-induced aggregation of integrin receptors at focal adhesion sites led to the identification of phosphorylated 125 kDa protein known as focal adhesion kinase (FAK). It has been characterized as a protein tyrosine kinase that demonstrates increased kinase activity and tyrosine phosphorylation in response to integrin activation. FAK has an important role in the tumor cell invasion and metastasis since FAK expression was correlated with more invasive human tumors, although it is not clear whether FAK expression in these tumors was a cause or a consequence of their increased invasiveness. There was no direct evidence to demonstrate a role of FAK in regulating tumor cell invasion and metastasis. The carboxy-terminal domain of FAK, an 41/43 KDa protein, is also expressed as a separate protein called FRNK (FAK-related non-kinase). It has been reported that expression of the FRNK promoted FAK dephosphorylation at Tyr-397 and potently blocked FAK-mediated cell migration in mouse fibroblast. So we examined whether FRNK played a role as an inhibitor of cell migration by blocking the tyrosine phosphorylation in two different chondrosarcoma cell lines. Through this experiment, we demonstrated that the expression of FAK correlated with tumor cell invasiveness.

Methods: Adenovirus transfection: Human chondrosarcoma cell lines, JJ102 and 105 KC, were infected with AdFAK-CD or AdLacZ as a negative control at an optimal concentration of virus for each cell line. The adenoviruses construct contains C-terminal domain of FAK (FAK-CD). We measured the FAK and FAK-CD expression using western blotting. Also tyrosine phosphorylation of FAK was checked by immunoprecipitation of FAK followed by western blot analysis with anti-phosphotyrosine antibody. Cell migration was measured using boyden chamber assay (Chemicon. CA) and wound healing assay. Cells were used 36 hours after transfection with the indicated constructs following overnight serum starvation (0.5% FBS). Quantitative Boyden chamber assay: Exponentially growing cells were plated on collagen type I coated-chambers to give 100 cells per 24-well culture plates in complete growth media. After 8 hours, the monolayer of cells were wounded by scratching with a pipet tip, washed with PBS, photographed (0 hour point) and measured the interval distances. Results: We observed that the inhibition of FAK phosphorylation through the transfection of FAK-CD. The level of FAK expression was not changed, but FAK-CD was expressed only in transfection group. The tyrosine phosphorylation of FAK was significantly inhibited by the FAK-CD. By using boyden chamber assay, we found that the cell migration was inhibited by transfection of FAK-CD in FAK-overexpressed chondrosarcoma cell lines (Figure 1). Cell migration was significantly inhibited by the FAK-CD transfection in both KC (75.5%) and JJ (79.9%) cell lines. It was supposed that the tumor cell invasiveness could be controlled by the modification of FAK phosphorylation. In addition, the same inhibitory effects were detected by wound healing assay (Figure 2). At 18 hours after the scratch, the KC cells showed migration into the cleared area whereas JJ cells had become dense along the wound edge at 9 hours. Significantly, the difference of wound closure was observed in KC cells at 30 hours. Also JJ cells showed the similar finding at 15 hours. This result was due to difference in the rate of proliferation between KC and JJ cells. To examine the proliferation for wound healing assay, cell counting and hexoaminidase assay was done at each time point. Between KC and JJ cells showed no significant difference in proliferation within 30 or 15 hours between AdLacZ and AdFAK-CD transfected groups. Eventually, we concluded that FAK-CD can inhibit cell migration through dephosphorylation of FAK.

Discussion: Several functions have proposed for FAK. FAK functions or requirements may well differ among different cell types. More generally, increased FAK expression has been correlated with invasive and metastatic potentials in human tumors, lending clinical significance to the elucidation of its function in malignant cells. As FAK is activated and undergoes autophosphorylation on tyrosine following integrin occupancy and cell spreading, we found that the migration through transfection of C-terminal domain of FAK was inhibited by inducing tyrosine dephosphorylation. It suggests that cell migration can be modified by the control of FAK phosphorylation through transfection of FAK-CD. We sought to elucidate the mechanism, by which the more aggressive chondrosarcoma cell lines are able to resist attachment inhibition, thereby exhibiting the ability to invade local tissue and metastasize. The FAK-CD can effectively reduce the tumor cell invasiveness through inhibiting the overexpressed FAK activity. Consequently, the FAK overexpression is directly related to the tumor cell invasiveness. Therefore, the FAK will be the new target for anticancer therapy.

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