**THE C-TERMINAL DOMAIN OF FOCAL ADHESION KINASE REDUCES TUMOR CELL INVASIVENESS IN CHONDROSARCOMA CELL LINES**

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**Introduction:** The coordination of the steps involved in the invasion of normal tissue by tumor cells requires the regulation of a number of complex cytoprotic processes which include the binding of specific cell-surface molecules on the plasma membrane to the extracellular matrix (ECM), the elaboration of targeted proteases to degrade ECM components. 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, integrin 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 protein species and cyclin dependent kinases. 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 because 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. So we proposed a hypothesis that FAK had a significant role in tumor cell invasiveness. Through this experiment, we demonstrated that the expression of FAK correlated with tumor cell invasiveness and the cell attachment to the extracellular matrix in chondrosarcoma cell lines.

**Methods:** The adenovirus containing C-terminal domain of FAK (FAC-CD) was constructed. Adenovirus carrying the LacZ gene which expresses β-galactosidase protein served as a control. We used bovine articular chondrocytes as a normal control and human chondrosarcoma cell lines – JJ012 and 105KC. Chondrocytes were isolated by enzymatic digestion. Chondrocytes were cultured in the monolayer for 7 days with DMEM/F-12 medium containing 10 % fetal bovine serum (FBS). Chondrosarcoma cell lines were cultured in monolayer with DMEM containing 10% FBS. Cells were plated at 1.5X10⁶ per 100 mm culture plates and allowed to attach for 24 hours, and then infected with AdFAK-CD or AdLacZ at an optimal concentration of virus for each cell line. We measured the FAK and FAK-CD expression using Western blot analysis. Analysis of tyrosine phosphorylation of FAK was accomplished by immunoprecipitation of FAK followed by Western blot analysis with anti-phosphotyrosine antibody. Proteins were visualized using the ECL detection system. For evaluating the change of FAK expression in malignant cells, we used the monoclonal antibody to integrin β₁ subtypes and cyclic RGD peptides, we were able to inhibit chondrosarcoma cell and chondrocyte attachment to extracellular type-II collagen. However, in the more aggressive chondrosarcoma cell line (JJ), integrin β₁ monoclonal antibody inhibited cell adhesion by only 68 %, while cyclic-RGD peptides exhibited no inhibitory effects. We therefore conclude that the overexpression of FAK increases the ability of malignant chondroid cells to adhere the ECM, leading to local tissue invasion and metastasis. The FAK-CD can effectively reduce the tumor cell invasiveness through inhibiting the overexpressed FAK activity. So the FAK will be the new target for anticancer therapy.

**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 potential in human tumors, lending clinical significance to the elucidation of its function in malignant cells. In these chondrosarcoma cell lines we used, the FAK was related to the cell attachment behavior and the degree of tumor cell differentiation. We were able to demonstrate increased expression of FAK in chondrosarcoma cell lines in comparison to that in chondrocytes. We believe that the increased expression of FAK in malignant cells may play a role in their increased ECM-adhesion characteristics. 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. Using the monoclonal antibody to integrin β₁ subtype and cyclic RGD peptides, we were able to inhibit chondrosarcoma cell and chondrocyte attachment to extracellular type-II collagen. However, in the more aggressive chondrosarcoma cell line (JJ), integrin β₁ monoclonal antibody inhibited cell adhesion by only 68 %, while cyclic-RGD peptides exhibited no inhibitory effects. We therefore conclude that the overexpression of FAK increases the ability of malignant chondroid cells to adhere the ECM, leading to local tissue invasion and metastasis. The FAK-CD can effectively reduce the tumor cell invasiveness through inhibiting the overexpressed FAK activity. So the FAK will be the new target for anticancer therapy.

**Results:** Using Western blot analysis, we observed increased expression of FAK by the chondrosarcoma cell lines (JJ and KC) in comparison to that by chondrocytes (Fig. 1). JJ cells were found to have the highest level of FAK expression. The degree of tumor cell differentiation was correlated with the level of FAK expression. Following the inhibition of FAK expression through the transfection of FAK-CD, the level of FAK expression was not changed. But, FAK-CD expressed only in transfection groups and tyrosine phosphorylation of FAK was significantly inhibited by the FAK-CD transfection. So we can effectively inhibit the overexpression of FAK through transfection of the inhibiting FAK expression, cell attachment tests were performed with or without treatment of integrin β₁ monoclonal antibody (ITGB1). Both chondrosarcoma cell lines and chondrocytes exhibited 95 % attachment inhibition following transfection and treatment with ITGB1. Without treatment with ITGB1, cell attachment to extracellular type-II collagen was inhibited in JJ cells only. The integrin β₁ and β₃ mRNA expression was not changed by transfection. The integrin α₂ mRNA expressions were slightly changed, but in KC cell, integrin α₂ mRNA expression was upregulated by transfection of FAK-CD. In the protein level, α₂ and α₅ integrin was not changed by transfection.

**Figure 1.** Results of Western and Immunoprecipitation. The overexpression of FAK can be successfully inhibited by transfection with FAK-CD.

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