PHOSPHORYLATION OF FOCAL ADHESION KINASE IS INVOLVED IN MOTILITY AND MORPHOLOGY OF HUMAN FIBROSARCOMA CELLS.

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Introduction
Tumor invasion consists of multiple and sequential steps including tumor cell adhesion, migration and degeneration of extracellular matrix. We have shown that with the treatments of cAMP analogues, human fibrosarcoma cells, HT1080 reduced the various capacities related to invasion, such as adhesion, motility, and typeIV collagenolytic activity.

Recently, it is reported that a non-receptor tyrosine kinase, Focal adhesion kinase (FAK) plays critical roles in the integrin-mediated signal transduction. FAK localizes to focal adhesion, and is involved in cell survival, cell cycle progression, and cell motility signals. Integrin engagement and a number of growth factors induce phosphorylation of FAK. FAK phosphorylation at Tyr397 (pY397-FAK) correlates with catalytic activity of FAK. The phosphorylation of FAK might be important for cell migration, since the expression of a protein-phosphatase PTEN caused dephosphorylation of FAK and inhibited cell motility. It is also reported that FAK deficient fibroblast migrated poorly in response to chemotactic signals.

We have found that pY397-FAK was decreased after treatment with 8-Br-cAMP in HT1080 cells. In this study, we investigated the biological function of FAK phosphorylation in HT1080 cells and surgical samples of malignant fibrous histiocytoma (MFH), the most common high-grade soft tissue sarcoma.

Methods
HT1080 cells were maintained in DMEM supplemented with10% FBS.

The C-terminal, non-catalytic domain of FAK, designated as FRNK (FAK-Related-Non-Kinase) is known to be a dominant-negative inhibitor of endogenous FAK function. A replication-defective adenovirus harboring FRNK and an empty vector (Adv-SR) were infected into HT1080 cells. Twenty four hours after the infection, the cell lysates were subjected to Western blot analysis, and expression of FAK and pY397-FAK were evaluated. For staining of actin filaments, the infected cells were seeded on cover-slips and cultured for 24h. The specimens were then treated with TRITC-labeled phalloidin and observed using a confocal microscopy. In vitro chemotaxis assay was carried out using type IV collagen-coated polycarbonate filters and boyden chambers.

Ten primary and 4 recurrent samples of surgically resected MFH were used in this study. Histological subtypes of primary MFH comprised 5 cases of storiform-pleomorphic type, 4 cases of myxoid type, 1 case of giant cell type. Histological subtypes of the 4 recurrent samples were identical to those of corresponding primary samples. Follow-up period ranged from 6 to 58 months (mean, 20.1months). Samples were subjected to Western blot analysis, and expression of FAK and pY397-FAK were evaluated. The levels of expression of pY397-FAK and total FAK were quantitatively estimated by densitometric scanning with NIH image 1.62.

All statistical analyses were carried out according to Student’s t test.

Results
Expression of FRNK protein by Adv-FRNK infection was confirmed by Western blot analysis. Although forced-expression of FRNK in HT1080 cells appeared to have no significant effect on endogenous FAK levels, pY397-FAK was reduced in a dose dependent manner. Adv-FRNK infection also inhibited the spreading of HT1080 cells, and the infected cells were rounded up. FBS-induced chemotaxis of HT1080 cells was remarkably reduced by the expression of FRNK (Fig.1).

In clinical samples, all surgical specimens expressed FAK and pY-397 FAK. Expression levels of pY397-FAK in primary tumors were not associated with the recurrence. However, the expression levels of pY397-FAK in recurrent tumors were stronger than those in corresponding primary tumors (n=4).

Discussion
We have previously reported that HT1080 cells treated with 8-Br-cAMP exhibited dephosphorylation of FAK and inhibition of cell motility. In the present study, direct inhibition of the phosphorylation of FAK at Tyr397 by Adv-FRNK caused reduced motility, impaired cell spreading and rounded morphology. These data suggest that the phosphorylation of FAK at Tyr397 might be critical in motility and morphology of HT1080 cells.

It is demonstrated that FAK expression is elevated in several cancers, such as breast, colon, and thyroid cancer. On the other hand, little is known about the FAK status in sarcomas. In this study, all clinical cases of MFH expressed FAK and pY397-FAK. Interestingly, pY397-FAK levels in the recurrent MFH samples were elevated compared with those in the corresponding primary samples. These results suggest that FAK phosphorylation might be correlated with the recurrences of MFH.

![Fig.1. Effects of infection with Adv-SR (vector) or Adv-FRNK on migration of HT1080 cells.](image-url)