ROLE OF TUMOR NECROSIS FACTOR ALPHA, INTERLEUKIN 8 AND DEXAMETHASONE IN THE FAK EXPRESSION BY HUMAN NUCLEUS PULPOSUS CELLS

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Introduction: Focal adhesion kinase (FAK) is a non-receptor type protein tyrosine kinase that might be involved in a wide variety of physiological and pathological processes which includes tissue formation, cell migration and tumor metastases. Although many studies have shown the various roles of FAK in nerve cell lines and others, none of the studies have defined the role of FAK in human nucleus pulposus cells. We examined whether inflammatory cytokines (TNF-α and IL-8) can activate FAK expression in human nucleus pulposus cells, as they may cause cell-to-cell disassembly in the nucleus pulposus and they might play a role in the disc degeneration. We also studied whether steroids, which are a commonly prescribed medication for discogenic pain, have an influence on the FAK expression induced by inflammatory cytokines in human nucleus pulposus cells.

Methods: Human nucleus pulposus cells were obtained from nine patients who underwent discectomy for lumbar intervertebral disc prolapse. And the disc material was digested with collagenase. After enzymatic digestion, the suspension was filtered through a 70 µm mesh filter and washed with Dulbecco’s modified Eagle’s medium (DMEM) and a primary culture of these cells was started. Isolated nucleus pulposus cells were cultured in DMEM that was supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 U/mL streptomycin at 37 °C in 5% CO2. The cultured nucleus pulposus cells were examined three times in succession to assess the effect of cytokines (TNF-α and IL-8) and steroids (dexamethasone, DEX) on the FAK expression by these cells. Then, Equal numbers of nucleus pulposus cells were either left untreated in serum free media (SFM) or they were treated with TNF-α, IL-8 and DEX for one day at 37 °C in 5% CO2. After incubation, the cells were washed. To obtain the cellular lysate, the cells were lysed on ice for thirty minutes in RIPA buffer that contained a protease inhibitor mixture. Fifty micrograms of the supernatant were separated with the use of 10% polyacrylamide gel that contained 10% sodium dodecyl sulphate (SDS). 15 M Tris-HCl, 0.035% N, N, N', N'-tetramethylenediamine and 7 mg ammonium persulphate. The separated proteins were transferred to a nitrocellulose membrane at 36 mA in a transfer buffer that contained 39 mM glycine, 48 mM Tris base, 0.037% SDS and 20% methanol. The membranes were sequentially incubated with anti-FAK mouse monoclonal antibody (mAb) or phospho-specific FAK (pY397) mAb at 1:1000 dilutions. Horseradish peroxidase-conjugated anti-mouse immunoglobulin was used as a secondary antibody at 1:1500 dilutions. The detections were performed using electrochemiluminescence detection reagent. In some cases, the Western blots were stripped and re-blotted with mAb according to the manufacturer’s instruction. And immunofluorescence analysis of the cultured cells was done too.

Results: Following stimulation of the human nucleus pulposus cells with TNF-α, IL-8 and Dexamethasone, the FAK expression intensity was increased with TNF-α and IL-8 treatment as compared to treatment with serum free media (SFM) and Dexamethasone. When comparing TNF-α and IL-8, the intensity of the FAK expression was found to be more significantly increased with IL-8 treatment than with using TNF-α treatment (P < 0.01) (Figure 1A). We further investigated whether there was FAK activation by using phospho-specific FAK (pY397) mAb, the activated phospho-FAK was faintly detected following stimulation with TNF-α; however, the level of activated phospho-FAK was significantly increased following stimulation with IL-8 (P < 0.01) (Figure 1B). TNF-α and IL-8 were found to induce an activated FAK expression in human nucleus pulposus cells. Then, we studied the FAK expression levels by performing Western blot analysis after treating the human nucleus pulposus cells with varying concentrations of TNF-α and IL-8. Treatment with TNF-α at 0.5, 1 and 2 µg/ml for 24 hours showed only slight increases in the FAK expression by 103 ± 11%, 107 ± 23% and 125 ± 18%, respectively, compared to the control (0 µg/ml). Meanwhile, IL-8 treatment at 0.5, 1 and 2 µg/ml for 24 hours showed a significant increase in the FAK expression levels by 136 ± 18%, 150 ± 20% and 178 ± 43%, respectively, relative to the control (0 µg/ml). These results suggest that IL-8 produces greater FAK activation in human nucleus pulposus cells, as compared to TNF-α, in a dose dependent manner and this was found to be more statistically significant (P< 0.01). In western blotting and immunofluorescence to evaluate the effect of Dexamethasone co-stimulation with TNF-α, it was found that the FAK expression levels in cells that were treated with Dexamethasone were similar to that of the cells treated in serum free media (SFM).

Conclusion: The activated FAK expression by the degenerated human nucleus pulposus cells was found to be positively correlated with the levels of TNF-α and IL-8. The FAK expression in response to inflammatory cytokines may act during the initial stages of disc degeneration by signaling cell-to-cell disassembly in the nucleus pulposus cells. FAK might be considered as a target for therapeutic intervention to prevent disc degeneration, although further studies are needed to confirm this hypothesis. The current study also reveals that Dexamethasone is capable of decreasing FAK expression by the human nucleus pulposus cells. Similar studies that would study the response of nucleus pulposus cells to cytokines may help us achieve a better understanding about the stepwise progression of disc degeneration.

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