The small proteoglycan biglycan regulates growth factor/cytokine–induced signaling in annulus fibrosus and nucleus pulposus cells of the intervertebral disc

Youwen Deng1, David Gerard1, Howard S. An2, Gabriella Cs-Szabo1,2
1Biochemistry, Rush University Medical Center, Chicago, IL; 2Orthopedic Surgery, Rush University Medical Center, Chicago, IL
gcassabo@rush.edu

Introduction: Intervertebral disc (IVD) cells produce a variety of extracellular matrix (ECM) molecules, including fibril-forming collagens, large proteoglycans (PGs), such as aggrecan, and small PGs, such as biglycan. Biglycan plays a regulatory role in the assembly of the ECM by binding to different matrix molecules, including fibrillar collagens, fibronectin and growth factors. We previously showed that biglycan expression increased in degenerated human discs [1], and also showed an age-related increase in normal IVDs [2]. The majority of biglycan in degenerated and aged disc tissue accumulated in close proximity to the cells [2-3] and was shown to down-regulate gene expression levels of aggrecan and collagen by binding to human disc cells through the EGF receptor [4]. Since in degenerated IVD, not only biglycan but cytokine and growth factor levels are elevated, it is important to determine whether biglycan can influence the action of these factors and thus, the repair process. For this purpose, bovine IVD cells from the annulus fibrosus (AF) and nucleus pulposus (NP) were cultured separately in confluent monolayer and the binding characteristics of biglycan to the cells were assessed. The action of biglycan on the epidermal growth factor-, osteogenic protein-1 (OP-1) - and interleukin-1 (IL-1)-induced signaling was determined.

Materials and Methods: Ten bovine tails were purchased from the local slaughterhouse. The IVDs were aseptically dissected and the AF and NP were separated. Cells were released by sequential enzymatic digestion and were used freshly or after a maximum of one passage in culture.

Receptor binding: Biglycan (Sigma) was labeled with Rhodamine-red (RR) according to the manufacturer’s instructions (Molecular Probes). Cells were cultured overnight in chamber slides; and then were subjected to RR-labeled biglycan at different concentrations (20-80 μM) for 30 min to 3 hr at 37°C. Competition assays were performed using RR-labeled biglycan (20 μM) in competition with non-labeled biglycan (20-80 μM); EGF receptor blocking antibody (Sigma; 20-80 μM) and EGF (20-80 μM) for 3 hr at 37°C. The amount of fluorescent label was observed under a fluorescent microscope (Nikon Eclipse E600) equipped with Metamorf software.

For signaling studies, IVD cells were cultured in monolayer and treated with different concentrations of biglycan (5-40 μM); between 5 minutes and 2 hours. Cells were also treated with EGF, IL-1 or OP-1 and inhibitors of several signaling pathways (Calbiochem) in the presence or absence of biglycan. Cells were lysed and signaling molecules were detected using specific antibodies (Cell Signaling) on Western blots. These results were confirmed in cells that were encapsulated in alginate beads.

Results: Both AF and NP cells were able to bind biglycan in a concentration- and time-dependent manner. Non-labeled biglycan demonstrated a definite ability to compete with the RR-biglycan in binding to both cell types by completely abolishing the binding of the RR-biglycan at the 80 μM concentration. Competition of biglycan with EGF and the EGF receptor blocking antibody led to almost complete abolishment of the fluorescent signal. This proves that the majority of biglycan binds to these cells through the EGF receptor.

In both AF and NP cells, biglycan, like EGF, was found to be signaling through the ERK (MAPK) and the Akt (PI3K) pathways. The biglycan signal was weaker for the ERK pathway and stronger for the PI3K pathway than in the case of EGF. Signals were inhibited by pathway-specific inhibitors. OP-1 slightly, while IL-1 significantly activated the ERK pathways. Combined treatments with biglycan and growth factor/cytokine resulted in an inhibition of the ERK pathway. The inhibition was more pronounced in each case with increasing biglycan concentration. The least reduction was found in case of EGF+Biglycan treatment, while OP-1 abrogated biglycan induced ERK signaling completely. Very strong inhibition of the ERK pathway as well as the NFkB pathway was found when cells were treated with biglycan and IL-1.

Discussion: In this study, we demonstrated that biglycan can bind to the cell surface of both AF and NP cells through the EGF receptor, activating the ERK and Akt pathways. The ERK pathway was also activated by EGF and slightly by OP-1. In addition to the NFkB pathway, the ERK pathway was also strongly activated by IL-1. While EGF and OP-1 promote matrix synthesis, therefore contribute to the cell’s ability to repair, IL-1 is believed to play a critical role in anti-anabolic and catabolic processes in tissue degeneration. Our results showed that biglycan interfered with both EGF and OP-1 signaling, through which, biglycan may regulate the effects of these growth factors. By reducing the signal in both the ERK and NFkB pathways activated by IL-1, biglycan may play a beneficial role in IVD degeneration counteracting the effect of this cytokine. The mechanism of action for biglycan may partially be explained by our previous findings that biglycan can bind and sequester growth factors as well as interfere with receptor expression and/or internalization [4,5].

When biglycan is present in the degenerated IVD in high levels, this regulatory role of biglycan may be important in restoring balance in metabolism. Due to this function, biglycan could be beneficial for the health and repair of IVD.


Acknowledgements: This work was supported by the NIH (PO1AR-48152)