THE ROLE OF THE INDIAN HEDGEHOG AND FIBROBLAST GROWTH FACTOR RECEPTOR 3 SIGNALING PATHWAYS IN CHONDROSARCOMA

Oji GS, Stevens J, Dolan L, Bonaldo F, Soares B, Morcuende J.
University of Iowa, Iowa City, IA
Joose-morcunde@uiowa.edu

Introduction
Survival rate for grade III chondrosarcoma is low and most deaths are caused by metastasis. Unfortunately, chondrosarcoma respond poorly to chemotherapy and radiotherapy. Thus a novel agent that will effectively treat patients systemically will improve prognosis. Recent investigations have focused on the Indian Hedgehog (Ihh)– Parathyroid related protein (PTHrP), & Fibroblast Growth Factor Receptor 3 (FGFR3) signaling pathways because of their regulatory role in endochondral bone formation. In the growth plate cartilage, Ihh and PTHrP are involved in a feedback loop to increase proliferation and delay differentiation of chondrocytes. Fibroblast Growth Factor Receptor 3 (FGFR3) conversely decreases proliferation and hastens differentiation with an agonist. Currently it is thought that FGFR2 is the agonist for FGFR3. We sought to investigate the role of these signaling pathways in the rat chondrosarcoma (RCS) cells utilizing expression and functional studies

Material and Methods

Isolation of total RNA and Semiquantitative reverse-transcription polymerase chain reaction
Sprague-Dawley rats were obtained from Harlan (Indiana, U.S.) and killed between 2-5 weeks of age. The cartilage from the head of the femur and proximal tibia was obtained. Tumor cells used was from a strain of RCS rats received from Indiana University, Indiana, US. Different cycles were used to determine optimal condition for analysis and were all in the linear range of the polymerase chain reaction. B-actin was used as the internal control. We compared the intensity of the bands on 2% agarose gel for each primer set

Immunohistochemistry
Standard peroxidase-labelled streptavidin-biotin detection method was used. Rat tissues with implanted chondrosarcoma were observed for 10 to 14 days to allow growth of tumor into the marrow. Sections were incubated overnight at 4°C in a humid chamber with appropriate dilution of primary antibodies (FGFR3, 1:200, Santa Cruz Biotechnology, Inc., California, US; and Ptc-1receptors, 1:500, Orbigen Inc., California, US). Next day, incubation was done in biotinylated goat anti-rabbit (Sigma, Missouri, US) diluted 800 in blocking solution. Negative control slides were incubated without primary antibodies. The conditions of immunohistochemical staining were evaluated using a modified Mankin scoring system adopted from Xu et al. 2003. Statistical significance was calculated using the Repeated Measure Analysis of Variance.

Cell Culture and Cell Count
The rat chondrosarcoma cell line LTC (King KB and Kimura JH) was cultured in high glucose Dulbecco’s Modified Eagle medium (DMEM) supplemented with fetal bovine serum (10%), and gentamycin (50µg/ml) and grown in humidified air with 5% CO2 at 37°C. Chondrocytes were obtained from femoral cap cartilage of 2 week old rats. For testing the effect of agonists/antagonists on cell proliferation, LTCs or chondrocyte cells were plated in 24-well plates (100,000 - 150,000 cells/well) in a total volume of 1 ml of culture medium per well. For studies utilizing FGFR2, a serum-free medium containing DMEM, 1% HEPES (25mM), and 100X ITS (1x, Sigma, MO, US), BSA (1%, Amresco, OH, US), and gentamycin (50µg/ml) was used. Afterwards, variable amounts of agents were added to six replicate wells and incubated for a total of 48 hours. The agents used were FGFR2 (PepTech Inc., NJ, US), Cyclopamine (BIOMOL Research Laboratories Inc., PA, US), FGFR3 (Santa Cruz Biotechnology, Inc. California US). The cells were counted with a hemocytometer. For cell viability, the trypan blue exclusion assay (0.4%) was used. A mean and standard deviation of cell number per well was calculated for each group and compared for analysis.

Discussion
These results suggest that RCS cells are responding correctly to uncontrolled proliferation by up-regulating the FGFR3 signaling pathway to decelerate proliferation and initiate differentiation. However, the process is not working effectively. This could be due to abnormality in the quality or quantity of endogenous FGF2 or a defect in the FGFR3 receptor and its signaling pathway, or a combination of both. There also seems to be a deregulation of the Indian hedgehog pathway in chondrosarcoma since addition of cyclopamine effectively induced stasis of proliferation in GPC cells but ineffectively in RCS cells. Further investigations are needed to determine the exact mechanism of action. Preliminary data suggest that agents that specifically target these pathways may have some value as novel approaches for the treatment of this disease.

References