Diagnostic and therapeutic implications of the oncogene LRF in chondrosarcoma

Yik, JHN; Kumari, R; Li, H; Carlson, CS; Haudenschild, DR; and Di Cesare, PE

1Department of Orthopaedic Surgery, UC Davis Medical Center, Sacramento, CA
2Department of Veterinary and Population Medicine, University of Minnesota, St. Paul, MN

e-mail: paul.dicesare@ucdmc.ucdavis.edu

Introduction
Chondrosarcoma is the third most common primary bone cancer of cartilaginous origin. Since chondrosarcoma is highly resistant to chemotherapy and radiotherapy, prognosis is poor for unresectable and metastatic diseases. Therefore, it is necessary to search for new targets for therapeutic intervention. The Leukemia/Lymphoma-Related Factor (LRF) is an oncogene that suppresses p53 expression and is aberrantly expressed in human cancers (Maeda et al., 2005). We have previously shown that LRF stimulates proliferation of bone marrow-derived stem cells and prevents their premature chondrogenic differentiation (Liu et al., 2004). In this study we explored LRF’s potential as a diagnostic and therapeutic marker for chondrosarcoma, by correlating its expression with tumor malignancy, and examining the effects of LRF depletion on chondrosarcoma cell growth in vitro.

Materials and Methods:
Human chondrosarcoma tissue microarray was obtained from Cybridi. FS090 chondrosarcoma cell line was a kind gift from Dr. Joel A. Block (Rush University, Illinois). The pKLO lentiviral-based expression system was used to knockdown LRF expression, with siRNA against EGFP as negative control. Antibodies against LRF, p53, and p21 were from Santa Cruz Biotechnology. WST reagent and senescence detection kits were from Biovision.

Results and Discussion:

Elevated LRF expression is associated with advanced grade chondrosarcoma. We first examined LRF expression by immunohistochemical staining in human chondrosarcoma tissue microarrays. Nuclear staining of LRF was readily detectable in chondrosarcoma of various grades (representative images are shown in Fig. 1), but not in benign enchondroma (not shown). Manual counting revealed that the percentage of LRF positive cells was highest in grade III chondrosarcoma (53.2 +/- 4.7%; mean +/- SEM) and lower in grade II (22.7 +/- 8.5%) and grade I (12.9 +/- 3.5%) tumors (Fig. 2). Thus, LRF expression correlated with the malignancy of chondrosarcoma.

Fig. 1 LRF expression is detected in various grades of chondrosarcoma.

LRF depletion decreases proliferation of chondrosarcoma cells. To investigate the role of LRF in chondrosarcoma growth, LRF expression was depleted in FS090 chondrosarcoma cell line through lentiviral transduction of siRNA (Fig. 3 inset). The knockdown of LRF suppressed proliferation of FS090 cells as reflected by the decrease in mitochondrial dehydrogenase activity in WST assays (Fig. 3, top panel), and the reduced abilities of these cells to form colonies after 2 weeks (lower panel), indicating that LRF is essential for proliferation.

Fig. 2 Tumor malignancy is associated with LRF expression.

LRF knockdown increases sensitivity of chondrosarcoma to doxorubicin. Finally, we tested whether high LRF expression contributed to the enhanced chemo-resistance in chondrosarcoma. The sensitivity of FS090 cells to doxorubicin, a common chemotherapy against sarcoma, was determined by WST assays and expressed as percent survived cells (Fig. 4). We also examined senescence in FS090 cells by double staining with LRF antibody and β-galactosidase. The results showed that effective LRF-depletion by siRNA (compared to the intense brown nuclear staining in wildtype and EGFP siRNA control cells) induced β-galactosidase expression (blue staining) that is indicative of senescence (Fig. 5). Moreover, in LRF-depleted cells, there was elevation of p21 and p53 (Fig. 6), which are known to promote cell cycle arrest and thus could be the mechanism by which LRF controls cell growth.

Fig. 4 LRF depletion leads to G1 cell cycle arrest.

LRF depletion induces cell cycle arrest and senescence. We next investigated the mechanism by which LRF inhibits cellular proliferation. FACS analysis revealed that LRF depletion induced G1 cell cycle arrest (Fig. 4). We also examined senescence in FS090 cells by double staining with LRF antibody and β-galactosidase. The results showed that effective LRF-depletion by siRNA (compared to the intense brown nuclear staining in wildtype and EGFP siRNA control cells) induced β-galactosidase expression (blue staining) that is indicative of senescence (Fig. 5). Moreover, in LRF-depleted cells, there was elevation of p21 and p53 (Fig. 6), which are known to promote cell cycle arrest and thus could be the mechanism by which LRF controls cell growth.

Fig. 5 LRF depletion induces β-galactosidase expression & senescence.

LRF knockdown increases sensitivity of chondrosarcoma to doxorubicin. Finally, we tested whether high LRF expression contributed to the enhanced chemo-resistance in chondrosarcoma. The sensitivity of FS090 cells to doxorubicin, a common chemotherapy against sarcoma, was determined by WST assays and expressed as percent survived cells (Fig. 7). The results showed that in LRF-depleted cells, there was an increase in doxorubicin sensitivity at lower dosages (0.02 and 0.13 μM) but not at IC50 (0.67 μM), indicating that targeting LRF could potentially enhance chondrosarcoma response to chemotherapy. It is important to note that all of the above results were reproducible in another chondrosarcoma cell line SW1353 (not shown), indicating that our observations are not cell line specific.

Conclusion and Significance
Our data show that expression of the oncogene LRF correlates with chondrosarcoma malignancy, and that LRF is indispensable for chondrosarcoma cell growth in vitro. Thus, LRF has the potential to be a diagnostic marker and therapeutic target for chondrosarcoma.

References: