2.3 Presentations, Abstracts or Publications to Date

Best Basic Science Presentation: AO Spine Fellows Forum, Banff, Alberta, March 14-16 2013:
“Canine Notochordal Cell-Secreted Factors Protect Murine and Human Nucleus Pulposus Cells From
Apoptosis by Inhibition of Activated Caspases -9, and -3/7”

(see abstract attached, appendix 3).

2.4 Has the funding of this project led to the receipt of other funds?
Not to date.

2.5 Abstract

Introduction: A minimally invasive method through which nucleus pulposus (NP) cell
viability and function could be maintained or even enhanced would revolutionize the
treatment of degenerative disc disease (DDD). Here we have investigated the effects of
nonchondrodystrophic (NCD) canine intervertebral disc (IVD)-derived notochordal cell
conditioned medium (NCCM) and chondrodystrophic (CD) canine IVD-derived
conditioned medium (CDCM) upon human NP cells under pro-death and degeneration
conditions.

Materials and Methods: We developed NCCM and CDCM from hypoxic culture of
freshly isolated NPs from NCD and CD canines, respectively. We obtained human NP
cells from 30 patients undergoing spinal surgery for discectomy and/or spinal fusion. 21
of the 30 donor tissues produced cells that expanded well in culture and these cells
were cultured with ADMEM/F-12 (control media), NCCM, or CDCM under hypoxic
conditions (3.5% O2) and treated with IL-1β+FasL or Etoposide (all supplemented with
2% fetal bovine serum). We determined the levels of activated caspase-8, caspase-9,
and caspase-3/7 activity in all donors and then using array-based gene expression
methods determined changes in extracellular matrix and apoptosis-related genes of
donors in which NCCM suppressed activated caspase 3/7 activity as compared to those
that did not. Cytokine ELISA assays were performed on responders vs non-responders
and then using Western blots we, probed for the expression of proteins as defined by
our genomic expression arrays (X-linked inhibitor of apoptosis proteins or XIAP).

**Results:** In untreated cells, NCCM suppresses activation of caspases -8, -9 and -3/7 however; CDCM does not. In cells co-treated with the powerful chemotherapeutic drug ‘Etoposide’ NCCM alone is capable of decreasing the expression of activated caspase-3/7. In cells co-treated with NCCM and etoposide, apoptosis is mediated by upregulation of of the powerful apoptosis inhibitor ‘XIAP’ and the pro-survival factor ‘Rab25’. NCCM also upregulates a number of important extracellular matrix molecules including collagens and TGFβ1 and down regulates a number of matrix metalloproteinases. Furthermore, responder cells secreted low levels of IL-6 into the tissue culture medium but elevated levels of IL-8 whereas non-responder cells secreted elevated levels of both of these cytokines. NCCM also confers increased cell viability at 24 hours when co-treated with etoposide as compared with etoposide alone.

**Conclusions:** NCCM obtained from the notochordal cell-rich NCD disc in contrast to CDCM from relatively notochordal cell-deficient IVDs, is able to suppress apoptosis of human NP cells in a caspase-dependent fashion when otherwise untreated. However, in the presence of the powerful death inducing chemotherapeutic drug etoposide, NCCM, but not CDCM suppresses cell death via inhibition of activation of caspase -3/-7. Furthermore, in the presence of etoposide NCCM induces an increased expression of the powerful inhibitor of apoptosis XIAP plus the pro-survival factor Rab25 and increased ECM protein expression plus that of TGFβ1. NCCM-responsive NP cells exhibit elevated levels of secreted IL-8 but low levels of IL-6 whereas non-responders secrete elevated levels of both cytokines suggesting a possible biochemical profile for NP cells that may be suitable for biologic therapy. The components of NCCM remain incompletely identified, however here we identify the mechanisms whereby NCCM suppresses cell death and rescues the expression of ECM genes suggesting that the essential components of factors secreted by notochordal cells could lead to a novel cellular and molecular strategy for the treatment of DDD.

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