Culture with live annulus cells, and culture with annulus cell conditioned media, significantly increase neurite growth

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INTRODUCTION:

The relationship between neurotrophins produced by human annulus cells and nerve ingrowth into the disc is poorly understood but highly relevant to low back pain. Neurotrophin (NT)-4 and brain-derived neurotrophic factor (BDNF) are signaling molecules active in neurite survival and outgrowth. Here we tested neurite growth using control media, media + NGF (nerve growth factor), media conditioned with previous growth of annulus cells, and during culture with live annulus cells. Our objective was to perform functional, cell-based kinetic assays of neurite dynamics which will help provide insight into whether disc cells play a direct role in signaling repulsion or ingrowth of nerve cells into the disc. We hypothesize that instead of being passive bystanders, annulus cells actively participate in signaling nerve cells into the disc.

METHODS:

IRB-approved study using cultured human annulus cells. Study design utilized annulus cells cultured from 3 normal Thompson grade I discs (Cooperative Human Tissue Network), 1 surgical grade I disc, 4 grade II, 3 grade III, 10 grade IV and 1 grade V discs. F11 nerve cells (a hybridoma from mouse neuroblastoma cells and rat DRG neurons, available from Sigma-Aldrich) were utilized. Prior to use, F11 nerve cells (1) underwent differentiation in 10 μM forskolin (Sigma) and were used immediately after this in experimental tests. Cell co-culture 4-day studies were performed using a cell well-membrane insert system (Corning Transwell polycarbonate membrane cell culture inserts for 24-well plates, 8.0 mm pore (Sigma)). F11 cells were always cultured in monolayer on top of the insert porous membrane in Dulbecco's Modified Eagle Medium (DMEM1) with 1% fetal bovine serum (FBS). Human annulus cells were always cultured in Minimal Essential Media with 20% FBS (MEM20) on the plastic well bottom. NGF (nerve growth factor, 100 ng/ml) added to the MEM20 in the larger lower well served as a positive neurotrophin control; no annulus cells were present in this NGF control. For tests using conditioned media (CM, media previously conditioned by culture of human annulus cells for 4 days), CM only was present in the larger well; no live annulus cells were present in CM tests. Membranes onto which neurites migrated were harvested and stained with modified Giemsa so that neurites could be visualized and measured using OsteoMeasure software (OsteoMetrics, Inc); both the longest extent of a neurite, and lengths of branches were measured and results averaged following the methods of Lie et al (3). Standard statistical methods were utilized with GraphPad Instat 3 (GraphPad Software, Inc., San Diego, CA); means $\pm$ S.D., ANOVA, and correlation coefficients were calculated; $p \geq 0.05$ was considered significant.

RESULTS:

Figure 1 illustrates images of neurite migration under control (Fig.1A) and NGF-treated (Fig.1B) conditions . Representative neurite outgrowth following co-culture with Thompson grade IV annulus cells (Fig. 1C) and following exposure to CM from a grade II annulus culture (Fig. 1D) show much greater outgrowth. Mean neurite lengths are shown in Figure 1E presenting data from control, NGF, co-culture with annulus cells or exposure to CM (*, $p<0.05$; ** $p<0.01$, and *** $p<0.0001$ vs Control). Following exposure to CM, a significant positive correlation was present between BDNF (brain-derived neurotrophic factor, pg/ml) and mean neurite length ($p = 0.047$, $r^2 = 0.45$; Figure 2A). Following exposure to co-culture with live annulus cells, a significant positive correlation was present between NT4 (neurotrophin-4, pg/ml) and neurite length ($p = 0.0005$; $r^2 = 0.69$; Figure 2B).

DISCUSSION:

F11 cells are well validated in the literature (2) for utility in studies of neural regeneration, as a model of peripheral sensory neuron growth for tissue scaffold design, and as a nociceptor model for examining regeneration-associated gene upregulation. The F11 cell line is a homogenous population, which exhibits markers and receptors of nociceptive sensory neuron linage, and produces electoral responses to nociceptive stimuli. F11 cells retain many features of native DRG neurons, including neurotransmitter synthesis, neuropeptide receptor expression and voltage-gated calcium channels. Both NT4 and BDNF are known to be produced by disc cells. Findings presented here show the dramatic increase in neurite lengths which result following F11 exposure to either CM or co-culture with live disc cells. In the analysis of the correlation of BDNF levels in CM with neurite length, the r^2 value suggests that 45% of the increase in length is related to BDNF. In addition, in the analysis of the correlation of NT4 levels during co-culture and neurite length, the r^2 value suggests that 69.9% of the increases in neurite length is related to NT4.

SIGNIFICANCE:

This novel research has direct clinical relevance because it addresses the primary clinical issue with disc degeneration, which is low back pain. These dynamic cell-based findings indicate that NT-4 and BDNF are key neurotrophins which play a critical role in disc-related neurite ingrowth in vitro. Results highlight the significant role of annulus cells in vitro in promoting nerve ingrowth as reflected by the increased neurite lengths when F11 cells were cultured with either annulus cell conditioned media or with live annulus cells. Results have implications for pain and nerve ingrowth in vivo into the outer annulus (where disc cell numbers are high) and nerve ingrowth into annular tears.

REFERENCES: 1) Wieringa P et al. Nanotechnology 2012; 23:275102. 2) Jow F et al. ASSAY and Drug Development Technol. 2006; 4:49-56. 3) Lie M et al. Neuroscience 2010; 169:855-862.

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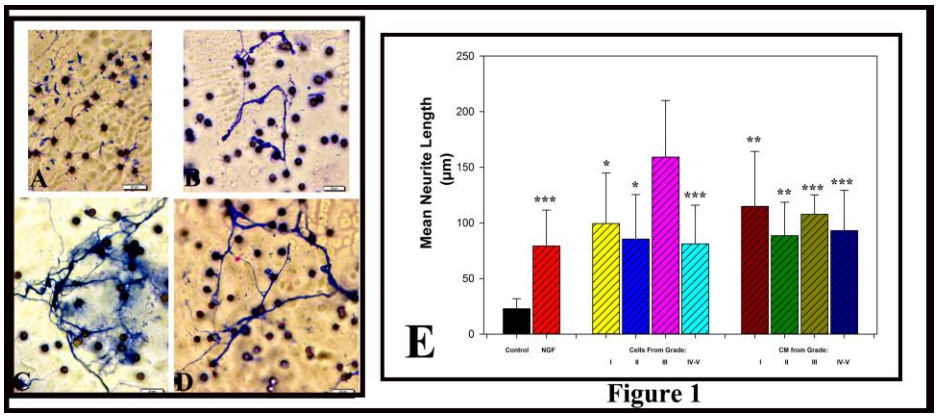

Figure 1

Figure 2