Introduction: Immature nucleus pulposus (NP) cells of rat and porcine intervertebral disc have cells of both notochordal-like and chondrocytic morphology [2]. In contrast, NP cells are nearly chondrocyte-like in juvenile human tissue and eventually become fibroblast-like in mature or aged NP tissue [10]. A limitation in understanding how human NP cells lose their notochordal-like morphology during aging is the absence of molecular features that can be used to distinguish human immature NP cell phenotypes. In this study, we propose that specific laminin subunits, and their receptors and binding proteins, are distinguishing features of these immature NP cells in the human. Laminins are composed of α, β, γ chains that together form at least 15 different laminin isoforms [4]. Laminins mediate many biological functions, including cell adhesion, proliferation and survival through binding to multiple integrins and non-integrin receptors (e.g., Lu, CD239), and other proteins (i.e., tetraspanin, CD151) [1,7,8]. The immature rat and porcine disc have been previously shown to express the laminin γ1 chain that is common to most laminin isoforms [5,6]. The α5 laminin chain and laminin receptors (e.g., integrin α6 and CD239) have also been identified in the NP, but not anulus fibrous (AF) regions of the immature rat and porcine disc [3,5,9]. The objective of this study was to evaluate expression of relevant laminin subunits and their binding proteins in human immature disc cells towards the goal of finding unique molecular markers for the immature NP.

Materials and Methods: Human lumbar disc samples (non-degenerate only) were obtained as discarded surgical tissues from pediatric patients (2-15 years) undergoing procedures for treatment of scoliosis. Tissues were prepared for cryosectioning, and also cells were isolated from AF and NP regions and cultured for 2-4 days. After fixation, both sections and cells were blocked and immunostained with antibodies to human laminin α5, α1 and γ1 chain (Santa Cruz), integrin subunits α3, α6, α1, β4 (BD), a laminin-related tetraspanin (CD151, Santa Cruz) and Lutheran blood glycoprotein (Lu, CD239, Serotec). All sections were counterstained with propidium iodide to label cell nuclei. Specimens were imaged using confocal microscopy with consistent settings to allow for comparison between samples.

Results: Region-specific expression of laminin subunits in the disc tissue and isolated cells. More intense staining for laminin α5 and γ1 chain was observed in the immature and young NP region as compared to AF (Fig.1, top). However, the laminin α1 chain was not detected in any NP or AF tissues (data not shown). Laminin 10 (LN10 or Lam 5-1-1) and laminin 11 (LN11 or Lam 5-2-1) are both formed from the α5 and γ1 subunits, while Laminin 1 (LN1, Lam 1-1-1) and Laminin 3 (LN3, Lam 1-2-1) are formed from the α1 and γ1 subunits. These findings suggest that LN10/11 may be the major laminin isoforms present in the NP region of immature human tissues. These results are consistent with our previous finding in rat and porcine NP tissues [3]. There was an evidence of intense cell-associated expression of the laminin α5 and γ1 chain in the isolated young NP cells (Fig.1, bottom), consistent with a higher tissue-level expression for this subunit in immature and young human NP tissues.

Discussion: New findings for the unique expression of the laminin α5 chain, laminin receptors (integrin α6, CD239) and related proteins (CD151) in NP tissues and cells of immature and young human discs suggest that these proteins may be useful to distinguish immature cells and to phenotype regenerated disc matrix. Future work will address the biological functions of these laminins and binding proteins in the immature NP.


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