Matrix remodeling during intervertebral disc growth and degeneration implicated by FAST staining

INTRODUCTION

Various imaging techniques have been widely used to study intervertebral disc (IVD) biology, among which histological methods are most common as they are indispensable tools to retrieve structural information. Stainings using Alcian blue-fast red and safranin-O-fast green are popular but they have limitations in delineating the complex structures of the IVD comparing to their use for articular cartilage. Triple dye methods, including picrosirius red-alcian blue-hematoxylin and Masson’s trichrome have been recently used with some success. As proteoglycan is a major component of the IVD, we hypothesize that a synergistic staining with dyes suggestive of glycoprotein interaction may generate a structural image of IVD with enhanced resolution. We have established a novel multi-chromatic staining protocol for assessment of the IVD using a combination of Fast green, Alcian blue, Safranin-O, and Tartrazine stains (FAST staining). The aim of this study is to test if the FAST staining protocol can reveal fine structure of the IVD as well as provide insight about changes of extracellular matrix (ECM) during disc growth and degeneration using animal models.

METHODS

Lumbar IVD, including the endplates and vertebral bodies, of 6 month old New Zealand White rabbits were harvested to investigate the FAST staining pattern. Disc degeneration was induced in 6 months old rabbits at the L2/L3 and L4/L5 levels by annulus puncture at 5mm depth with a 19G needle through an anterolateral retroperitoneal approach and the operated lumbar discs were harvested at 3 to 12 months post-operation to study the FAST staining pattern during the degenerative changes. Coccygeal intervertebral discs from C57BL mice from birth to 8 weeks of age were collected to study the FAST staining pattern during postnatal growth. All tissues were fixed in 4% paraformaldehyde and were decalcified to prepare sagittal paraffin sections. The sections were stained by 1% alcian blue (pH1.0), 0.1% safranin-O, 0.25% tartrazine, and 0.001% fast green. All animal experiments were performed according to the protocols approved by the local health department and institutional ethics committee.

RESULTS

The FAST staining protocol labeled and differentiated all major vertebra-disc compartments with distinctive color (Fig.1a-c): The nucleus pulposus (NP) matrix was dominantly stained by alcian blue (Fig.1a). Inner and outer annulus fibrosus (AF) were preferentially stained orange by safranin-O with intercalated alcian blue-positive matrix (Fig.1b). The cartilage of the vertebral growth plate was stained red and bone in yellowish green (Fig.1c). Under prolonged staining of alcian blue, the cartilaginous endplate (EP) could be visualized (Fig.1d).

In the puncture-induced degeneration model, the boundary between NP and AF deteriorated progressively. The NP became indistinguishable and the disc center was occupied by fibrous tissue. The IVD became uniformly positive for alcian blue which then progressively reduced and disappeared in the course of degeneration. In growing mouse IVD, continual changes in matrix volume and staining pattern were observed. At birth, the NP was only slightly alcian blue positive while the IA and OA contained alcian blue and safranin-O positive matrix respectively. During growth, the alcian blue positive matrix in NP continued to expand and the AF gradually transformed into safranin-O-positive lamellae along with the formation of tartrazine/fast green-positive superficial annulus (SA).

DISCUSSION

The FAST staining method has provided unique histological tool to study glycoprotein- or proteoglycan-rich tissues. This integrated staining method allows labeling of different disc compartments with a spectrum of colors and enable differentiation of the NP and AF matrix, visualization of the EP, and distinct differentiation of the disc compartments (AF/NP/EP) from hyaline cartilage and bone in a simultaneous manner. Analysis of the disc degeneration model has revealed gradual but profound alteration of FAST staining pattern in the NP and AF, suggesting the degeneration has initiated a dynamic matrix remodeling event in the IVD. We propose that the overall content and distribution of alcian blue stain, which preferably interacts with sulfated glycosaminoglycan, may provide an additional cue to the severity of disc degeneration. The IVD has relatively primitive annulus structure at birth. The FAST staining suggests that the construction of mature annulus architecture requires vigorous matrix remodeling events rather than sole matrix deposition during growth of IVD.

ACKNOWLEDGEMENTS

This work was supported by the University Grants Committee of the Hong Kong SAR of China (grant HKU7496/05M).