INTRODUCTION

Cell cultures remain an indispensable in vitro tool to investigate and understand the physiologic and metabolic properties of the intervertebral disc (IVD) cells, as well as identifying and testing potential therapeutic targets. Nucleus pulposus (NP) cell cultures typically involve gross isolation of NP tissue from the disc followed by a series of enzymatic digestion leading to a mixture of cell population that has historically been called “IVD cell culture”. Fundamental information on the general growth characteristics of this IVD cell culture, possible existence of cell types and their relative abundance and function, and potential changes in cell composition during disc degeneration are lacking. Here we reported the growth characteristics and unique gene expression profile of human NP cells in monolayer culture as well as detection of several distinct cell subpopulations. This information provides a framework essential for guiding future disc basic research. Moreover, the existence of cell subpopulations opens up the possibility of identification and characterization of cell subpopulations with high therapeutic potentials, i.e., the cell types which primarily involved in producing either matrix catabolic enzymes associated with degeneration or matrix structural proteins important for regeneration.

METHODS

Global gene expressions were acquired using the Affymetrix GeneChip Human Genome U133 Plus 2.0 Arrays. Comparison of gene expression to other human primary cells, also maintained in monolayer cultures, were made using the same Affymetrix probe sets and normalized to GAPDH control. Cell growth characteristics, including cell motility, cell area, population doubling time were determined from time-lapse video tracking experiments. Collagen and aggrecan protein expression were examined by immunofluorescence and phalloidin co-stain to visualize the cellular actin cytoskeleton. All analyses were performed using monolayer cultures of primary cells isolated from multiple (4-6) surgical samples of human grade 3 degenerated discs.

RESULTS

Sub-profile of gene expression of selected proteoglycans, and collagens and metalloproteinases of NP cells is distinctive from the other cell types but is most similar to that of chondrocytes ((Fig. 1)). Human NP cells maintained in monolayer cultures exhibited multiple morphologies, with the majority of cells polygonal and chondrocyte-like in appearance. A fraction (~15%) of the cells are elongated and fibroblast-like, and a few cells (<5%) are unusually large and rounded, termed giant cells (Fig. 2). Morphologically heterogeneous cells were also detected in vivo by H&E general stain of human NP tissue (Fig. 2D, yellow arrows). Time-lapse video tracking experiment revealed an average population doubling time of 52 hours as well as the presence of slow moving (0.22 µm/min) ~4000 µm giant cells in comparison to fast moving (0.22 µm/min) but smaller ~400 µm polygonal cells (Fig 3C, 3D). About 60% of the initial cell population composed of very slowly dividing cells, within which a third of this population did not divide within a five day period (Fig. 3B). Immunofluorescence analyses also revealed cell heterogeneities, with only a small subpopulation immunopositive for collagen type II (Fig 2B) and aggrecan (Fig. 2C).

DISCUSSION

The lack of biologic characterization of the cells that comprise the intervertebral disc complicates our understanding of the underlying disc pathobiology. Due to scarcity of tissue, cells isolated from humans routinely require expansion on monolayer cultures before growth in culture for subsequent studies. Cellular properties of these monolayer cultures are still poorly defined. Our studies demonstrated that human NP monolayer cultures showed unique gene expression profile and slow growth characteristics. These basic information will be useful in the verification of phenotypes of NP cell cultures, particular those of established disc cell lines. Existence of cell subpopulation expressing collagen II and aggrecan also have important implications as they could be potential therapeutic targets in the design and development of effective cell-based IDD therapy. Currently these subpopulations are being isolated and characterized for their ability to maintain synthesis of key matrix proteins important for disc regeneration. Establishment of a

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