Stem cells for intervertebral disc regeneration and basic study aimed for the identification of disc-progenitor cells and their niche

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In order to achieve a maximal effect in stem cell therapy for intervertebral disc disease, knowledge on cells, extracellular matrix and the microenvironment of the native disc must be extensively studied. Fully committed adult cells are cells that actively function as main cell population eventually going into apoptosis. Other than committed adult cells are the tissue specific somatic progenitor/stem cells which are an undifferentiated cells found among differentiated cells in tissue or organ that can renew itself. Stem cells are distributed around the body in various other 'niches'. Evidence of existence of the small stem cell population has not been well studied in the intervertebral disc.

In order to identify the somatic stem cell population that reside in the intervertebral disc, so called "stem cell markers" were immunohistochemically analyzed in rat, beagle and human intervertebral discs. These markers included CD11a, b, c, CD24, CD49f, CD56, CD63, CD73, CD90, CD105, CD120a, CD124, CD166, MHC class I, etc. The immunohistochemical analysis in rat intervertebral disc specimens revealed that most of these classical stem cell markers may not be useful in identifying endogenous stem cells in the disc. Especially, positivity of the hall mark markers CD90, CD105 and CD 166 was 85, 92 and 98 percent (n=5). This was far different from the standard characteristics of stem cells which reside in small population with inactive cell viability at rest and after initiation, shows high self renewal and proliferative ability with multi-potent differentiation. Regarding other markers, no significant expression (0-10percent) was detected for CD56, CD120a CD124 MHC class I, etc in the rat disc, which may remain these markers as potential candidates. To note, negative expression in some of these markers may be a result of non-cross reactivity of the antibody to the rat.

Result of human disc specimens showed that CD56, 90 105,166 was expressed in some young disc cells in sparse areas, such as NP cells forming clusters but in old aged disc where cells are isolated in single cells, merely none of these markers were positive in most discs.

To overcome the lack of stem cell population indicated by the immuno-histochemistry results, identification of progenitor cells by marker analysis was continued through FACS analysis. By plotting the positivity of these markers through serial culture periods, we are able to detect cell markers which correlate with cell proliferation. As mentioned, stem/progenitor cell populations show little or decreased cell number at primary culture and give rise to rapid proliferation several weeks after, meaning potential for high self-renewal. We also found that by culturing beagle NP cells in gels, several different colonies can be induced. These included sphere shaped colony and few adherent colonies. Number of adherent colonies increased with time with no change with culture period. On the other hand, change in number of sphere colonies formed showed correlation with proliferation curve pattern of progenitor cells. This meant that cells forming sphere colonies derive from small cell population that is resting in the beginning and rapidly proliferate after several weeks of culture. Investigation of these colonies which are regarded as candidates for disc progenitor cells will be presented.

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