ADULT AND LATE FETAL EQUINE TENDONS CONTAIN CELL POPULATIONS WITH WEAK PROGENITOR PROPERTIES IN COMPARISON TO BONE MARROW DERIVED MESENCHYMAL STEM CELLS

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Introduction

Tendon frequently heals poorly following injury. It is uncertain to what extent intrinsic tendon progenitor cells contribute to the repair process, or whether extrinsic cells from surrounding tissues of the bone marrow may be required to effect repair. The failure of satisfactory tendon repair could occur due to a lack of tendon progenitor cells or due to a lack of necessary signals to effect satisfactory progenitor cell differentiation. In this study we tested the following hypotheses:

1). Adult and late fetal equine tendons contain progenitor cells with stem cell like properties.

2). Cells recovered from different compartments of the tendon, released using different extraction techniques, have differing progenitor properties.

3). Progenitor ability is enhanced by cell expansion in basic fibroblast growth factor (FGF-2) supplemented media.

4). Equine mesenchymal stem cells (MSCs) derived from adult bone marrow have greater progenitor properties than the resident tendon cell progenitor.

Material and Methods

MSCs from adult equine bone marrow were isolated using a percoll density centrifugation and selected by attachment on a plastic surface. To isolate adult skeletonally mature (aged 4-9 years) and fetal tenocytes (last 2 months gestation), we either used collagenase digested superficial digital flexor tendon or we cultivated outgrowing cells from tendon explants. To investigate the effect of FGF-2 on the differentiation potential of adult tenocytes and MSCs, we proliferated the cells with or without 5ng/ml FGF-2. After 2 passages, the cells were tested for differentiation along chondrogenic, osteogenic and adipogenic pathways. Differentiation was confirmed by histological analysis (Safranin O, Von Kossa, Alizarin Red S, Oil Red O), biochemical assay (GAG content and alkaline phosphatase activity) and real-time PCR quantification of gene expression for relevant differentiation markers genes (Collagen-I, -II, Sox9 and Bone sialoprotein (BSP)). Statistical comparisons were made by Mann-Whitney non-parametrical tests

Results

Equine marrow derived MSCs were able to undergo chondrogenic, osteogenic and adipogenic differentiation in a FGF independent manner although FGF-2 treatment of cells prior to differentiation significantly enhanced the proliferation rate of the cells (p<0.01). For all three differentiation pathways, adult and foetal tenocytes had a considerably lower differentiation potential compared to the marrow derived MSCs. Adult tenocytes cultivated by outgrowth from explants had significantly enhanced chondrogenic ability in comparison to adult tenocytes obtained by collagenase digestion. Proliferation of adult tenocytes in FGF-2 had a positive effect in enhancing some markers of chondrogenic differentiation. Results for osteogenic differentiation differed from chondrogenic differentiation. Alkaline phosphatase activity and gene expression levels of BSP indicated a higher osteogenic potential of adult and foetal tenocytes isolated by collagenase digestion in contrast to cells obtained from outgrowth of explants. FGF-2 had no effect on the osteogenic differentiation potential in adult tenocytes. In the adipogenic pathway, only outgrowing adult tenocytes from explants showed a weakly positive differentiation effect without a positive effect of FGF-2. Fetal derived tenocytes appeared to have no difference in multi-potential progenitor ability in comparison to adult derived tendon cells

Conclusion

In this study it was possible to differentiate equine MSCs into the chondrogenic, osteogenic and adipogenic lineage in a manner that was not dependent on the cells being initially proliferated in FGF-2. This is in contrast to the situation observed in human MSCs, where FGF pre-treatment of such cells considerably enhances their chondrogenic potential. Equine tendons in vitro contain only a weak stem cell like progenitor population. Populations of cells with differing progenitor abilities are obtained from tendons depending on how the cells are initially isolated from the tendon for culture purpose confirming that these isolation methods may yield cells from different compartments within the tissue. FGF-2 enhanced proliferation of equine adult tenocytes appears to have different effects on progenitor cell behaviour depending on the differentiation pathway. Somewhat surprisingly, late fetal tendon tissue had similar level of progenitor cell ability to adult tendon, indicating that by this stage of development most cells within tendon have become committed to this particular mesenchymal lineage. In conclusion, equine MSC have a considerable better multilineage differentiation potential than equine tenocytes. We have shown that equine tendons contain cells with only a relatively weak stem cell like properties (multipotency). The failure of equine tendon injuries to repair adequately may be due to a lack of intrinsic progenitor cells which would be necessary to undergo relevant differentiation to effect tissue healing. Further experiments are now required using clonally derived populations of cells to identify whether this lack of tendon progenitor cells is due to either all tendon cells having weak multipotency, or whether there are differing populations of cells within normal tendon with varying progenitor ability and there being only minority cell populations of truly multipotent cells.

52nd Annual Meeting of the Orthopaedic Research Society

Paper No: 1113

References


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