Control of Mesenchymal Stem Cell Chondrogenesis in Micromass and on Collagen Scaffolds by Autocrine Transglutaminases

Shobana Shanmugasundaram and Maria Nurminskaya

Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD 21201.

Statement of Purpose: The increasing incidence of articular cartilage pathology poses a challenging goal for researchers and surgeons to regenerate articular cartilage. Today there are no strategies that reliably reproduce the biological composition and biomechanical properties of hyaline cartilage. Mesenchymal stem cells (MSC) represent a beneficial cell source for bioengineering articular cartilage should their hypertrophic differentiation pathway be prevented. Recent data implicate two transglutaminase enzymes – TG2 and FXIIIa – as autocrine regulators of chondrogenesis in the growth plate and in osteoarthritis. We postulate that chondrogenic differentiation in MSCs may be controlled by modulating endogenous levels of transglutaminases (TGases). We demonstrate that ablation of TGases in differentiating MSCs results in increased number and area of the chondrogenic nodules, and in induction of chondrogenic molecular markers. We further show that enzymatic activity of TG2 negatively regulates chondrogenesis in MSC through cross-linking of the cartilaginous collagens types II and XI. The effects of incorporating TGase inhibitors into biopolymer scaffolds for cartilage tissue engineering utilizing human MSC are being tested. These results outline a novel approach to foster MSC chondrogenesis and, at the same time, to prevent chondrocyte maturation via endogenous autocrine regulators of chondrogenesis - the transglutaminase enzymes.

Methods: To examine chondrogenic differentiation in MSCs with ablated TGases we employed limb bud mesenchymal cells from TG2 null mice and wild type controls as described previously[1]. Expression of FXIIIa in both types of MSCs was downregulated with virally expressed siRNA. Quantitative RT-PCR on three 9 days old micromasses from independent experiments was analyzed in triplicates for chondrogenic and hypertrophic markers using Roche SYBR green mix in a Lightcycler 480. The data were normalized to the average of housekeeping genes-Actin and RPL19. To determine the input of the TGase-mediated cross-linking of the cartilage-specific collagens on MSC chondrogenesis, $10\text{-}50^4$ cells/cm^2 human MSC were seeded on films of bovine Collagen II and XI (0.5mg/ml). Chondrogenic differentiation was induced by TGF-b3 and dexamethasone with and without 0.01U/ml purified recombinant human $TG2_{active}$ (hTG) or human $TG2_{inactive}$ (hTGinact). Glycosaminoglycan (GAG) content in both the mice and human MSCs were quantified with Alcian Blue and normalized to DNA stained with crystal violet. Western blot was employed to analyze cross-linking of collagen films by TG2 by incorporation of biotinylated pentylamine substrate, and the retention of TG2 protein in collagen films.

Results: Genetic ablation of TG2 results in a 3-fold increased deposition of the GAG-rich extracellular matrix, enhancement of early chondrogenic markers (Fig.1A) and formation of more chondrogenic nodules (Fig. 1B) in MSC undergoing spontaneous chondrogenesis. Further, down-regulation of FXIIIa in TG2-/- MSCs chondrogenic differentiation is even more pronounced with GAG deposition being almost 8-fold increased compared to TG2-/- cells.

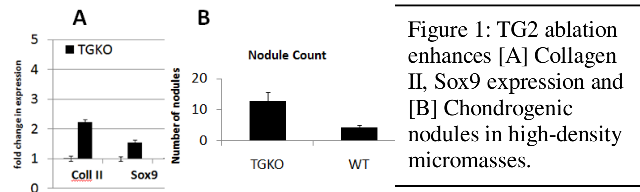

Figure 1: TG2 ablation enhances [A] Collagen II, Sox9 expression and [B] Chondrogenic nodules in high-density micromasses.

These data support the hypothesis that TGases are negative autocrine regulators of chondrogenic differentiation. Importantly, down-regulation of FXIIIa expression results in a dramatic increase in expression of collagen type X – a marker of chondrocyte hypertrophy (Fig. 2A, B), suggesting that FXIIIa suppresses terminal chondrogenic differentiation. This seems to be a specific function for FXIIIa since ablation of TG2 does not induce collagen type X (Fig. 2C).

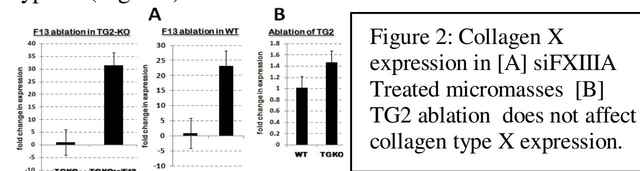

Figure 2: Collagen X expression in [A] siFXIIIA Treated micromasses [B] TG2 ablation does not affect collagen type X expression.

Therefore, isozyme-specific approaches should be considered in MSC-based cartilage bioengineering to promote matrix deposition while inhibiting cell differentiation. Lastly, we tested whether cartilage-specific collagens are involved in TG2-mediated inhibition of chondrogenesis. Treatment of collagens type II and XI films with TG2 inhibited chondrogenesis in hMSC, detected by expression of chondrogenic markers. Enzymatic activity of TG2 is required for this effect (Fig. 3A). A plausible mechanism accounts for TG2-induced crosslinking of collagen film (Fig. 3B) in inhibition of MSC chondrogenesis. In addition, collagen films retainTG2 proteins (Fig. 3C). TRetained protein may affect intracellular signaling in MSC to inhibit chondrogenesis.

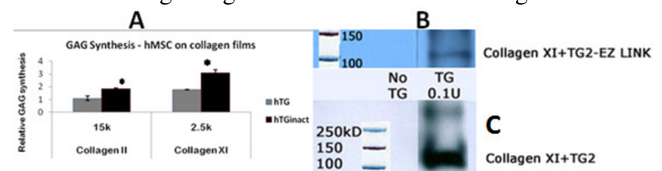

Figure 3: [A] hMSC GAG synthesis [B,C] Western blot of TG2.

Conclusions: Together, our results identify TGases as novel autocrine regulators of chondrogenesis and suggest that incorporation of slow-released TGase inhibitors in biopolymer scaffolds may benefit cartilage engineering from MSCs.

References: Stanton LA. Biochem J. 2004; 378:53-62.

©2011 Society For Biomaterials