Introduction

Transforming growth factor beta (TGF-β) superfamily members play diverse roles in cartilage development and maintenance. TGF-β not only up-regulates a number of molecules associated with prechondrogenic condensation, it also up-regulates chondrogenic gene expression through several signaling pathways. Moreover, TGF-β has been reported to inhibit osteoblast differentiation by repressing runt-related transcription factor 2 (Runx 2) [1]. However, our previous study indicated that TGF-β1 alone is not sufficient to fully differentiate mesenchymal stem cells (MSCs) into chondrocytes [2]. Other factors are necessary for the chondrogenic differentiation process. Histone deacetylases (HDACs) are the enzymes catalyzing the deacetylation of histone and non-histone proteins and regulating cell proliferation, differentiation, and apoptosis. Recently, it was reported that HDAC4 acts as a negative regulator of chondrocyte hypertrophy [3]. We hypothesize that HDAC4 may promote TGF-β1-induced stem cell-based chondrogenesis.

Methods

Experimental design. Adenovirus mediated HDAC4 (Ad.HDAC4) and Ad.LacZ were from Vector Biolabs. After amplification, Ad.HDAC4 and Ad.LacZ were quantified using plaque-forming unit (PFU) titration. Synovial cells were isolated from synovial tissue of 3-month-old pig knees. SDSCs were negatively isolated from primary culture by Dynal® Magnetic beads [4]. Passage 3 SDSCs were infected with Ad.HDAC4 at multiplicities of infection (MOI) of 100 PFU/cell. Ad.LacZ served as a control. The infected 0.5 × 10^6 SDSCs were centrifuged at 500 g for 5 min, and incubated for 24 h to form day 0 pellets. Day 0 pellets were incubated in chemically defined medium with or without the treatment of TGF-β1 in a low oxygen incubator (5%O2, 5% CO2, 37°C). Four groups were set up as “Ad.LacZ”, “Ad.HDAC4”, “Ad.LacZ + TGF-β1”, and “Ad.HDAC4 + TGF-β1”. The pellets were collected at days 3, 9, and 15.

Analytical methods. (1) Histology (n=2), i.e. alcian blue staining for sulfated glycosaminoglycans (GAGs), and immunostaining for collagens I and II; (2) Biochemistry analysis (n=4) for the amounts of DNA and GAG per pellet; (3) TaqMan® PCR (n=3) for mRNA levels of collagens I and II, X, Sox 9, and aggrecan; (4) Statistical significance was assessed using single factor ANOVA (p <0.05).

Results

HDAC4 Enhances SDSC-Based Chondrogenesis Mediated by TGF-β1

[Histology data at day 15 – Fig.1] Ad.LacZ-transformed SDSC (L-SDSC)-pellets incubated with TGF-β1 underwent chondrogenesis in the peripheral regions at days 9 and 15, despite no obvious GAG synthesis at day 3. More extensive staining was seen at day 15 as opposed to day 9, confirming our previous results that TGF-β1-mediated SDSC chondrogenesis was time-dependent [4]. Ad.HDAC4-transformed SDSC (H-SDSC)-pellets exhibited intense expression of GAG in response to TGF-β1 compared to L-SDSC-pellets. For treatment without TGF-β1, no GAG was detectable in either L-SDSC-pellets or H-SDSC-pellets, which indicates that Ad.LacZ or Ad.HDAC4 alone are insufficient to initiate SDSC chondrogenesis. Immunostaining showed a ring of intense collagen II expression similar to GAG staining.

[Biochemistry analysis data - Fig.2] To facilitate comparison, day 0 DNA content was used to adjust the DNA content from days 3, 9 and 15. There was no statistical difference in DNA contents at day 3 among the four groups. However, compared to the control groups, the groups treated with TGF-β1 can minimize the decrease in cell number at days 9 and 15; of them, H-SDSCs yielded pellets maintaining the highest cell number. The chondrogenic marker, GAG/DNA, was shown to increase in all groups with time, particularly for the pellets treated with TGF-β1. The H-SDSC-pellets with treatment of TGF-β1 exhibited the highest chondrogenic differentiation.

Discussion

Our study demonstrated that adenovirus-mediated HDAC4 enhances and maintains chondrogenesis of SDSCs induced by TGF-β. Further, it indicates that growth factors and transcription factors may be required to sustain chondrogenic differentiation of MSCs. This study is the first case reporting that HDAC4 enhances chondrogenesis induced by TGF-β; however, the mechanisms underlying this process are still not clear and require further investigation.

References