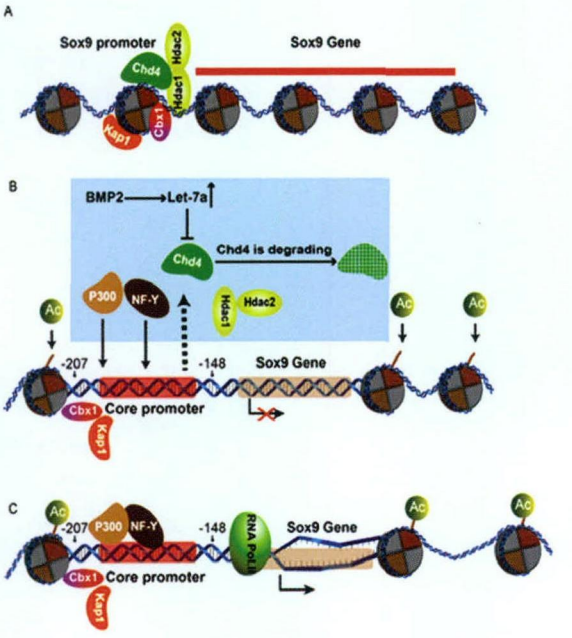
Repressive roles of Chd4 in BMP2-induced chondrogenesis

Chondrogenesis is a tightly regulated process in which multipotential mesenchymal cells differentiate into chondrocytes to form cartilage. And MEFs are able to enter and complete the program of chondrogenic differentiation *ex vivo*, changing from undifferentiated progenitor cells to mature hypertrophic chondrocytes. However, no data focusing on the comprehensive proteomic aspect of BMP-2 induced chondrogenesis have been reported. Funded by the Ministry of Science and Technology of China and the National Natural Science Foundation of China, Prof. Pan Qiuhui, member of Tenth People's Hospital of Tongji University and her team workers published their findings en titled "Chd4 and associated proteins function as corepressors of Sox9 expression during BMP - 2-induced chondrogenesis" in the *Journal of Bone and Mineral Research* (**2013**, 28(9):1950—61).

On the basis of iTRAQ labeling coupled with on-line 2D LC/MS/MS proteomic technology, the researchers firstly identify a series of proteins downregulated in BMP-2 induced chondrogenesis. Interestingly, biological analysis reveals that Chd4 and its associated proteins in an interaction network function in the control of transcription, chromatin modification, and RNA splicing, directly or indirectly regulating cell differentiation. Knockdown of Chd4 results in a remarkable upregulation of Sox9 and promotes chondrogenic differentiation, illustrating that Chd4 is a repressive regulator of chondrogenesis. Then given Sox9 is a key positive player modulating chondrogenic differentiation, promoter studies and mutant assays reveal that Chd4 negatively controls the expression of Sox9 through the TGGCTG box at-207/-148 adjacent to the transcription start site of Sox9 (Figure A). Further study shows that Chd4 interacts with Hdac1 and Hdac2 to competitively inhibit the binding of NF-Y-p-300 complex to the promoter of Sox9 and prevent chondrogenic differentiation by downregulating the expression of Sox9.

Nuclease hypersensitivity assays indirectly indicating the status of the chromatin structure display that knockdown of Chd4 leads to the open status of the chromatin. Additionally, analysis of the associated proteins of Chd4 in the network using the ChIP and Re-ChIP assays demonstrates that Cbx1 and Kap1 synergistically promote the binding of the histone protein H3 with the Chd4 complex and simultaneously cooperate with other proteins in the network to transcriptionally regulate the expression of Sox9. To identify the upstream regulator of Chd4 in BMP-2 induced chondrogenesis, regulation of miRNAs comes to mind. The Chd4 3'UTR reveals a highly conserved domain for let-7, and data from luciferase assays confirm the direct interaction of let-7a with Chd4 3'UTR region (Figure B). Let-7a is involved in promoting chondrogenic differentiation by transcriptionally inhibiting Chd4 and its associated proteins. Interestingly, deletion of chd4 leads to a slightly increased binding of the NF-Y-p 300 complex (Figure C).



The findings of this work illustrate an epigenetic regulation of the chondrogenic differentiation process as well as expand the understanding of the intracellular mechanisms that regulate chondrogenic differentiation and self-renewal of MSCs. Furthermore, these results promote the application of MSCs in the treatment of human cartilage disease.