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COMMENTARIES

The Family of Osteoblast Transcription Factors is Growing

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Commentary on: Hong JH, Hwang ES, McManus MT, Amsterdam A, Tian Y, Kalmukova R, Mueller E, Benjamin T, Spiegelman BM, Sharp PA, Hopkins N, Yaffe MB. TAZ, a transcriptional modulator of mesenchymal stem cell differentiation. *Science*. 2005 Aug 12;309(5737):1074-8.

Osteoblasts are derived from mesenchymal stem cells that are also capable of forming adipocytes, chondrocytes and myocytes (1). Prior to 1997, it was surmised, based on indirect evidence, that several master genes were responsible for the derivation of each lineage. That a single gene, Runx2 (also known as Cbfa1, Osf2 and AML3), could be responsible was first indicated by the absence of osteoblasts in Runx2 null-mutation mice (2;3;4). These studies were followed by the elegant demonstration that osterix$^{-/-}$ mice completely lacked bone formation, showing for the first time that the osterix transcription factor is essential for osteoblast differentiation (5).

Regulators of mesenchymal stem cell differentiation can have competing effects. For example, peroxisome proliferator-activated receptor γ (PPARγ), which promotes differentiation to adipocytes, inhibits osteogenesis by physically blocking the activity of Runx2 (7;8). PPARγ insufficiency enhances osteogenesis by promoting formation of osteoblasts from bone marrow progenitor cells (9). Runx2, on the other hand, inhibits adipocyte differentiation in vitro (6).

In "TAZ, a transcriptional modulator of mesenchymal stem cell differentiation" (10), a new transcriptional coactivator is described which appears to play a key role in the differentiation of mesenchymal cells into osteoblasts. TAZ was identified in a screen of 14-3-3 binding proteins. It has a WW domain, a protein module known to mediate protein-protein interactions with proline-rich peptide motifs such as Pro-Pro-X-Tyr (where X is any amino acid). Transcriptional activators PPARγ and Runx2 also contain Pro-Pro-X-Tyr motifs, suggesting they may interact with TAZ, and leading the authors to explore the role of TAZ in osteoblast versus adipocyte differentiation. This study shows that TAZ functions as a type of "rheostat" in determining whether mesenchymal stem cells differentiate into osteoblasts or adipocytes.

The authors used several lines of evidence to establish a role for TAZ in promoting osteoblast differentiation. In the first set of experiments, they used TAZ small interfering RNAs (siRNAs) to decrease TAZ expression in murine C2C12 cells. C2C12 cells treated with BMP-2 differentiate into osteoblasts and secrete osteocalcin. C2C12 cells transfected with TAZ siRNAs, showed decreased osteocalcin expression, indicating a decreased degree of osteoblastic differentiation. Given the interaction of WW domains with Pro-Pro-X-Tyr motifs, the presumed mechanism of this effect is through Runx2. Indeed, the authors show that TAZ can be co-immunoprecipitated with Runx2. Furthermore, experiments in which a Runx2-dependent luciferase reporter gene was cotransfected with TAZ into C2C12 cells yielded increasing levels of luciferase activity with increasing levels of TAZ expression, suggesting that TAZ functions as a transcriptional coactivator of Runx2. The authors also show that TAZ is bound to

the endogenous osteocalcin promoter in BMP-2 treated C2C12 cells. Together, these results suggest that TAZ increases osteoblastic differentiation as a result of its transcriptional coactivation effects on Runx2.

In vivo evidence for the role of TAZ in osteoblast differentiation was obtained in zebrafish. Antisense morpholino oligomers were used to decrease levels of TAZ in zebrafish, which were then examined at 8 days post-fertilization. The zebrafish with decreased TAZ levels had no demonstrable bone, as assessed by Alizarin Red S staining and histologic examination.

An alternate fate for mesenchymal stem cells is adipogenesis. The authors reasoned that if TAZ has a role in promoting osteoblast differentiation, it might also be expected to inhibit adipocyte differentiation. In support of this hypothesis, they find that TAZ interacts with PPARγ *in vitro*, and can be coimmunoprecipitated with PPARγ from cotransfected 293T cells. Next, the authors expressed a luciferase reporter under the control of the PPARγ-dependent aP2 promoter in U2OS cells. When those cells are treated with rosiglitazone, a PPAR-activating ligand, to induce aP2 expression, coexpression of TAZ results in decreased luciferase activity, suggesting inhibition of adipocyte differentiation. In addition, in 3T3-L1 cells TAZ expression was correlated with decreased AP2, PPARγ, and C/EBPα expression.

If TAZ overexpression inhibits adipocyte differentiation, then decreasing TAZ activity should enhance adipocyte differentiation. In the next series of experiments, the authors show that this is the case. 3T3-L1 cells were transfected with TAZ siRNA or control RNA, and transferred to adipocyte differentiation medium. There was decreased TAZ expression in the cells transfected with TAZ siRNA, and these cells had higher rates of adipocyte differentiation, as shown by Oil Red O staining and aP2 expression. As in osteoblastic cells, TAZ was found bound to the aP2 promoter in the 3T3-L1 cells that were induced to undergo adipocyte differentiation. In addition, mouse embryo fibroblasts in which the TAZ gene was deleted by homologous recombination had higher rates of adipocyte differentiation in the presence of rosiglitazone and differentiation medium.

Finally, the authors used short hairpin RNAs to decrease TAZ expression in freshly isolated bone-marrow derived mesenchymal stem cells. This resulted in decreased bone formation, as measured by Alizarin Red S staining, and increased fat formation, as measured by Oil Red O staining. Again, these results place TAZ at a key position in the determination of bone versus fat differentiation.

In summary, the authors show that TAZ plays an important role in the decision of mesenchymal stem cells to become osteoblasts or adipocytes (Fig. 1). The work suggests several interesting lines of future research. First, while the authors show that TAZ is necessary for osteoblast formation, they have not shown whether it is sufficient to promote osteoblastogenesis. Second, the authors show that 14-3-3 can be coimmunoprecipitated with TAZ, and that a point mutation decreasing TAZ binding to 14-3-3 increases TAZ nuclear localization. It will be interesting to learn more about the role 14-3-3 plays in this process, as well as how and when TAZ interacts with Runx2 versus aP2. Finally, it will be exciting to explore the clinical implications with regards to the role of TAZ in bone biology and disease.

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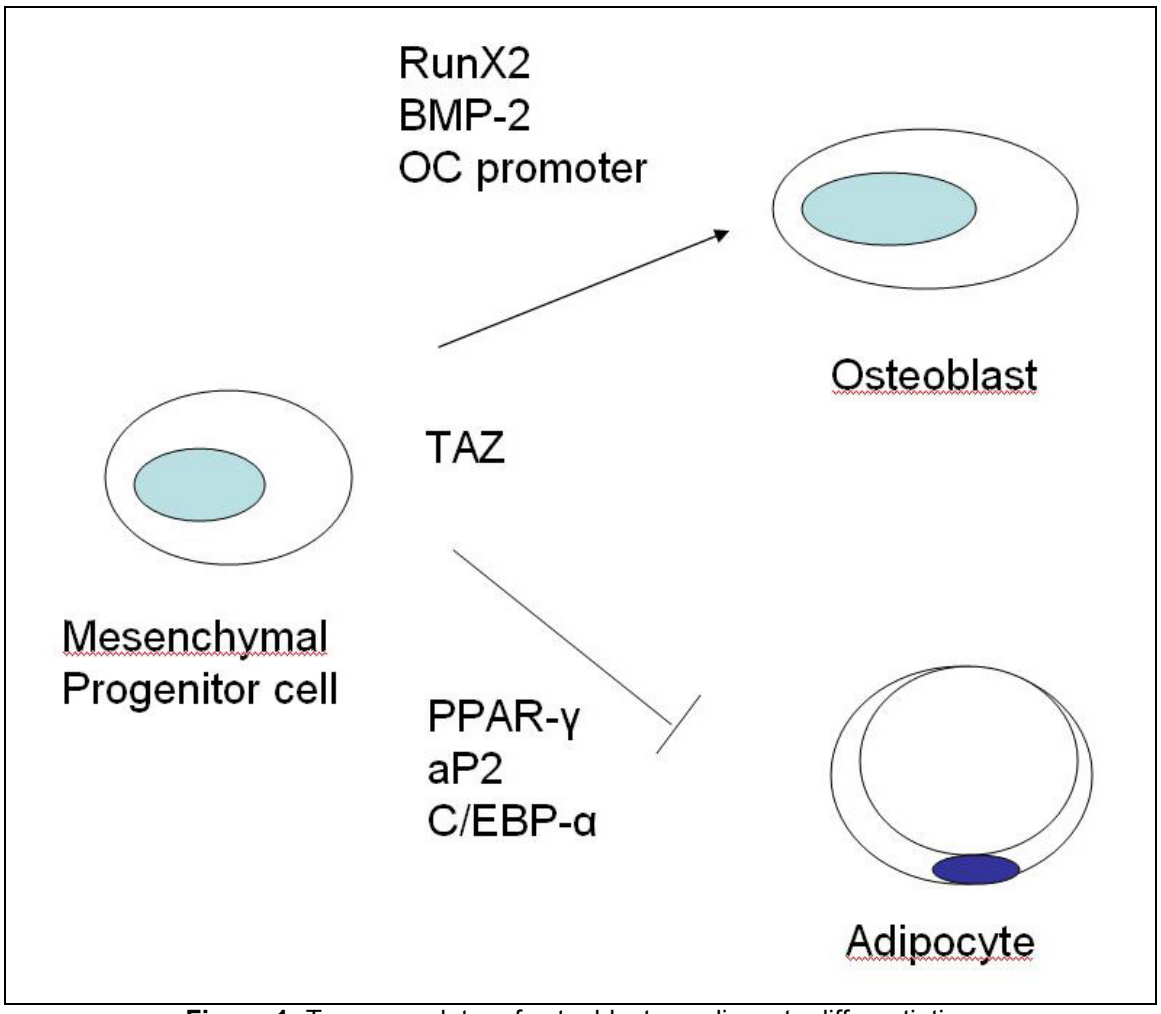

Figure 1: Taz, a regulator of osteoblast vs adipocyte differentiation

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