Review

TAZ

A β-Catenin-Like Molecule That Regulates Mesenchymal Stem Cell Differentiation

Jeong-Ho Hong
Michael B. Yaffe*

Center for Cancer Research; Division of Biological Engineering and Department of Biology; Massachusetts Institute of Technology; Cambridge, Massachusetts USA

*Correspondence to: Michael B. Yaffe; Center for Cancer Research; Division of Biological Engineering and Department of Biology; Massachusetts Institute of Technology; 77 Massachusetts Ave.; Cambridge, Massachusetts 02139 USA; Tel.: 617.452.2103; Fax: 617.452.4978; Email: myaffe@mit.edu

ABSTRACT

Regulating the switch between proliferation and differentiation of mesenchymal stem cells is critical for the development of normal tissues, and the prevention of tumors. How mesenchymal stem cells exit from the cell cycle and differentiate into alternative cell fates such as bone, fat, and muscle, is incompletely understood. We recently discovered that a WW domain-containing molecule, TAZ, functions as a transcriptional modulator to stimulate bone development while simultaneous blocking the differentiation of mesenchymal stem cells into fat. These developmental effects occur through direct interaction between TAZ and the transcription factors Runx2 and PPARγ, resulting in transcriptional enhancement and repression, respectively of selective programs of gene expression. We propose that TAZ, as well as a highly related molecule YAP, are functionally, though not structurally, similar to β-catenin and integrate extracellular, membrane, and cytoskeletal-derived signals to influence mesenchymal stem cell fate.

The formation and maintenance of differentiated tissue types requires a precise balance between cell proliferation and differentiation. This occurs in most tissues during embryogenesis, and perhaps throughout adult life, by maintaining a population of pluripotent stem cells that can alternatively give rise to either additional stem cells, or to cells that are committed to undergo differentiation with limited replicative potential. In adults, this process has been best delineated in self-renewing tissues such as the hematopoeitic system, where stem cells are obvious and abundant. For other cell and tissue types, such as pancreatic β-cells, the search for a normal adult stem cell has proven elusive.[1] Stem cells for mesenchyme-derived tissues, such as bone, muscle, cartilage, and fat, lie somewhere between these two extremes. Mesenchymal stem cells constitute a small population of pluripotent cells within the bone marrow that are functionally defined by virtue of their ability to (1) adhere to tissue culture plastic and proliferate ex vivo, and (2) differentiate into adipocytes, chondrocytes, myocytes and osteoblasts under the influence of particular culture conditions.[2,3] These cells are believed to spontaneously differentiate into different tissue types when placed in the correct microenvironment in vivo, and their migration from the bone marrow followed by differentiation into various cell lineages is thought to contribute to tissue maintenance throughout the lifetime of an organism. In addition, mesenchymal stem cells retain their phenotypic character when cells that were differentiated in culture are reimplanted into adult animals suggesting that such cultured cells could be used in various therapeutic settings.[4] How cell cycle regulatory proteins such as Rb and E2F family members, and cyclin-dependent kinase inhibitors such as p21 and p27 control the extent of mesenchymal cell proliferation and the transition from proliferation to differentiation is incompletely understood. A molecular understanding of the process of mesenchymal stem cell differentiation is important since many cancers are thought to arise from excessive proliferation of stem cells, or of mutant cells with certain stem cell like properties.[5]

Terminal differentiation of mesenchymal stem cells appears to be largely controlled by the selective activation of specific programs of gene expression.[6,7] These genetic programs, which are triggered by a small number of key transcription factors, appear to be mutually exclusive—that is, once a mesenchymal stem cell becomes committed to the osteoblast lineage, it seems to lose the ability to switch to the adipocyte lineage, and vice versa.[7-9] How the transcription factors that control these cell fate decisions are themselves regulated remains relatively unclear.

We recently discovered that a β-catenin-like molecule, TAZ, sits at the convergence point of multiple signaling pathways that control mesenchymal stem cell differentiation into bone and fat. TAZ acts as a transcriptional modulator by regulating the functional

interaction of specific transcription factors with chromatin.[10] In addition to bone and fat, TAZ also appears to be involved in stem cell differentiation of myocytes, as well as various epithelial tissues. Zebrafish deficient in TAZ, for example, show abnormal ventral curvature, cardiac and pigmentation abnormalities, and a complete failure of bone formation prior to death at around 8.0 days post-fertilization.[10]

TAZ was originally identified during a series of control experiments in a proteomic screen looking for 14-3-3-interacting proteins.[11] TAZ is very similar to a related molecule, YAP, which was found during a screen for binding partners of the SH3 domain of the Src-family kinase Yes.[12] Both TAZ and YAP contain (1) a 14-3-3 binding motif; (2) a single or duplicated WW domains (1 in TAZ, 2 in YAP); (3) an extended coiled-coiled region within a larger transcriptional regulatory domain; (4) multiple sites of phosphory-lation, and (5) a C-terminal motif that can interact with PDZ domain-containing proteins (Fig. 1A). How these domains and motifs function together to regulate cell differentiation in response to extra-cellular signals is poorly understood.

The WW domains of TAZ and YAP bind strongly to the sequence motif Pro-Pro-X-Tyr. Surprisingly, this motif can be found within the regulatory regions of a large number of transcription factors, including Runx2 and PPARγ, as well as members of the Sox, SMAD, and Forkhead families (Fig. 1B), suggesting that TAZ and YAP may function as general transcriptional modulators during the execution of many developmental programs.

The C-terminal transcriptional regulatory domain of both TAZ and YAP, in isolation, can strongly coactivate gene expression when corecruited with Runx1 to an artificial promoter in a GAL4-driven luciferase reporter system.[11,13] In vivo, however, TAZ and YAP appear, instead, to regulate Runx2-driven genes during osteoblast differentiation in exactly opposite ways. Stein and colleagues found that YAP was a specific inhibitor of Runx2-driven genes during osteoblast differentiation in a manner that required Src kinase activity.[14] We found that TAZ strongly activates Runx2-driven genes during terminal osteoblast differentiation.[10] In addition to interacting with Runx2, both YAP and TAZ bind to TEAD/TEF transcription factors involved in muscle differentiation,[15,16] although whether this binding stimulates or inhibits endogenous TEF-driven gene expression remains to be determined. TAZ also binds to the transcription factor TTF-1 that is involved in formation and differentiation of the lungs and respiratory epithelia, and stimulates the production of pulmonary surfactant.[17] In contrast to these stimulatory effects, TAZ binds to, but markedly inhibits, the ability of the adipocyte transcription factor PPARγ to drive the expression of fat cell genes such as aP2, and depletion of TAZ in mesenchymal stem cells dramatically increases their adipogenic potential.[10] Thus, the global function of TAZ appears to be as a context-dependent transcriptional modulator during cell fate selection by mesenchymal stem cells (Fig. 2A).

The relatively acidic C-terminus of TAZ likely interacts directly with core transcriptional machinery to stimulate gene expression.[11] However, the mechanism of transcriptional repression is not clear. TAZ might directly recruit transcriptional corepressor complexes containing HDACs, for example, or might inhibit recruitment of critical transcriptional coactivators for fat cells such as PGC1α.[18] Regardless of these mechanistic details that remain to be worked out, by stimulating bone development and blocking fat cell differentiation, TAZ acts as a rheostat to control the extent of mesenchymal stem cell differentiation down these two opposing pathways. This has
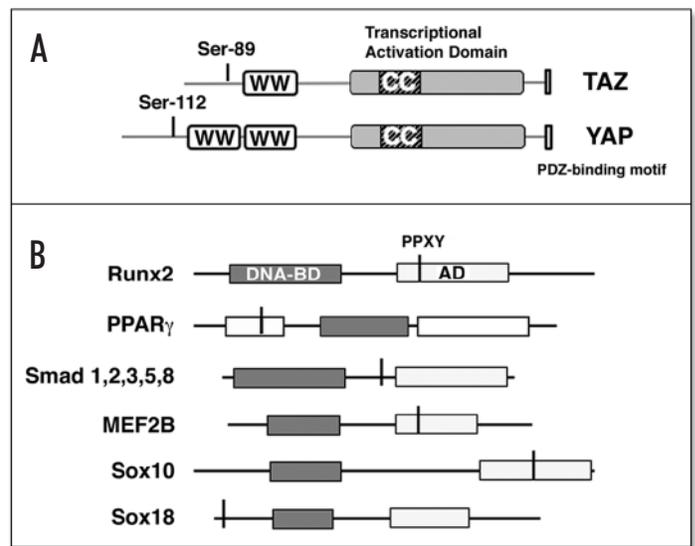

Figure 1. TAZ domains and motifs. (A) Domain architecture of TAZ and YAP. WW domains and coiled-coiled domains (CC) are indicated. Phosphorylation of Ser-89 in TAZ and Ser-112 in YAP regulate 14-3-3-binding and seques-tration in the cytoplasm. (B) Many transcription factors contain PPXY sequences (vertical line) that match the binding motif for the TAZ and YAP WW domains. Of the proteins shown, only Runx2 and PPARγ have been shown to bind to TAZ and/or YAP.

important clinical implications not only for understanding abnor-malities of differentiation seen in cancer and human developmental diseases, but also for understanding tissue changes associated with aging and diseases such as cirrhosis. During aging, the progressive loss of bone mass is accompanied by an equally progressive replace-ment of the bone marrow with fat.[19,20] Both of these phenomenon could be explained by a progressive reduction in TAZ-driven gene expression with age. Likewise, hepatic fibrosis culminating in cir-rhosis results from the proliferation of, and extracellular matrix production by, mesenchymal stem cells and hepatic stellate cells. Quiescent hepatic stellate cells express numerous markers of adipocytes, including PPARγ, while activated stellate cells involved in fibrosis express myocyte markers including MEF-2.[21] Thus, upregulation of TAZ in response to liver injury, if it occurs, might underlie the switch in gene expression programs responsible for the fibrotic response.

Emerging data suggests that, in response to various extracellular cues, developmental signaling pathways act directly on TAZ to control the extent of mesenchymal stem cell proliferation or differentiation into various cell types. The processes by which TAZ is regulated are poorly understood, but appear to involve changes in TAZ expression, phosphorylation, and subcellular localization (Fig. 2B). Subpopulations of both TAZ and its paralogue YAP can be found in close association with the membrane and actin cytoskeleton,[11,22] within the cytosol bound to 14-3-3 proteins,[11,23] and within punctate foci in the nucleus where transcriptional regulation is occurring.[10,11,14,24] Localization of TAZ and YAP at the membrane/cytoskeleton, as well in these punctate nuclear foci, appears to require the C-terminal PDZ binding motif. At the membrane, this motif in TAZ and YAP binds to the PDZ domain-containing proteins NHERF-2, and NHERF, respectively.[11,22] NHERF and NHERF-2 also bind to the cyoplasmic tails of transmembrane receptors and to members of the MERM family (moesin, ezrin, radixin and merlin) of actin binding
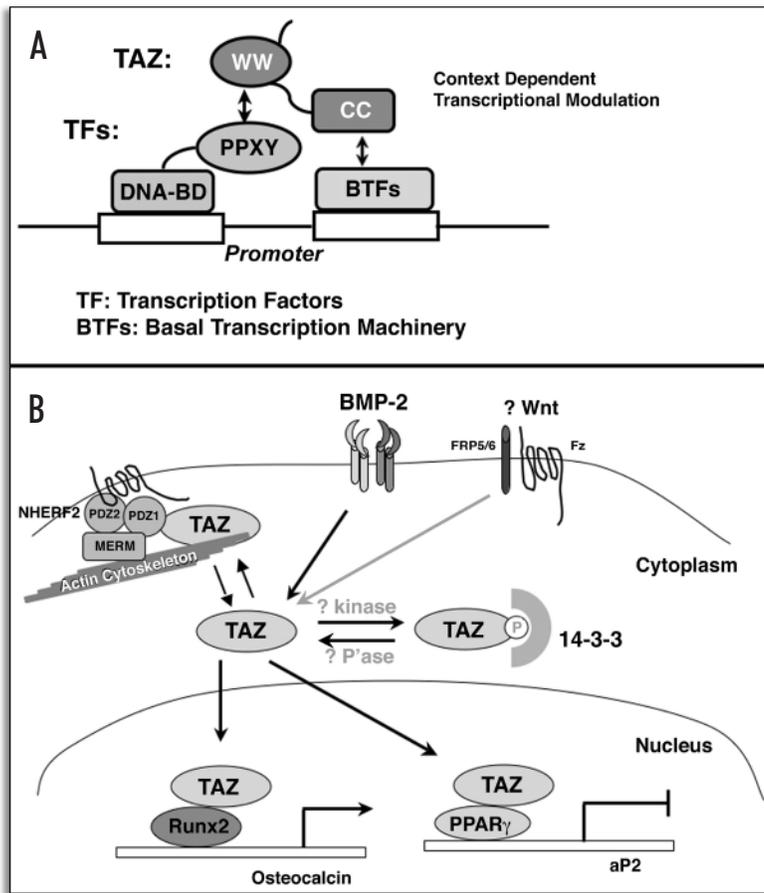
Figure 2. Mechanism and regulation of TAZ function. (A) Interactions between the coiled-coiled/transcriptional activation domain of TAZ and the basal transcriptional machinery are modulated by interactions of the WW domain with specific transcription factors to stimulate or repress gene expression. (B) Multiple signaling pathways regulate the level and subcellular localization of TAZ to control mesenchymal stem cell differentiation. Many of the details remain to be elucidated.

(our unpublished observations). The BMP-2 driven increase in TAZ protein levels appears to be due, at least in part, to transcriptional upregulation of TAZ mRNA in positive feedback loop involving TAZ itself.

Besides BMP-2 signaling, the Wnt signaling pathway is likely to play an important role in regulating TAZ function, since the phenotypic effects of TAZ manipulation on bone and fat development recapitulate similar phenotypes seen in mice in which the levels of Wnt-10b have been genetically manipulated.[26,27] A major target of Wnt signaling is the stabilization of β-catenin, an Armadillo repeat-containing molecule that acts as a transcriptional coactivator to enhance osteogenic differentiation by increasing the expression of early markers of bone differentiation such as alkaline phosphatase,[28] while also acting as a transcriptional repressor of adipocyte differentiation,[29,30] much like TAZ. The function of β-catenin in bone differentiation is complex; binding of β-catenin to one of its transcription factor targets, TCF-1, leads to enhanced expression of Runx2,[31] while Lef-1, another β-catenin target, seems to repress the ability of Runx2 to promote late osteoblast differentiation.[32] Thus, the process of mesenchymal stem cell differentiation involves both membrane receptor-mediated changes in β-catenin, YAP and TAZ expression, as well as dynamic changes in the interactions of β-catenin, YAP and TAZ with specific transcription factors. The most parsimonious summary of all the published findings is to posit that both β-catenin and TAZ function together to repress the differentiation of mesenchymal stem cells into adipocytes. β-catenin and YAP specifically facilitate early osteoblast differentiation of mesenchymal stem cells by increasing the expression of a selected set of early genes, while priming Runx2 levels for completion of osteoblast differentiation driven by TAZ:Runx2-dependent transcription of late genes. Intriguingly, like TAZ and YAP, β-catenin levels are subject to tight regulation, distinct pools of β-catenin localize to the plasma membrane (bound to cadherins) and to the nucleus, and, β-catenin also contains a C-terminal PDZ-domain binding motif. Interactions between the β-catenin PDZ-binding motif and a number of PDZ domain-containing proteins have been shown to modulate β-catenin-driven gene transcription,[33-35] though whether these events are relevant to mesenchymal stem cell differentiation remains to be explored. Despite their divergent structures, the functional similarities between TAZ, YAP and β-catenin, together with hints about their common targets and regulation, suggests that these proteins may be members of a large superfamily of membrane/cytoskeleton-associated transcriptional regulators that globally control the switch between cell proliferation and differentiation.

proteins, potentially transmitting signals from the membrane and actin cytoskeleton to regulate TAZ- and YAP-mediated transcriptional changes in the nucleus depending on the status of cell-cell adhesion.[25] Intriguingly, phosphorylation of YAP by Src-family kinases occurs at the membrane, and has been shown to required for its binding to, and inhibition of, Runx2, and for YAP localization into subnuclear compartments.[14] Whether similar signaling events at the membrane regulate TAZ function is not yet known.

In addition to their membrane and nuclear localization, both TAZ and YAP can be sequestered in the cytoplasm and rendered functionally inactive by binding to 14-3-3 proteins in a phosphorylation-dependent manner.[11,23] The protein kinase AKT has been proposed as the candidate kinase that regulates YAP-14-3-3 interactions.[23] Our unpublished observations suggest that AKT does not regulate the interaction of TAZ with 14-3-3, and the relevant kinase is currently unknown.

TAZ mRNA and protein levels change substantially as cells cease proliferating and initiate differentiation. TAZ levels increase several fold when mesenchymal stem cells are stimulated to undergo osteoblast differentiation by treatment with Bone Morphogenic Protein-2 (BMP-2), while conversely, TAZ levels decrease by ~50% in mesenchymal stem cells during the process of adipocyte differentiation

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