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miR-34s keep osteoblasts bone idle

An in vivo study reveals how an miRNA family inhibits osteoblast proliferation and differentiation.

The development of bone-forming osteoblasts is controlled by transcription factors such as Runx2, Osterix, and ATF4, which, in turn, are regulated by a variety of nuclear proteins that inhibit or activate these factors. miRNAs have also been implicated in osteoblast differentiation, though little is known about the effects of individual miRNAs on skeletogenesis in vivo. Wei et al. now identify a family of miRNAs that restrict bone formation by targeting a key regulator of osteoblast differentiation (1).

Embryonic mice whose osteoblast progenitors lack the miRNA-processing enzyme DICER have severe defects in skeletogenesis, whereas loss of the enzyme in adulthood causes enhances bone formation (2). Several studies have identified individual miRNAs that regulate the differentiation of cultured osteoblasts in vitro (3, 4), but, says Gerard Karsenty, from Columbia University in New York, "there hasn't been a systematic analysis in vivo for the loss-of-function of a given miRNA."

Karsenty and colleagues, led by Jianwen Wei, therefore looked for miRNAs specifically expressed in differentiating osteoblasts. "We realized that two members of the miR-34 family—miR-34b and miR-34c—were highly enriched in osteoblasts but poorly expressed in many other cell types," explains Karsenty. Moreover, these miRNAs were predicted to target the mRNA encoding Satb2, a nuclear matrix protein that promotes osteoblast differentiation by increasing the activity of Runx2 and ATF4 (5). "That caught our attention," Karsenty says.

To investigate the miR-34 family's function in bone formation, Wei et al. generated mice whose osteoblasts overexpressed miR-34c. These mice had fewer osteoblasts, slower rates of bone formation, and lower overall bone mass. Knocking out the miRNAs had the opposite effect: mice whose osteoblasts lacked both miR-34b and miR-34c had increased numbers of osteoblasts and enhanced bone formation. Indeed, embryonic mice initiated skeletogenesis earlier in the absence of the miR-34 family, indicating that the miRNAs usually inhibit osteoblast differentiation.

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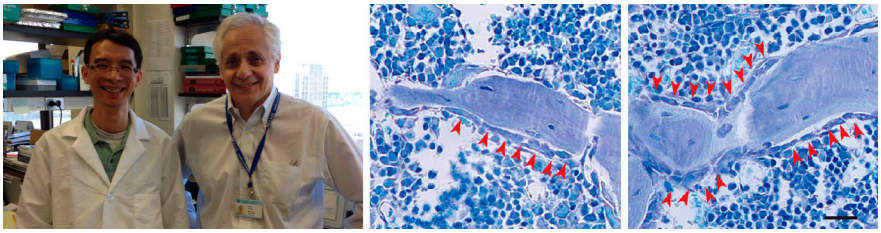
Wei and colleagues confirmed that miR-34b and -c target a sequence in the 3′ untranslated region of the *Satb2* mRNA, blocking its translation in osteoblasts. Satb2 protein levels were thus reduced in the bones of mice overexpressing miR-34c and elevated in mice lacking the two miRNAs. To determine whether the targeting of Satb2 explained the miR-34 family's effects on osteoblast differentiation, the researchers removed one copy of the *Satb2* gene from miR-34 knockout mice. Satb2 protein levels returned to normal, and the mice accumulated bone at the same rate as wild-type animals.

The effects of miR-34b and -c go beyond differentiation, however. Wei et al. found that the miRNAs also reduce osteoblast proliferation. "We thought—based on the fact that Satb2 was a good target—that miR-34b and -c would affect differentiation," says Karsenty. "But we didn't expect there to be an effect on proliferation as well."

Wei et al. therefore searched for cell cycle proteins that might be regulated by the miR-34 family and found target sequences in the mRNAs encoding Cyclin D1, CDK4, and CDK6. The levels of all three of these proteins were elevated in osteoblasts lacking miR-34b and -c and suppressed when miR-34c was overexpressed.

"miRNAs are often considered to have a minor, fine-tuning effect on gene expression that is best seen when you challenge the mice," says Karsenty. miR-34b and -c, however, have a significant impact on bone development and homeostasis, even in unchallenged animals. Karsenty and colleagues now want to investigate how the expression of miR-34 family members is regulated and to look for additional miRNAs that regulate skeletogenesis. "My guess is that there are other miRNAs—that may not be osteoblast specific—that have the opposite effect to miR-34s on osteoblast differentiation and proliferation," Karsenty says.

Focal Point



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Jianwen Wei (left), Gerard Karsenty (right), and colleagues (not pictured) identify two miRNAs that inhibit the differentiation and proliferation of bone-forming osteoblasts. miR-34b and miR-34c decrease bone formation by reducing levels of the osteoblast differentiation factor Satb2 and the cell cycle proteins Cyclin D1, CDK4, and CDK6. Compared with control animals (center), mice whose osteoblasts lack the two miRNAs (right) have increased numbers of osteoblasts (red arrowheads) as well as earlier induction of skeletogenesis and greater bone mass in adulthood.

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