CLINICAL IMPLICATIONS OF BASIC RESEARCH

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How Stem Cells Turn into Bone and Fat

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Stem cells and progenitor cells reside in various tissues of the body and are capable of generating daughter cells of different lineages. Mesenchymal stem cells (MSCs) are multipotent stromal cells that reside in tissues such as bone marrow, adipose tissue, liver, and skeletal muscle. MSCs are known to contribute to tissue repair and are capable of differentiating into osteoblasts (bone), adipocytes (fat), or chondrocytes (cartilage).[1] How a single MSC generates cells of completely different phenotypes has been a mystery. A recent study by Rauch et al.[2] provides some clues that have ramifications for our understanding of obesity and disorders of bone mineralization.

In their study, Rauch et al. isolated human MSCs from bone marrow and adipose tissue and then treated the MSCs in the laboratory with a cocktail of chemicals that is known to induce differentiation of MSCs into either fat or bone. They observed that adipogenic differentiation was associated with a change in expression of a greater number of genes than was osteoblastic differentiation, and the magnitude of induction of the adipogenic-specific genes was far greater than that of osteoblast-specific genes. The authors attributed these differences to the fact that the undifferentiated MSC resembled the osteoblast more than the adipocyte. Most of the gene-expression machinery harnessed by the undifferentiated MSC for differentiation into an osteoblast was already turned on; hence, morphing into an osteoblast required amplification of gene networks that were already active. In contrast, differentiation into an adipocyte involved the expression of a completely new set of genes along with silencing of genes that were active in the undifferentiated MSC.

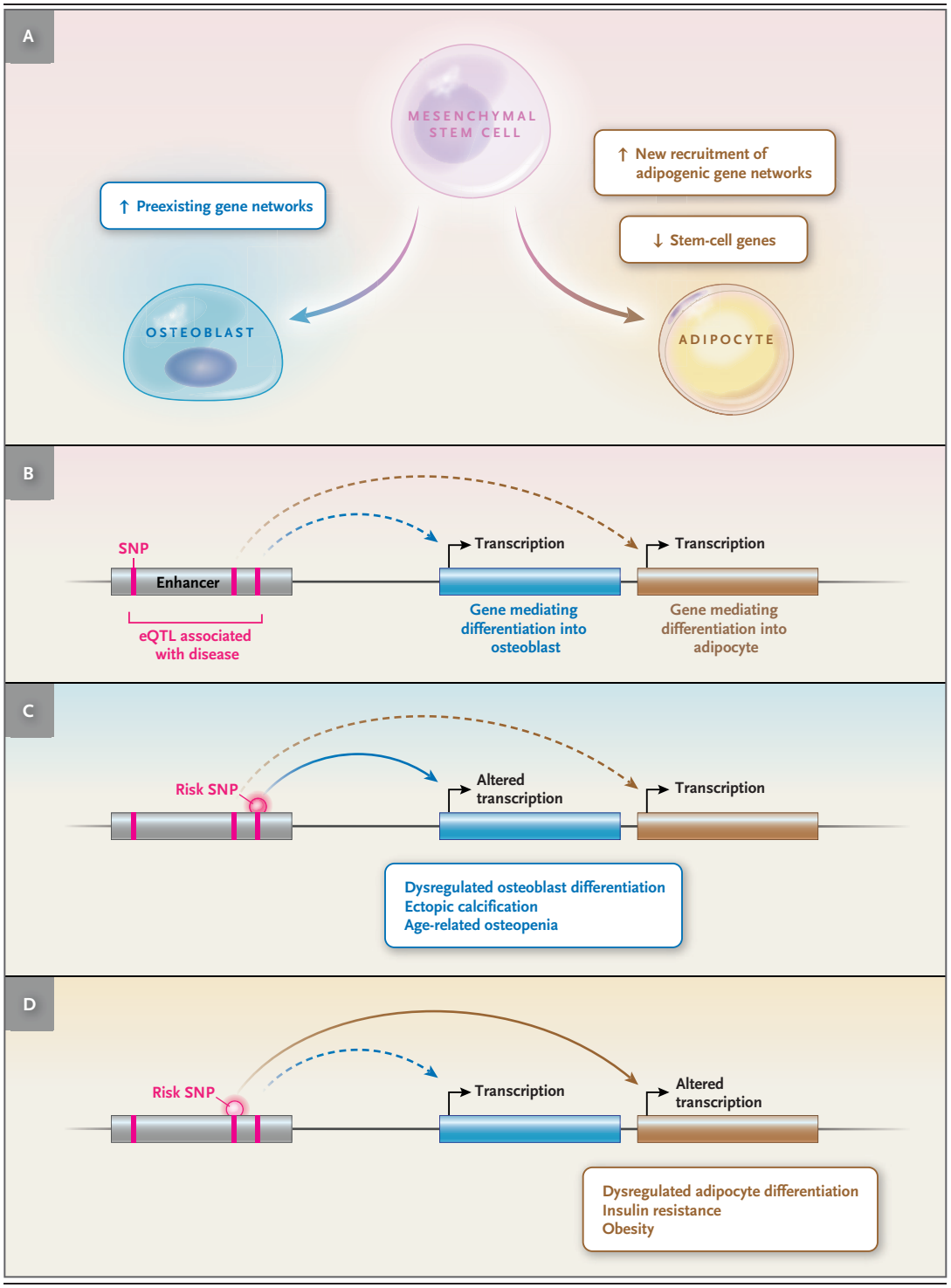
To obtain an insight into the molecular mechanisms of specification and induction of adipogenic or osteogenic genes, Rauch et al. looked at segments of DNA called enhancers, which regulate transcription and thus expression of a gene. Enhancers can be present hundreds or thousands of bases upstream or downstream from the gene and can be thought of as amplifiers of gene expression. When enhancers are in an open position, they readily allow binding of proteins called transcription factors, and the enhancer elements along with the attached proteins interact with elements of the gene to augment expression. Conversely, when enhancers are in a closed position, transcription factors cannot bind to them, and gene expression is attenuated. The authors observed that enhancers regulating the expression of osteoblast-specific genes such as alkaline phosphatase, biomineralization associated (*ALPL*), were already in an open position in undifferentiated MSCs, thus allowing easy access to transcription factors and differentiation into an osteoblast. In contrast, enhancers regulating the expression of key adipogenic genes were in a closed position, and adipogenic differentiation involved extensive remodeling of

Figure 1 (facing page). Differentiation of Human MSCs into Osteoblasts or Adipocytes and Implications for Human Disease.

Panel A shows the differentiation of human mesenchymal stem cells (MSCs) into osteoblasts, a process that primarily involves activation of preexisting gene networks, and adipogenic differentiation, a process that involves chromatin remodeling with new enhancer activation, recruitment of adipogenic genes, and simultaneous silencing of stem-cell genes. Panels B through D show models of how dysregulated MSC differentiation can be associated with human diseases of bone and adipose tissue: Panel B shows how disease-associated single-nucleotide polymorphisms (SNPs) and expression quantitative trait loci (eQTL) affecting enhancer regions can inhibit or augment adipogenic or osteogenic differentiation; Panel C, how dysregulated osteoblast differentiation can be associated with ectopic calcification or age-related osteopenia; and Panel D, how dysregulated adipogenic differentiation can be associated with insulin resistance or obesity.

chromatin to bring these enhancers to an open position. Thus, the hurdles or the genetic barriers that the MSC must overcome to become an adipocyte are far greater than those it must overcome to become an osteoblast.

The enhancers identified by Rauch et al. appear to be clinically relevant. Sequence variations in the human genome can be associated with quantitative differences in the degree of expression of genes in various tissues. Such genomic



loci that determine differences in the degree of expression of a particular gene are known as expression quantitative trait loci. The authors observed that expression quantitative trait loci known to affect the expression of osteogenic and adipogenic genes in humans were abundantly enriched in the enhancers regulating adipogenic and osteogenic differentiation. In this regard, single-nucleotide polymorphisms (SNPs) associated with human diseases of adipose or bone function were also substantially enriched in enhancers that showed dynamic activation during adipogenic or osteogenic differentiation. Thus, SNPs that disrupt enhancer function or gene-expression networks driving the adoption of adipogenic or osteogenic fates could cause or affect the disease phenotype. Using computational approaches, the authors identified 12 key transcription factors that were highly expressed and could bind specific motifs (DNA sequences) in the enhancer regions and regulate expression of key genes driving adipogenesis or osteogenesis. Silencing these transcription factors significantly inhibited differentiation of the MSC into an osteoblast, decreased expression of *ALPL*, and attenuated mineralization. At the same time, silencing these transcription factors augmented adipogenesis and lipid accumulation; this shows how specification of a human adult stem cell to adopt a target cell fate can be simply altered by modulation of transcription factors. The authors observed varying expression of these transcription factors in biopsy specimens of human bone or adipose tissue or in MSCs and preadipocytes obtained from patients with obesity, osteoporosis, or age-related dysfunction — a finding that corroborates the clinical relevance of their overall observations.

The principal conclusions of the study by Rauch et al., when placed in clinical context, have implications for human MSC biology and the physiology of bone and adipose tissue in health and disease. Ectopic calcification is a common pathologic condition that results in mineralization of soft tissues such as the heart valves, blood vessels, and skeletal muscle. In affected tissues, stromal cells are thought to upregulate osteogenic genes, differentiate into an osteogenic phenotype, and contribute to mineralization.[3,4] The conclusions by Rauch et al. suggest that the gene-regulation machinery is already primed in such cells to adopt osteogenic fates, and this creates a permissive environment for the stromal cells to assume osteogenic fates in response to environmental cues. Conversely, defects or a delay in fracture healing could be caused by the inability or malfunction of such gene networks to efficiently drive MSCs to adopt osteogenic cell fates. Aging and obesity induce ectopic adipocyte accumulation in the bone marrow, a process that impairs hematopoietic and osteogenic regeneration.[5] More specifically, aging is known to inhibit the osteogenic lineage (age-related osteopenia), whereas a high-fat diet promotes adipogenic expansion.[5] Rauch et al. provide a rational platform to understand and target such age-related changes, in which environmental cues or genetic factors affecting a core group of enhancers or transcription factors would be sufficient to drive bone marrow MSCs to a specific fate and contribute to a pathologic phenotype (Fig. 1). The transcription factors that activate osteogenic or adipogenic enhancers, highlighted by Rauch et al., show changes in expression in various pathophysiologic states such as osteoporosis, obesity, insulin resistance, and aging. A detailed investigation of such transcription factors and lineage-committing genes provides an experimental path for the potential identification of therapeutic targets for obesity, age-related osteopenia, and ectopic calcification.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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