Traditionally, it has been considered the province of proteins to regulate cell lineage determination in both embryonic and adult cellular differentiation. Homeodomain proteins, the products of homeobox genes, are the classic rulers of overall embryonic body patterning with its axial symmetries and asymmetries. These homeodomain transcription factors along with other morphogenetic proteins also influence identity in adult tissues, through downstream targets, some of which remain unknown.

In blood and tissue, lineage determination seems to occur through a hierarchical organization. The best-characterized differentiation hierarchy is that of the hematopoietic lineages in bone marrow. Precursors of blood cells are guided to differentiate along sequentially branching myelocytic or erythrocytic lineages by proteins collectively termed the “colony-stimulating factors.” Precursors of connective tissue and other mesenchymal cells also seem to follow a lineage hierarchy, remarkably similar to that of neural crest cells in the embryo. Just as hematopoietic precursor cells persist as stem cells in adult marrow, there is now evidence that pluripotent mesenchymal precursor cells, possibly qualifying as “stem” cells, also persist in adult tissues. Most importantly, these so-called mesenchymal stem cells actively re-populate connective tissue. This has important implications for human health, given the potential to harvest and harness these cells for cultivation and replacement at sites of non-healing injuries. Implementation of such a novel potential therapy will require detailed knowledge of the factors that determine commitment to a specific lineage. Some of the regulatory factors orchestrating mesenchymal differentiation have been identified, but many remain unknown.

A particularly interesting aspect of the mesenchymal differentiation hierarchy is that it seems to be regulated not only by protein factors but also by lipids and lipid-derived molecules. Sphingolipids, gangliosides, and ceramides have known roles in differentiation. Sterols also influence cellular differentiation. Calcitriol is a classic regulator of osteoblastic and osteoclastic differentiation, and it promotes expression of I-mfa, an inhibitor of the MyoD family. Certain oxysterols signal differentiation through the liver X receptor. The modified lipoprotein, oxidized LDL, induces osteoblastic differentiation of vascular cells and inhibits differentiation of bone-derived preosteoblasts. In bone, osteoblastic differentiation is regulated by the LDL receptor–related protein, LRP5. By preliminary report, loss of bone in mice with defective LRP5 is reversed by treatment with inhibitors of the cholesterol biosynthetic pathway (statins). Sonic hedgehog (Shh) is a critical developmental factor in embryonic development, particularly for membranous bone formation. It directs commitment of mesenchymal precursor cells to the osteoblastic versus adipocytic lineage. Facial deformities including cyclopia occur in sheep whose mothers ingest a weed that blocks cholesterol biosynthesis. It is now understood that Shh function requires covalent binding with cholesterol. Thus, when the cholesterol substrate for this modification is lacking, craniofacial development would be disrupted, leading to premature fusion of the centerline and a single eye.

Even neuronal survival is regulated by fat-related molecules. The prostaglandin derivative 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) regulates neurogenesis. In addition, recent evidence indicates that both oleic acid and cholesterol provided by neighboring astrocytes regulate the final stages of neurogenesis including synapse formation and maintenance. Interestingly, this process depends on endogenous cholesterol biosynthesis, a finding with important potential implications for long-term use of statins, at least some of which cross the blood-brain barrier.

Many of the lipid factors that regulate differentiation, including eicosanoids such as 15d-PGJ2, fatty acids, oxidized LDL and its components such as HETE and HODE, and other fatty acid derivatives are known activators or ligands of a family of nuclear receptor transcription factors, the peroxisome proliferator-activated receptors (PPARs), which are highly expressed in fat tissue. Additional lipid factors may yet be found to act through PPARs. Thus, in many instances, lipid regulation of differentiation may be through PPARs. One of the PPAR subtypes, PPARγ, is already established as a master regulator of adipogenic differentiation, comparable to MyoD for myocyte differentiation. Shh-cholesterol acts in part by inhibition of PPARγ. PPAR activation also induces caspase-dependent apoptosis in neurons.

In this context, it is intriguing that Oyama and colleagues report in this issue of Circulation Research that PPARγ activation inhibits osteopontin (OPN) expression by interference with binding at a site in the OPN promoter that can bind homeodomain transcription factors. This observation adds to the evidence for possible crosstalk between PPARs and homeoprotein binding sites. It was previously shown that PPARγ2 inhibits Runx2 (Cbfa-1), the master regulator for
osteoelastic identity, as well as the inhibition of Runx2 and the osteocalcin fibroblast growth factor response element–binding protein by the homeodomain protein Msx2.\textsuperscript{23,24} These findings link lipid factors directly to fundamental lineage determination.

OPN is a multifunctional matrix protein that regulates migration, adhesion, survival, and inflammation.\textsuperscript{25} Overall, these functions seem to orchestrate response to injury or wound healing.\textsuperscript{26} OPN is a component of the differentiation pathways for both osteoblasts and osteoclasts. Osteoclasts anchor to extracellular OPN within bone matrix, allowing these bone-resorbing cells to cling to the surface of mineralized bone matrix and create a tight seal, hence the origin of its name “bone-bridge.” Cancer cells also use OPN to block attack by phagocytic cells, trapping them in the extracellular matrix and inhibiting their production of cytotoxic radicals such as nitric oxide.\textsuperscript{27} OPN is also produced in the matrix of atherosclerotic lesions and is likely to have a role in vascular calcification. Thus, downregulation of OPN by treatment of OPN in bone matrix and inhibiting their production of cytotoxic radicals such as nitric oxide.\textsuperscript{27} OPN is also produced in the matrix of atherosclerotic lesions and is likely to have a role in vascular calcification. Thus, downregulation of OPN by treatment of PPAR activators including promotion of reverse cholesterol transport via ABCA1 in vitro,\textsuperscript{31} inhibition of monocyte adhesion to endothelial cells via inhibition of endothelial VCAM-1 expression in vitro,\textsuperscript{32} and reduction of blood pressure and beneficial changes in lipid status in vivo.\textsuperscript{33}

The ability of PPAR activators to determine cell lineage raises other implications for lipid differentiation factors in disease processes. Differentiation gone awry in response to excess lipid derivatives may explain the formation of ectopic tissue, including cartilage, bone, fat, and even marrow, in atherosclerotic lesions. Lipids may also have a role in osteoporosis in which bone stroma is replaced with fat. Stromal cells represent about 1% of the cells in the bone marrow. These immature mesenchymal precursor cells can differentiate into either adipocytes or osteoblastic cells. The decision between the two lineages is regulated, in part, by PPAR\(\alpha\), with activation inhibiting osteoblastic in favor of adipogenic differentiation of these stromal cells.\textsuperscript{34} Osteoporosis also results from excess osteoclastic resorption relative to osteoblastic formation of bone, and this is also regulated by lipids. Monocytes may differentiate along the macrophage and osteoclast lineage, and this choice is also influenced by PPAR activators.\textsuperscript{35} PPAR activation also inhibits myogenic differentiation.\textsuperscript{36}

By clever design, these powerful lipid-like molecules and their precursors are readily accessible from a large reservoir surrounding the cell, the plasma membrane. Cytosolic phospholipases can readily clip these molecules from the lipid bilayer, as needed, and they may be converted to a variety of active PPAR agonists by cytosolic cyclooxygenases and lipoxygenases. The potent activity of lipid-like molecules in adult tissue differentiation leads to speculation about a higher-ranking position in embryonic development as well as importance in aging. Even the very first developmental event in the fertilized ovum, asymmetric orientation into vegetal and animal poles, may derive from a gradient of phospholipid oxidation in the plasma membrane radiating from the site of fertilization. At the other end of the life span, accumulation of lipids in tissues may generate unintended differentiation signals and confused identities in mesenchyme, ultimately leading to the painful changes of old age—hardening of soft tissue and softening of hard tissue.

References

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