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Jawing about TNF: New Hope for Cherubism

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Mutations in the SH3-domain binding protein 2 (SH3BP2) are known to cause a rare childhood disorder called cherubism that is characterized by inflammation and bone loss in the jaw, but the mechanism has remained unclear. In this issue, Ueki et al. (Ueki et al., 2007) now demonstrate that a cherubism mutation activates mouse Sh3bp2 resulting in enhanced production of the cytokine TNF-α by myeloid cells, leading to both bone loss and inflammation.

Cherubism is a dominantly inherited disorder that is characterized by bone destruction in the upper and lower jaws and is accompanied by inflammation and fibrosis. The disease typically begins in children before the age of four and regresses at puberty. The cherubic appearance of children with the disorder, characterized by an upward gaze and round face, stems from facial swelling. Although the disease was first described in 1933, its pathogenesis has remained elusive. Genetic analysis has established several point mutations in the *Sh3bp2* gene, which encodes a widely expressed scaffold protein (Imai et al., 2003; Ueki et al., 2001). However, the function of this multidomain protein, the nature of the mutations (activating or dominant-negative), and the primary cells involved in pathogenesis have been unclear. The disease is disfiguring during the active phase, and there are permanent dental complications. Although surgical intervention is sometimes successful, most patients do not receive any disease-modifying treatment. Thus, the discovery by Ueki et al. (2007) that cherubism is caused by enhanced cytokine tumor necrosis factor α (TNF-α) production by myeloid cells due to an activating mutation in Sh3bp2 not only represents a major advancement in our understanding of the pathogenesis of the disease but suggests new potential options for its treatment.

Although the heritable nature of cherubism is well accepted, Ueki et al. are the first to establish an experimental model of the disease, recapitulating the most common human Sh3bp2 mutation (P418R) in mice. They created a knockin mouse model in which the disease causing proline to arginine substitution is introduced in the mouse *Sh3bp2* gene (P416R). They find that there is inflammation and bone resorption in the jaw in mice homozygous for the mutation but also a global decrease in bone mass and systemic myeloid inflammation. Thus, although the model reproduces the classical phenotypes of cherubism, it is not restricted to the jaws and behaves primarily as an autosomal-recessive disease, leaving open the possibility that additional factors contribute to the human disease.

The histology of affected tissue from cherubism patients is very similar to fibrous dysplasia (a disease involving cells of the mesenchymal lineage), with a mixture of fibroblasts, bone-resorbing osteoclasts, and inflammatory cells. The course of cherubism is coincident with tooth eruption, and many patients fail to develop second and third molars. This has led to the proposal that cherubism is due to defective signaling in mesenchymal cells associated with tooth development, with enhanced activation of osteoclasts (the cells that resorb bone) as a secondary consequence (Hyckel et al., 2005). The issue of the pathogenic cell has been resolved with the mutant *Sh3bp2* knockin model. Myeloid osteoclast progenitors from mice bearing one or two mutant *Sh3bp2* alleles formed more osteoclasts in culture, with higher resorptive activity. Furthermore, inflammation and bone loss were transferred by bone marrow transplantation of mutant *Sh3bp2* marrow into recipients with normal *Sh3bp2*, demonstrating that the disease is caused by mutant *Sh3bp2* in a hematopoietic cell. To further define the pathogenic cell type, the authors crossed the *Sh3bp2* knockin mice with mice deficient in *rag1*, which do not have functional lymphocytes, and with *op*-deficient mice, which lack myeloid cells. Mutant *Sh3bp2* caused bone loss in the absence of lymphocytes but not in the absence of myeloid cells. Thus, the primary target for disease therapy should be the myeloid lineage.

Osteoclasts are multinucleated, terminally differentiated cells derived from the myeloid lineage, which degrade both the organic and mineralized components of bone. The primary factor responsible for osteoclast differentiation is RANKL, which signals via its receptor RANK and leads to acti-

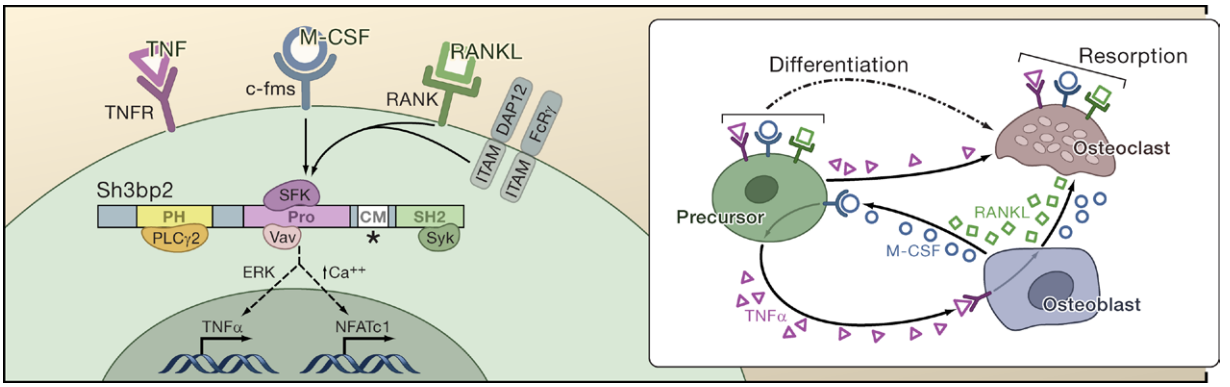

Figure 1. Involvement of Mutant Sh3bp2 in Cherubism

Mutations in Sh3bp2 cause cherubism, which is characterized by inflammation and bone loss in the jaw. Sh3bp2 is a multidomain scaffolding protein with a pleckstrin-homology domain (PH), a proline-rich domain (Pro), and a Src-homology 2 domain (SH2). Most of the known cherubism mutations (CM) lie in a stretching region between the Pro and the SH2 domains. Downstream of stimulation by the cytokines M-CSF and RANKL, the Sh3bp2 P416R mutant (Sh3bp2*) regulates the formation of a complex containing a Src family kinase (SFK), Syk, PLCγ2, and Vav. In mice expressing Sh3bp2*, TNF production by osteoclast precursors is elevated in response to M-CSF via activation of the ERK signaling pathway. Sh3bp2* also leads to increased osteoclastogenesis via upregulation of the calcium-dependent gene NFATc1 downstream of RANKL and the costimulatory receptors Dap12 and FcRγ that contain ITAMs (immunoreceptor tyrosine-based activation motifs). (Inset) In mice expressing Sh3pb2*, osteoclast precursors express more TNF than wild-type mice leading to upregulation of M-CSF and RANKL by osteoblasts. The combination of M-CSF, RANKL, and TNF promotes the differentiation of osteoclast precursors and augments the bone-resporptive activity of mature osteoclasts.

vation of NF-κB- and MAPK-dependent pathways. However, costimulatory signals emanating from FcRγ or Dap12 (transmembrane receptors harboring cytoplasmic immunoreceptor tyrosine-based activation motifs or ITAMs) are also required for osteoclastogenesis (Koga et al., 2004). RANKL-mediated phosphorylation of ITAMs occurs via Src family kinases and results in the recruitment of Syk kinase (Mocsai et al., 2004), activation of phospholipase Cγ2 (PLCγ2), and the mobilization of intracellular Ca^{2+}, which is critical for upregulation of NFAT-c1, the master transcription factor in osteoclasts (Mao et al., 2006). Interfering with any of the above molecules impairs the differentiation and function of osteoclasts. Sh3bp2 mediates ITAM-dependent activation of calcium signaling and NFATc1 upregulation in lymphocytes (Deckert et al., 1998). In these cells Sh3bp2 binds to Src family kinases, Syk, PLC-γ, and Vav guanosine exchange factors. Ueki and colleagues have now determined that the Sh3bp2 P416R cherubism mutation in the osteoclast lineage leads to hyperphosphorylation of Syk at Y346 and increases Syk activity in response to RANKL. Therefore, it is likely that Sh3bp2 also serves as an ITAM-dependent scaffold in osteoclast precursors, bringing together Src family kinases, Syk, and PLCγ2 (Figure 1).

The myeloid progenitors of osteoclasts are also dependent on signaling by macrophage colony-stimulating factor (M-CSF). Interestingly, the Sh3bp2 P416R mutation also leads to enhanced M-CSF signaling to the MAPK-ERK pathway. Consistent with this observation, a recent report showed that activation of ERK and of its upstream kinase MEK1 was impaired in B cells from mice lacking Sh3bp2 (de la Fuente et al., 2006), possibly due to impaired Ras activation mediated by PLCγ2. Further work will be necessary to determine the exact pathways by which Sh3bp2 mediates signaling downstream of M-CSF.

TNF-α potently activates osteoclasts and is responsible for a large proportion of bone loss in inflammatory arthritis, periodontal disease, and prosthetic implant loosening. Therefore, it is perhaps surprising that the role of TNF-α in cherubism has not previously been explored. In the mutant *Sh3bp2* knockin mice, increased levels of TNF-α in serum correlated with systemic inflammation and bone loss. When crossed onto a TNF-α null background, the mutant *Sh3bp2* knockin mice no longer lose bone nor show evidence of inflammation, demonstrating that TNF-α is required for these pathologies. Isolated myeloid cells from the knockin mice produced large amounts of TNF-α in response to M-CSF treatment (in an ERK-dependent manner), and transplantation of marrow from these same mice led to an elevation of serum TNF-α along with inflammation and bone loss. Thus, it appears that TNF-α generated by myeloid cells participates in a positive feedback loop, enhancing M-CSF and RANKL production by osteoblasts (Figure 1), which stimulates more TNF-α synthesis by the precursors. Acting on the precursors, the combination of M-CSF, RANKL, and TNF-α is potently osteoclastogenic (Kitaura et al., 2005), increasing the numbers of mature osteoclasts, which themselves are stimulated by the same set of cytokines for bone resorption.

Although cherubism was described more than 70 years ago, the rather sparse literature in this area has provided little insight on disease mechanisms or effective therapies. For patients with this rare disease, the identification of TNF-α as a major pathogenic factor is significant news. Anti-TNF therapies are already in clinical practice for the treatment of rheu-

matoid arthritis. Should these drugs also prove effective in the treatment of cherubism it can be hoped that the interval from laboratory discovery to clinical use would be short.

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Hairy Math: Insights into Hair-Follicle Spacing and Orientation

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Hair follicles in the skin have a characteristic spacing and orientation. Two recent papers (Sick et al., 2006; Wang et al., 2006) report the use of contrasting mathematical models and experimental manipulations to gain insight into the mechanisms underlying patterns of hair-follicle distribution and orientation.

Mathematical and computational models can play an important role in integrating the wealth of information generated by molecular biology and genetics. They are particularly useful for understanding how phenomena at the tissue or organismal level arise from networks of molecular interactions within and between cells. Ideally, such models should offer biological insight into complex phenomena and generate new testable hypotheses. The authors of two recent papers (Sick et al., 2006; Wang et al., 2006) appearing in *Science* and in the *Proceedings of the National Academy of Sciences* use contrasting approaches to model observed patterns of hair-follicle distribution and orientation. In one case the model is based on known and predicted properties of key signaling molecules, whereas the other model is more abstract and does not depend on specific biological parameters.

Hair follicles are found in regular arrays in which large primary follicles that develop first are interspersed with smaller, secondary follicles that develop at a later stage. Both primary and secondary follicles are uniformly oriented in an anterior-posterior direction (Figure 1). Turing first proposed a reaction-diffusion (R-D) model that explained how amplification of small initial fluctuations of an activator and inhibitor that interact could lead to development of a spatial pattern of morphogens (Turing, 1952). More recently, Jung et al. showed in skin explant cultures from chick embryos that the size, number, and distribution of developing feather buds could be altered by addition of specific morphogens (Jung et al., 1998). Insight into the molecular signals that direct initial patterning of the hair-follicle array has been provided by experiments showing that follicle induction is blocked by ectopic expression of Dickkopf1 (Dkk1), a secreted Wnt/β-catenin inhibitor (Andl et al., 2002), and is stimulated by constitutive activation of the Wnt/β-catenin pathway (Gat et al., 1998; Kuraguchi et al., 2006). These observations identify Wnt/β-catenin signaling as a key initiating