REVIEW ARTICLE

Molecular basis of cranial suture biology and disease: Osteoblastic and osteoclastic perspectives

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Abstract   The normal growth and development of the skull is a tightly regulated process that occurs along the osteogenic interfaces of the cranial sutures. Here, the borders of the calvarial bones and neighboring tissues above and below, function as a complex. Through coordinated remodeling efforts of bone deposition and resorption, the cranial sutures maintain a state of patency from infancy through early adulthood as the skull continues to grow and accommodate the developing brain's demands for expansion. However, when this delicate balance is disturbed, a number of pathologic conditions ensue; and if left uncorrected, may result in visual and neurocognitive impairments. A prime example includes craniosynostosis, or premature fusion of one or more cranial and/or facial suture(s). At the present time, the only therapeutic measure for craniosynostosis is surgical correction by cranial vault reconstruction. However, elegant studies performed over the past decade have identified several genes critical for the maintenance of suture patency and induction of suture fusion. Such deeper understandings of the pathogenesis and molecular mechanisms that regulate suture biology may provide necessary insights toward the development of non-surgical therapeutic alternatives for patients with cranial suture defects. In this review, we discuss the intricate cellular and molecular interplay that exists within the suture among its three major components: dura mater, osteoblastic related molecular pathways and osteoclastic related molecular pathways.
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Introduction

The human skull is formed from nine cranial bones, including two frontal bones, two parietal bones, two temporal bones, one ethmoid bone, one sphenoid bone, and one occipital bone. These bones articulate with one another at joints composed of fibrous tissue, also known as cranial sutures. The areas where several sutures come into contact are called fontanelles. The skull contains a number of sutures, including the sagittal suture, located between the two parietal bones, the coronal sutures, located between the two frontal and parietal bones, the metopic suture, located between the frontal bones, the lambdoid sutures, located between the supraoccipital and parietal bones, and the squamosal suture, located between the temporal, parietal, and sphenoid bones (Fig. 1A—C) During suture formation, the neighboring bone fronts of the calvarial bones come into close proximity to one another. The bones either abut at the suture site, as is the case at the sagittal and metopic sutures, or overlap, which occurs at the coronal and lambdoid sutures.[1] In addition to these cranial sutures, there are also a number of facial sutures present in the human craniofacial skeleton — however, the embryology, anatomy, and research pertaining to the facial sutures are outside the scope of this review.

The human skull begins to form between days 23—26 of gestation, from both mesoderm- and neural crest-derived tissues. During development, these sutures remain patent to allow for the expansion of the cranial vault and the underlying brain. The metopic suture is the first to undergo fusion at approximately 9 months of age, while the sagittal suture does not fully close until adolescence or later.[2,3] As the calvarial bones continue to grow, cranial sutures also act as important areas of new bone formation and bone turnover, and facilitate skull growth in the direction perpendicular to their suture orientation.

Bone can form in one of two ways: either through endochondral or intramembranous ossification. Most bones in the human body undergo endochondral ossification, whereby mesenchymal stem cells first differentiate into chondrocytes, which secrete a cartilage matrix that eventually undergoes osteoblast-driven ossification. The bones of the cranial vault, however, undergo intramembranous ossification, a process that does not have a cartilage intermediate. Instead, mesenchymal cells, located between the dermal mesenchyme and the meninges, differentiate directly into osteoblasts, which then form bone through the secretion of an osteoid matrix.

The cranial suture can be thought of as a complex, composed of the two osteogenic bone fronts on either side of the suture, the mesenchymal tissue of the suture, the underlying dura mater, and the overlying pericranium (Fig. 2).[4] The cells in the middle of the mesenchymal tissue of the suture remain undifferentiated during cranial vault development, while the cells near the two osteogenic bone fronts typically become bone through the process of intramembranous ossification.

The tissues of the cranial suture complex all interact with one another to allow for proper suture formation and patency throughout development. In functional terms, suture formation and development must be in tight synchronicity with underlying organ development to facilitate
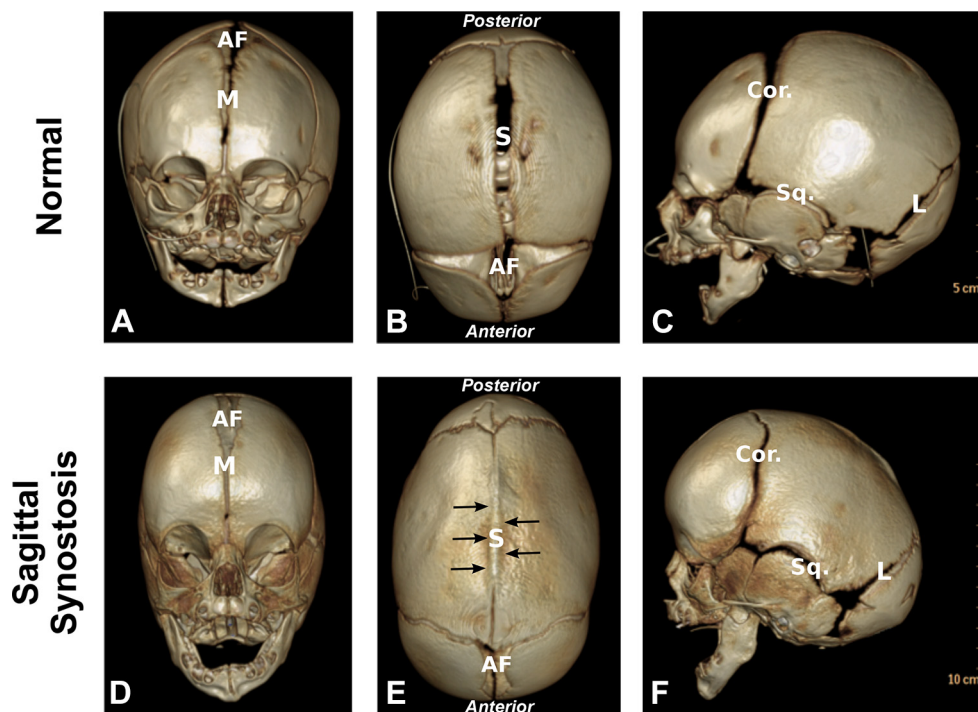

Figure 1   Three-dimensional computed tomography reconstructions of a 4-week-old patient with normal suture development (A—C) and an 8-week-old patient with sagittal synostosis (E—F). Anterior (A & D), vertex (B & E), and lateral (C & F) views. (A—C) Typical skull contour with patent sutures. (C—F) Elongated skull shape associated with fusion of the sagittal suture (arrows). AF, anterior fontanelle; M, metopic suture; S, sagittal suture; Cor., coronal suture; Sq., squamosal suture; L, lambdoid suture.
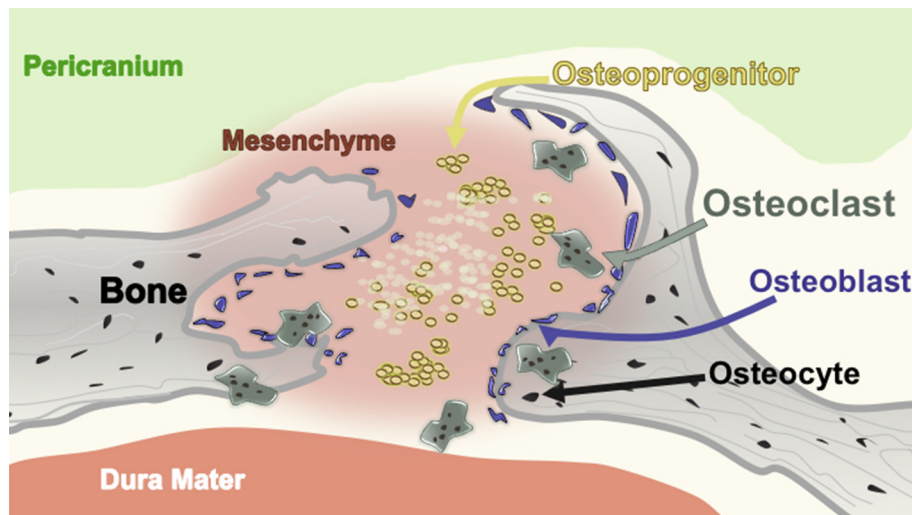
Figure 2 Schematic representation of the cranial suture complex. Two osteogenic bone fronts are bridged by mesenchymal tissue with associated osteoclasts, osteoblasts, and osteoprogenitor cells. The underlying dura mater and overlying pericranium also contribute to this functional complex (Figure courtesy of Justine C. Lee, MD, PhD).

growth. When it is not, functional impairment can ensue. A prime example is craniosynostosis, or premature fusion of one or more cranial and/or facial suture(s), which can occur in nonsyndromic or syndromic forms (Fig. 1D–F). Craniosynostosis is a condition affecting approximately one in 2500 live births worldwide.[5–7] Premature fusion of a cranial suture leads to failed expansion of the skull perpendicular to the affected suture and compensatory overgrowth parallel to it.[8] Such abnormal expansion can clinically manifest as craniofacial asymmetries, increased intracranial pressure, mental delay, severe proptosis, strabismus, visual and airway compromise, midfacial underdevelopment, and malocclusion. Currently, the only therapeutic measure for craniosynostosis is surgical correction.

However, from our growing knowledge about the etiopathogenesis of this disease, and more specifically the genetic basis of disease, a number of signaling pathways, cytokines, and growth factors have been shown to play important roles in proper cranial vault growth and expansion. Such knowledge may lead to new therapies that may be adjunctive to, or preclude, surgery.

We will discuss this delicate cellular and molecular interplay within the suture in its three major components: dura mater, osteoblastic related molecular pathways and osteoclastic related molecular pathways.

Dura mater related pathways

It has been long known that the dura mater plays some role in suture patency and closure. Classic studies in which murine cranial sutures typically programmed not to fuse, such as the sagittal suture, are transplanted to suture-fusing dura mater regions and then respond with fusion point to this critical relationship.[9] Similarly, Opperman and colleagues demonstrated that coronal suture devoid of underlying dura mater closed by 3 weeks, in contrast to its natural state to remain patent.[10] To this end, dura mater

cells have been shown to express FGF2, and varying concentrations of the TGFβ isoforms.[9,11] Despite this apparent relationship, no further elucidation of this interplay has been made in the clinical context.

Osteoblastic related pathways

Over the past several years, our knowledge of the molecular pathways governing suture patency and pathology from a dural and osteoblastic perspective has grown immensely. Principally, such discovery has stemmed from the identification of altered genetic expression, molecular pathways and environmental causes in forms of syndromic craniosynostosis. The most well-established signaling pathway associated with osteoblast differentiation in the context of suture maturation and disease is the fibroblast growth factor receptor (FGFR) pathway. This molecular signaling pathway is quite intricate, comprised of 4 different receptors and over 22 ligands.[12] During in utero development of the human fetus, expression of FGFR-1, -2, and -3 has been demonstrated within the cranial sutures.[13] Confirming the significant role of FGF signaling in this context, at least 3 point mutations in FGFR (FGFR2 S252W, FGFR2 C342Y, and P253R for example) correlate phenotypically with syndromic craniosynostoses such as Apert and Crouzon. Such mutations alter the level of constitutive receptor activation, ligand-receptor affinity, and the pattern of splicing and expression, which may in turn explain not only the mechanism of premature suture closure and disorder type, but also the phenotypic variability within each syndrome.[12,14] Lastly, this significant role has been corroborated in multiple genetically altered animal models.[15–18]

Other osteoblastic-related genes have been implicated in suture homeostasis and disease. Some of these genes have been elucidated from even more rare craniosynostosis syndromes, such as Boston-type craniosynostosis (MSX-2, a homeobox gene located on the long arm of chromosome 5),[19,20] Saethre-Chotzen syndrome (haploinsufficiency of

the Twist nuclear transcription factor gene),[21,22] and craniofrontonasal dysplasia (Eprhin B2),[23] to name a few. Owing to the contribution of osteogenesis genes in the importance of suture fusion, we more recently reported findings on a child with quadruplication of the RUNX2 gene, which encodes the Runt-related transcription factor 2 (aka core-binding factor subunit alpha-1),[24] a transcription factor essential for osteoblast differentiation.[25] It follows that our patient with overexpression of this gene exhibited multisutural craniosynostosis, midface hypoplasia and exorbitism akin to the classic dysmorphisms of the FGFR-related craniosynostoses.[24] In addition to these rare associations, transforming growth factor beta (TGFβ) superfamily genes such as TGFβ2, TGFβ3 and bone morphogenetic proteins (BMPs) all appear to play a role. More specifically, the expression of TGFβ2 has been shown to be important in a rabbit model of craniosynostosis,[26,27] whereas higher expression of TGFβ3 has been associated with suture patency. The same follows for BMP3. Nacamuli and colleagues found an inverse correlation of BMP3 expression and suture closure over time, suggesting that this factor aids in suture patency. Interestingly, rat calvarial osteoblasts stimulated with FGF2 or co-culture with nonsuture dural cells led to a decrease in BMP3 mRNA expression that ranged from 50% to 85%.[28] Despite experimental evidence for a role of TGFβ superfamily genes in these animal models, few human cases of TGFβ gene mutation have been described that supports this situation in the clinical scenario.

Osteoclastic related pathways

Dissimilar to our knowledge of molecular signaling relevant to suture osteoblast function, little information exists in regards to the role of osteoclasts in suture homeostasis and the signaling factors critical to their function within the suture. The sparse literature that exists, however, affirmatively indicates a role of osteoclast activity and suture morphology. Our major hypothesis is based upon the concept that suture homeostasis relies upon intricate crosstalk between osteoblasts and osteoclasts at the suture front. To this end, an exciting link has been made in our laboratory between immune cells and bone cells, thus establishing the field of osteoimmunology. The TNF superfamily signaling cascade involving TRANCE (RANK-L), its receptor RANK, and downstream adaptor proteins, TRAFs, have been shown to play an important role in osteoimmunology of skeletal homeostasis. RANKL is a factor expressed not only on activated T cells that mediates dendritic (antigen-presenting cell) survival, but also by osteoblasts, which modulates osteoclast development and differentiation. It follows that RANKL-deficient (KO) mice lack osteoclasts, have marked osteopetrosis with growth plate defects, resulting in growth retardation in the extremities, vertebrae and skull.[29] Moreover, mice that lack the cell receptor RANK have a similar phenotype to their ligand-deficient counterparts, as well as mice that lack one of the downstream signaling adaptor proteins, TRAF-6.[30] Conversely, mice lacking the soluble decoy-receptor, osteoprotegrin (OPG), which inhibits osteoclastogenesis in vitro, exhibit osteoporosis of the axial skeleton and have

an excess of osteoclasts.[31] In none of these reports were any descriptive or quantitative data produced regarding the suture morphology in these genetically altered animals. Additionally, although much research has investigated the role of immune modulators on skeletal homeostasis, little information existed in regards to the role of the RANKL-RANK-TRAF signaling cascade in regards to craniofacial development and suture biology.

More recently, we have identified a significant role of osteoclast activation and maintenance in suture biology through this signaling pathway (Fig. 3).[32] Specifically, sentinel work characterized a temporospatial relationship between RANK protein expression and physiologic cranial suture closure in a murine model. Further delineation of osteoclastic modulators through proteomic and candidate gene approaches may spawn the development of animal models for the investigation of nonsyndromic craniosynostosis, as well as molecular-targeted therapies to treat the clinical disease.

We further used whole organ culture experiments to determine the effects of RANK blockade on suture homeostasis. When explanted cranial sutures were maintained in culture, and infected with adenovirus bearing inhibitory RNA to RANK (siRANK), we found an increase in osseous bridging in such treated cranial sutures, as exhibited by an increase in Hounsfield units (HU) by microCT. Additional studies identifying overexpression of OPG in human craniosynostotic suture samples via Western blot analysis and failed posterofrontal suture closure in mice deficient in OPG via microCT, are the focus of another study from our work (unpublished data), which further implicates this signaling pathway in the context of suture homeostasis.

A critical feature to the RANK-RANKL-OPG axis is that it is highly dependent upon post-translational modification (ubiquitination and phosphorylation) to confer its downstream effects. Owing to this fact, Western blot analysis of protein extracts isolated from human cranial sutures demonstrated higher expression of ubiquitin-conjugated (osteoclast activating) TRAF-6 in patent sutures compared to craniosynostotic sutures (unpublished data).

Targeted molecular therapy for craniosynostosis: a new horizon?

Based on the discovery of multiple pathways described above, several molecular therapies have been proposed and tested in animal models of craniosynostosis. The overwhelming majority of these efforts have been made towards inhibition of osteoblastic activity and differentiation. To this end, Opperman and colleagues initially described a pro-patency effect of anti-TGFβ2 applied locally to suturectomy sites in rabbits, which would prevent the re-synostosis characterized in this animal model.[26,27] Interestingly, targeting another member of the TGFβ superfamily, landmark work by Warren et al demonstrated that Noggin, a BMP antagonist, was effective in inhibiting physiologic posterofrontal suture fusion in mice.[33] Furthermore, this inhibitory effect prevented, similar to the work of Opperman, re-synostosis in suturectomized mice.[34] This mechanistically echoes the pro-inhibitory effect of BMP3 on this signaling pathway and suture fusion. In
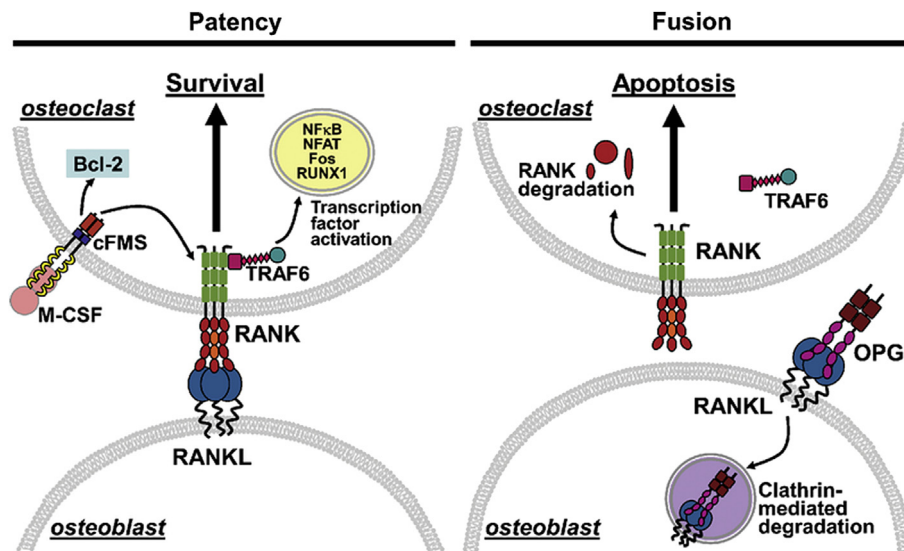
Figure 3 Model for RANK function in cranial suture biology. RANK receptors expressed on osteoclasts trimerize to interact with RANKL on osteoblasts or other involved cell types delivering survival signals to osteoclasts. In addition, the receptor—ligand complex or posttranslational modification of RANK prevent degradation of the receptor. A secondary role of macrophage colony stimulating factor potentiates RANK signaling by increasing the expression of RANK and Bcl-1. Decreasing activity downstream of RANK from protein degradation induces apoptosis. One of the possible mechanisms may involve the presence of the decoy receptor OPG. Reproduced from *J Craniofac Surg*. 2011; 22(2):699—705 with permission.

addition to these biological inhibitors, pharmacological inhibitors have been developed and shown to be relatively effective in abrogating the FGFR-[35] and MEK1-induced[36] pro-fusion suture morphology.

Stemming from our discussion above, it is of primary interest to our group to develop therapies that would stimulate osteoclast activation, whether through over-expression of RANK/RANKL or inhibition of OPG. In other words, we believe that not only osteoblast inhibition, but also osteoclast activation or both would be an effective anti-craniosynostotic strategy.

Conclusions and future directions

Cranial suture biology depends on a complex interplay between bone deposition and resorption. Elegant studies performed over the past decade have identified several genes critical for the maintenance of suture patency and induction of suture fusion. More recently, we have identified the potential significance of osteoclasts in the suture microenvironment. Specifically, in our recent studies, the essential regulators of osteoclastogenesis and osteoclast activation, RANK and the signaling active form of TRAF6, correlate to suture patency. Both RANK and TRAF6 are regulated post-transcriptionally. Furthermore, RANK knockdown via RNA interference is necessary and sufficient to induce increases in suture bone density consistent with fusion suggesting that dysregulation of RANK may serve a causal role in craniosynostosis. Lastly genetic abrogation of OPG expression correlates with failure of posterofrontal suture fusion in knockout mice. Future studies will be aimed towards proteomic analysis of post-translational differences between fused and patent suture samples. Molecular analysis will provide the basis for targeted

genetic/molecular alteration of osteoclasts and will provide a foundation for targeted therapies in nonsyndromic craniosynostosis.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

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