Molecular signaling in pathogenesis of craniosynostosis: the role of fibroblast growth factor and transforming growth factor–β

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The interplay of signals between dura mater, suture mesenchyme, and brain is essential in determining the fate of cranial sutures and the pathogenesis of premature suture fusion leading to craniosynostosis. At the forefront of research into suture fusion is the role of fibroblast growth factor and transforming growth factor–β, which have been found to be critical in the cell-signaling cascade involved in aberrant suture fusion. In this review, the authors discuss recent and ongoing research into the role of fibroblast growth factor and transforming growth factor–β in the etiopathogenesis of craniosynostosis. (DOI: 10.3171/2011.5.FOCUS1197)

Key Words • craniosynostosis • fibroblast growth factor • pathogenesis • transforming growth factor–β • molecular signaling

Regulation of suture fusion in craniosynostosis is a complex process involving the interplay of multiple signaling pathways, which results in premature and aberrant fusion of cranial sutures. Ongoing research in recent years has shown that most important among these pathways are those involving signaling mediated by fibroblast growth factor (FGF) and transforming growth factor (TGF)–β. In this review, we discuss the role of genetic mutations in the pathogenesis of craniosynostosis as well as recent and ongoing research into the physiological basis of normal and premature suture fusion mediated by FGF and TGF-β signaling.

Fibroblast Growth Factor Receptor Signaling

Genetics of FGFR Signaling

Fibroblast growth factor receptor (FGFR) signaling has been shown to be vital in many areas of skeletal development, with mutations in various craniosynostosis and dwarfism syndromes mapped to genes encoding for the extracellular domain, transmembrane domain, or tyrosine kinase domain of FGFRs. The majority of these mutations are gain-of-function mutations, suggesting a role for FGFRs in normal regulation of bone growth.

The 4 FGFRs (1–4) are part of a larger family of single-pass transmembrane tyrosine kinase receptors, with binding of FGF to FGFR in the presence of heparan sulfate proteoglycan as a cofactor leading to receptor dimerization at the cell surface and subsequent autophosphorylation that leads to phosphorylation of downstream signaling proteins.

FGFR2 is the most common receptor wherein mutations have been associated with craniosynostosis syndromes (Table 1). Crouzon syndrome was initially mapped to chromosome 10, position 10q25–26, in 1994 and linked to mutations in FGFR2. Most of these mutations lead to creation or destruction of cysteine residues. Other craniosynostosis syndromes subsequently linked to FGFR2 mutations include Apert syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome, Antley-Bixler syndrome, and Beare-Stevenson cutis gyrata syndrome. In Crouzon, Apert, and Pfeiffer syndromes, de novo mutations have been shown to arise only on the paternal chromosome. Interestingly, advanced paternal age was noted as a risk factor for mutations leading to Crouzon and Pfeiffer syndrome, suggesting that older men either accumulate or are more susceptible to a number of germline mutations.

Mutations in FGFR1 and FGFR3 have also been linked with craniosynostosis syndromes. Mutations in FGFR1 have been associated with Pfeiffer syndrome, while FGFR3 mutations have been linked with Crouzon syndrome with acanthosis nigricans and with Muenke syndrome.

Abbreviations used in this paper: FGF = fibroblast growth factor; FGFR = FGF receptor; TGF = transforming growth factor.
Table 1: Fibroblast growth factor receptor subtypes and synonyms associated with common syndromic craniosynostosis

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Synonyms</th>
<th>Syndromic Craniosynostoses</th>
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<tbody>
<tr>
<td>FGFR1</td>
<td>basic fibroblast growth factor receptor 1, Flg protein, N-SAM, OGD, HBGF, fms-related tyrosine kinase 2, FLT-2, CD331, CEK</td>
<td>Pfeiffer syndrome</td>
</tr>
<tr>
<td>FGFR2</td>
<td>Bek, K-Sam, CEK3, CFD1, BFR-1, CD332, ECT1, hydroxylprotein kinase, JW5, keratinocyte growth factor receptor, soluble FGFR4 variant 4, TK14, TK25</td>
<td>Crouzon syndrome, Apert syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome, Antley-Bixler syndrome, Beare-Stevenson cutis gyrata syndrome</td>
</tr>
<tr>
<td>FGFR3</td>
<td>Sam 3 protein, heparin-binding growth factor receptor, Mfr3</td>
<td>Crouzon w/ acanthosis nigricans, Muenke syndrome</td>
</tr>
</tbody>
</table>

 syndromes. The C749G (Pro250Arg) mutation in the gene for FGFR3 has also been attributed as a frequent cause of nonsyndromic coronal synostosis. While a number of mutations in FGFR1, FGFR2, and FGFR3 have been associated with craniosynostosis, analysis of FGFR4 showed that this receptor was unlikely to contribute significantly to craniosynostosis in humans.

A large number of mutations (more than 50, mostly missense mutations) have been identified in FGFR, which all lead via different mechanisms to gain-of-receptor function. These functions include constitutive activation of the receptor in a ligand-independent fashion or loss of ligand specificity, allowing the FGFR to be activated by splice variants, which do not normally have this function. Most mutations in FGFR2 localize to just 2 exons (IIia and IIIc), confirming the IgIIia/IIIC region as a mutation hotspot.

Loss of ligand specificity of FGFR2 has serious implications. Tissue-specific alternative mRNA splicing of FGFR2 normally creates 2 receptor isoforms, FGFR2b and FGFR2c, with specific ligand-binding properties. FGFR2b expression is restricted to epithelial lineages, with only FG7 and FG10 being able to activate FGFR2b. FGFR2c expression is normally restricted to mesenchymal lineages, with FG2, FG4, FG6, FG8, and FG9 being specific for FGFR2c. This specificity of ligand binding allows directional epithelial-mesenchymal signaling to occur during organogenesis and limb development. Loss of ligand-binding specificity toward different GFG isoforms may lead to varying severity of limb abnormalities. An example of loss of ligand specificity of FGFR2 was shown for Apert syndrome, with the S252W mutation allowing the mesenchymal splice form of FGFR2 (FGFR2c) to bind FG7 and FG10.

It is thought that limb abnormalities in Apert syndrome result from autocrine activation of FGFR2c by FG7 or FG10. Hence, inability to bind FG7 in other FGFR mutations for other craniosynostosis syndromes such as FGFR1 and FGFR3 mutations resulting in Pfeiffer and Muenke syndrome may explain mild limb pathology as opposed to Apert syndrome.

Mechanisms of Action Leading to Suture Fusion

Development of cranial sutures is highly reliant on cross-talk between different FGFRs, resulting in a balance between osteogenic cell proliferation and differentiation. At 6 weeks of development in human embryos, FGFR1 and FGFR2 are found in mesenchyme of the cranial vault, while FGFR3 is almost undetectable. Later in development, all three FGFR2s are coexpressed in pre-osteoblasts around osteoid and in osteoblasts forming mineralizing tissue. Signaling mediated through FGFR1 appears to regulate osteogenic differentiation, while signaling mediated through FGFR2 regulates stem cell proliferation. FGFR3 could play a cooperative role during the process of cranial suture development.

The exact mechanism by which mutations in FGFR result in aberrant suture fusion is as yet unclear. In vitro studies have shown that under different culture conditions FG signaling leads to either increased osteoblast differentiation or decreased osteoblast differentiation with subsequent apoptosis. One mechanism is suggested by a study focused on analysis of gene expression profiles, where downregulation of a number of Wnt target genes with simultaneous induction of the transcription factor Sox2 was found in osteoblasts expressing FGFR2-activating mutations as well as in osteoblasts treated with exogenous FG. Wnt signaling has been shown to promote osteoblast differentiation and function, while Sox has been shown to interfere with Wnt signaling. Wnt signals also cooperate with bone morphogenetic proteins to induce osteoblast differentiation. Hence, a block in Wnt signaling induced by FG might result in decreased osteoblast differentiation with subsequent apoptosis.

Other pathways have been implicated in pathogenesis of abnormal suture fusion downstream of FGFR. Noggin, an antagonist of bone morphogenetic proteins, has been found to be expressed in suture mesenchyme of patient but not fusing cranial sutures, with noggin expression suppressed by FG2 and syndromic FGFR signaling. Hence, syndromic FGFR-mediated craniosynostosis may result from inappropriate downregulation of noggin expression. Epidermal growth factor receptor (EGFR) and platelet-derived growth factor (PDGF)–α receptor expression were also recently found to be increased downstream of FGFR2 through activation of PKC-α-dependent AP-1 transcriptional activity in Apert craniosynostosis. FGFR signaling has also been shown to upregulate expression of the pyrophosphate generating enzyme (PC-1) and pyrophosphate channel (ANK), leading to increased mineralization of osteoblastic cells in culture. Clearly, a number of mechanisms must be responsible for downstream signaling of FGFR to result in craniosynostosis phenotype. However, at present, there is insufficient evidence...
to implicate one as a dominant mechanism of action in the pathogenesis of craniosynostosis.

Twist is a transcription factor that acts upstream of FGFR and has also been implicated in the pathogenesis of Saethre-Chotzen syndrome, with localization of a genetic mutation to the short arm of chromosome 7.5 Mutations resulting in altered protein-DNA binding lead to loss of protein function. Twist underexpression has been shown to result in differentiation of osteoblasts and premature fusion of sutures in a murine model.2 Increased osteoblast and osteocyte apoptosis has been found in coronal sutures of patients with Saethre-Chotzen syndrome,58 an effect of Twist haploinsufficiency resulting in increased TNF-α expression and caspase-2 activation. Mechanisms of action by which Twist regulates FGFR expression include one in which Twist interacts with NF-kB to control FGFR expression53 and also one in which Twist directly interacts with histone acetyltransferase p300/CBP, resulting in negative regulation of FGFR3 expression.13

Another transcription factor that may act upstream of FGFR signaling is muscle segment homeobox 2 (MSX2), which has been implicated in Boston-type craniosynostosis.5 MSX2 appears to maintain preosteoblastic cells of the osteogenic front in an undifferentiated condition and to stimulate proliferation. MSX2 mutation and overexpression in humans leads to premature suture fusion by increasing the pool of osteogenic cells.21

**Transforming Growth Factor–β Signaling**

Transforming growth factor-β consists of a superfamily of growth factors, 3 of which have been found to be relevant in cranial suture fusion and craniosynostosis. These growth factors include TGF-β1, TGF-β2, and TGF-β3. They exert downstream effects through binding to cell surface receptors with a single transmembrane domain and intracellular serine-threonine kinase domain.6 These receptors are divided into types I and II. Typically, TGF-β bind to TGF-β receptor II, which recruits TGF-β receptor I through phosphorylation, with subsequent activation of downstream cascades.

Different isoforms of TGF-β are upregulated to varying extents in patent and fused sutures. In rat models of suture fusion using the posterior frontal suture (the only suture that fuses physiologically), TGF-β1 and particularly TGF-β2 expression was upregulated.44,48 In contrast, TGF-β3 was found to be associated with patent sutures.41 In a rabbit craniosynostotic model, a similar pattern was seen, with TGF-β2 significantly upregulated in the osteogenic front, dura mater, and periosteum of nonfused sutures compared with normal ones.44

One mechanism postulated for regulation of suture fusion is by individual isoforms modulating access of other isoforms to cell surface receptor sites. TGF-β1, β2, and β3 all use the same cell surface receptors yet interestingly have opposite effects of suture patency and cell proliferation in the suture. TGF-β3, being a more potent competitor than TGF-β2 for binding to cell surface receptors, was found to bind and downregulate the number of TGF-β receptor I–positive cells in a rat suture model,40 suggesting that TGF-β3 could regulate tissue responsive-ness to TGF-β2 by modulating access of TGF-β2 to receptors.

A model has been proposed for downstream regulation of suture fusion whereby TGF-β2 induces suture closure through phosphorylation of Erk1/2 (Fig. 1).53 This effect is both direct and indirect, with indirect effects involving boosting Erk1/2 protein expression and inhibiting Smad2/3 protein expression. Smad2/3, conversely, has been associated with suture patency. As Erk1/2 is also a substrate for FGFR signaling, upregulation of Erk1/2 may also result in facilitation of FGFR-induced suture fusion. The importance of Erk1/2 in TGF-β2–mediated suture fusion has been demonstrated in a study where TGF-β2–mediated suture closure and cell proliferation were nearly completely inhibited by an Erk blocker, PD98059.29

The primacy of the TGF-β2 isoform in regulating suture fusion has been shown in studies in which treatment with TGF-β2 neutralizing antibodies inhibited postoperative resynostosis and enhanced growth of the cranial vault following surgical removal of the coronal suture in a craniosynostotic rabbit model.12,23 In TGF-β2 knockout mice, there was calvarial bone dysgenesis and sutural agenesis in addition to cardiac, lung, limb, eye, spinal column, and urogenital defects.58 There was no phenotypic overlap with TGF-β1 and TGF-β3 null mice, suggesting that noncompensated functions are mediated through individual TGF-β isoforms.

The mechanisms by which TGF-β regulate suture patency are becoming clearer from ongoing research. In the rodent model, the posterior frontal suture fuses during skeletal development, whereas all other cranial sutures remain patent. Using this model, alteration of normal cranial suture development has been achieved by manipulating TGF-β signaling in the tissues adjacent to the developing suture.60 Dura mater has been found to be essential in mediating suture fusion, likely through cellular mechanisms62 involving paracrine signaling mediated by FGF-2 and TGF-β1,11,26 among other cytokines. A model58 was proposed whereby approaching bone fronts secrete various growth factors to induce formation of a cranial suture. Once the bone fronts overlap, a signal arising from dura mater maintains patency of the newly formed suture. Upon stabilization of the suture, suture mesenchyme signals underlying dura mater not to produce osteogenic signals and maintains the suture in a patent state. A balance between TGF-β1 and TGF-β3, which likely secrete inductive or stabilizing suture signals, and TGF-β2, which secretes osteogenic signals promoting recruitment of osteoblasts and osteogenesis at the bone fronts, is essential for normal suture morphogenesis. These signals allow cranial vault growth to be coordinated with growth of the underlying brain. Therefore, an imbalance between the different TGF-β isoforms would result in craniosynostosis. Promising work has been done to demonstrate that TGF-β1 levels can be manipulated in vitro using short interfering RNA, which may provide a mechanism by which suture development can be modulated nonsurgically in the future.50,12

**Conclusions**

Craniosynostosis is a condition that has significant...
impact on the practice of neurological and craniofacial surgery. Advances in research have led to valuable insights into the critical role of FGFR- and TGF-β–mediated signaling in the pathogenesis of premature suture fusion. Normal suture fusion is dependent on a complex signaling cascade that can be disrupted by a large number of genetic mutations or perturbations in cell signaling. The present review has focused on cytokine regulation of suture fusion; however, many other mechanisms for sutural fusion and patency have been proposed, including increased apoptosis observed in patent sutures1 and the potential for force-induced craniosynostosis secondary to events in utero.52 Understanding how growth factors influence suture fusion allows development of new therapies for treatment.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: all authors. Acquisition of data: Cohen, Chim, Gosain. Analysis and interpretation of data: all authors. Drafting the article: all authors. Critically revising the article: Cohen, Manjila, Chim.

References

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44. Poisson E, Sciote JJ, Koepsel R, Cooper GM, Opperman LA, Mooney MP: Transforming growth factor-beta isoform expression in the perisutural tissues of craniosynostotic rabbits. Cleft Palate Craniofac J 41:392–402, 2004

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