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Mechanisms Underlying Cytokine-Mediated Cell-Fate Regulation in the Nervous System

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Abstract

Neurons, astrocytes, and oligodendrocytes, the three major cell types in the nervous system, are generated from common neural stem cells during development. Recent studies have provided evidence that neural stem cells are preserved in the adult brain, where, until recently, neurogenesis had not been considered to take place. The mechanisms that govern the fate of neural stem-cell determination have yet to be clarified. It is becoming apparent that soluble protein mediators referred to as cytokines play critical roles in cell-fate determination. For instance, bone morphogenetic proteins (BMPs) alter the fate of developing brain cells from a neurogenic differentiation to an astrocytic one. Different types of cytokines sometimes cooperate to modulate differentiation. For example, the interleukin-6 (IL-6) family cytokines and the BMP family cytokines act in synergy to elaborate astrocyte differentiation. In this review, we focus on recent progress that addresses the molecular mechanisms whereby cytokines regulate the fate of cells in neural lineages. We also discuss possible clinical applications of these findings to minimize the undesirable gliogenesis that occurs after neural stem-cell implantation and nerve injury.

Index Entries: IL-6 family of cytokines; LIF; BMP; STAT; Smad; HLH; neural stem cell; astocytogenesis; neurogenesis.

Signal Transduction Pathway Activated by Cytokines

Before reviewing recent progress of research on cytokine-mediated cell-fate determination in the nervous system, we discuss below the signal-transduction mechanisms of two representative and distinct types of cytokines, those in the interleukin-6 (IL-6) and BMP families.

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STAT3 Activation by IL-6 Family Cytokines

The IL-6 family of cytokines, i.e., IL-6, IL-11, leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin M (OSM), cardiotrophin-1 (CT-1), and cardiotrophin-like

cytokine (CLC) share the membrane glycoprotein gp130 in their functional receptor complexes as a receptor component critical for signal transduction (1–3). Upon binding of the IL-6 family cytokines to their specific receptor components, gp130 becomes dimerized either with itself or with another dimer partner like LIF receptor (LIFR) or OSMR, depending on the type of cytokine. This dimer formation of gp130 leads to the activation of associating cytoplasmic tyrosine kinases in the Janus kinase (JAK) family. Activated JAKs then phosphorylate tyrosine residues in the cytoplasmic region of gp130 and its dimer partners. Such phosphorylated tyrosine residues in turn attract downstream signaling molecules including a latent cytoplasmic transcription factor, STAT3 (for signal transducer and activator of transcription 3) harboring a Src homology 2 (SH2) domain, the structure of which recognizes phosphotyrosine-containing peptide. The receptor-docked STAT3 is then tyrosine-phosphorylated by neighboring JAKs, allowing it to homodimerize through intermolecular SH2 domain-phosphotyrosine interaction. The homodimerized and activated STAT3 is subsequently translocated into the nucleus to regulate expression of its target genes (Fig. 1).

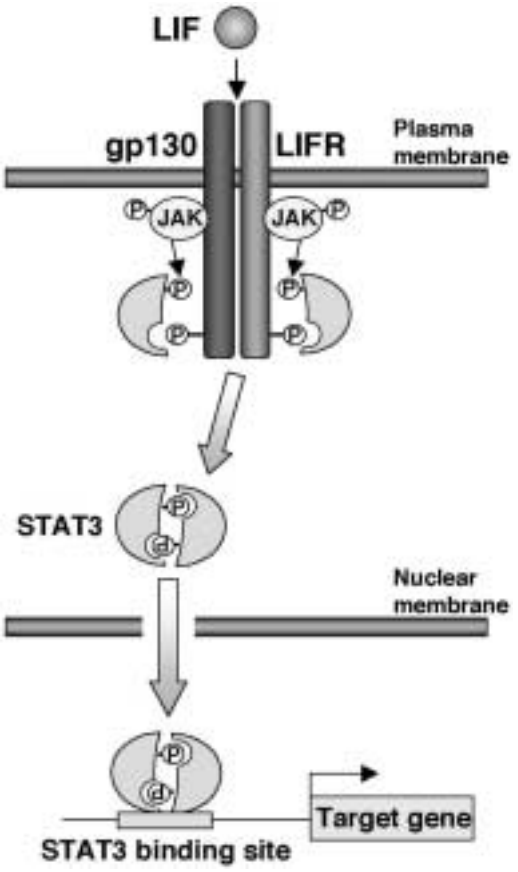

Fig. 1. Mechanism of STAT3 activation induced by IL-6 family cytokines. Representative LIF-induced activation of STAT3 is delineated. Upon binding of LIF to its specific receptor (LIFR), gp130 becomes dimerized with LIFR. This dimerization leads to the activation of associating JAK tyorisine kinases. Activated JAKs then phosphorylate tyrosine residues in the cytoplasmic region of gp130 and LIFR. Such phosphorylated tyrosine residues in turn attract a latent cytoplasmic transcription factor, STAT3 harboring a SH2 domain, the structure of which recognizes phosphotyrosine-containing peptide. The receptor-docked STAT3 is then tyrosine-phosphorylated by neighboring JAKs, allowing it to homodimerize through intermolecular SH2 domain-phosphotyrosine interaction. The homodimerized and activated STAT3 is subsequently translocated into the nucleus to regulate expression of its target genes.

Smad Activation by BMPs

BMPs, members of the transforming growth factor-β (TGF-β) superfamily, signal through a heterotetrameric serine/threonine kinase receptor complex composed of two type I and two type II receptor molecules *(4–6)*. Activated BMP receptors phosphorylate carboxy-terminally located serine residues in the downstream transcription factors Smad1, -5 and -8. After phosphorylation, these transcription factors bind with the common mediator Smad protein, Smad4, and the resultant complex accumulates in the nucleus to activate transcription of specific genes (Fig. 2). The inhibitory Smad proteins, Smad6 and Smad7, have been shown to repress the action of BMPs by inhibiting the receptor-mediated phosphorylation of Smad1 or Smad5 (Smad6 and

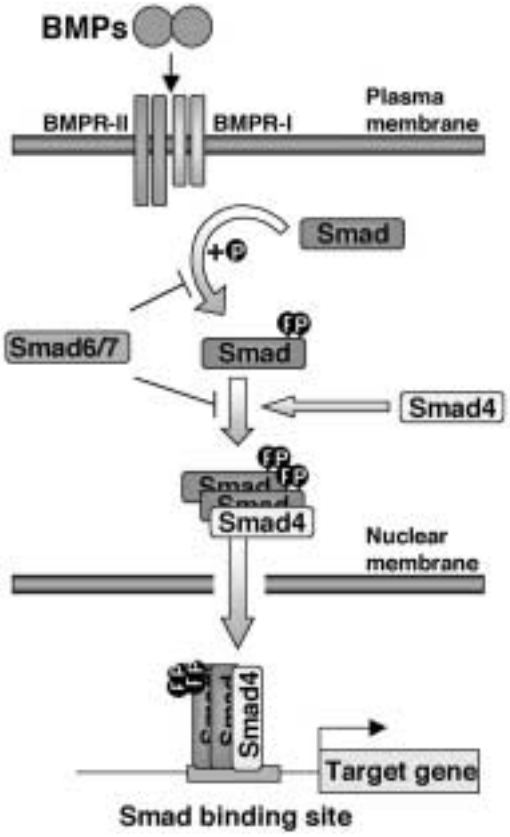

Fig. 2. Smad signaling pathway activated by BMPs. BMPs signal through a heterotetrameric serine/threonine kinase receptor complex composed of two type I (BMPR-I) and two type II (BMPR-II) receptor molecules. Activated BMP receptors phosphorylate carboxy-terminally located serine residues in the downstream transcription factors Smad1, –5 and –8. After phosphorylation, these transcription factors bind with the common mediator Smad protein, Smad4, and the resultant complex accumulates in the nucleus to activate transcription of specific genes. The inhibitory Smad proteins, Smad6 and Smad7, have been shown to repress the action of BMPs by inhibiting the receptor-mediated phosphorylation of Smad1 or Smad5 (Smad6 and Smad7), or by competing with Smad4 for binding to Smad1 (Smad6). Precise stoichiometry of the BMP-induced complex of pathway-restricted Smad (Smad1, –5 or –8) and common-mediator Smad (Smad4) has not yet been clarified.

Smad7), or by competing with Smad4 for binding to Smad1 (Smad6) *(4–9)*.

The Mechanism of Synergistic Astrocyte Differentiation Induced by the IL-6 and BMP Families of Cytokines

Synergistic Enhancement of Astrocyte Differentiation of Neuroepithelial Cells by IL-6 Type Cytokines and BMPs

Fetal telencephalic neuroepithelial cells contain neural precursors that give rise to both neuronal and glial lineages, the latter including astrocytes and oligodendrocytes *(10–12)*. The fate of neural precursors in the developing brain is believed to be determined by intrinsic cellular programs and external cues including cytokines *(10–12)*. Cytokines in the IL-6 and BMP families have been suggested as having the potential to induce astrocytic differentiation of neural precursors *(13–17)*, but each factor on its own is not sufficient to induce astrocytes from neuroepithelial cells cultured in vitro for 2 d *(18,19)*. However, a combination of two cytokines, each from two distinct cytokine families, for instance LIF and BMP2, has recently been shown to synergistically induce astrocyte differentiation in a 2-d culture of embryonic day (E) 14 mouse neuroepithelial cells *(18,19)*. The synergistic effect has been observed with any combination of any IL-6 family cytokines and BMP2, BMP4, or BMP7 *(18,20,21)*.

Complex Formation of STAT3 and Smads Bridged by Transcriptional Coactivator p300

The downstream transcription factors of IL-6 and BMP family cytokines, STAT3 and Smads, respectively, do not physically interact with each other. How then do IL-6 family cytokines and BMPs exert synergistic functions to induce astrocyte differentiation? As a mechanism to

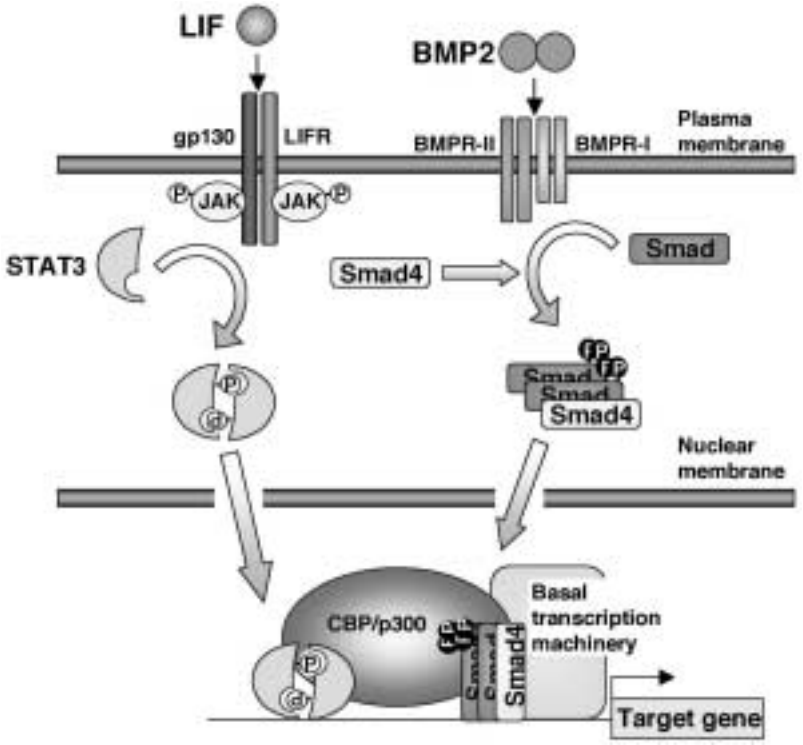

Fig. 3. Signaling cross-talk between two distinct cytokines. Synergistic integration of LIF and BMP2 signaling is achieved by the complex formation of their respective downstream transcription factors, STAT3 and Smads, together with p300. The basal transcription machinery is then activated by p300. Histone-acethylating property of p300 is thought to loosen chromatin structure.

explain this synergistic effect, it has been suggested that STAT3 and Smads form a complex bridged by the transcriptional coactivator, p300 (Fig. 3) *(18)*. The CREB-binding protein (CBP)/p300 family of transcriptional coactivators has been shown to interact with various transcription factors such as AP-1, Myb, and nuclear receptors, thus altering their activities *(22–24)*. It has been demonstrated that the NH_2- and COOH-terminal portions of p300 protein preferentially interact with STAT3 and Smad1, respectively, and that p300 acts as an adapter molecule linking these transcription factors *(18)*. The resultant complex is thought to be involved in synergistic transcriptional activation of the target genes such as those for astrocyte specific markers like glial fibrillary acidic protein (GFAP). The suggested mechanism for nuclear protein-complex formation is obviously important, considering that similar interactions between transcriptional coactivators and a variety of transcription factors have been reported. There could thus be some other types of nuclear-complex formation governing synergistic actions, with distinct types of cytokines in various cell systems, in addition to the one found in developing brain.

Cooperation of the Two Types of Cytokines in Developing Brain

Recognizing that all members of the IL-6 cytokine family, BMP2, BMP4, BMP7, and their cognate receptor components, are expressed in neuroepithelial cells *(19–21,25,24)*, it is conceivable that cooperation between the two types cytokines may take place in developing brain. In this regard, numbers of GFAP-positive astro-

cytes were severely reduced in brain of E18 gp130-deficient mice, even though expressions of BMPs and their cognate receptor components were normally detected *(19,27)*. Of note is that in contrast to the 2-d culture of neuroepithelial cells, as described earlier, either an IL-6 or a BMP family cytokine alone induced astrocyte differentiation of neuroepithelial cells when the culture duration was extended to, for example, 4 or 6 d *(19)*. This may be because exogenously administrated IL-6 family cytokines act in cooperation with their endogenously expressed and accumulated counterpart cytokines, BMPs, to induce astrocyte differentiation in long-term cultures, and vice versa. In support of this, BMP2 alone could not induce astrocyte differentiation in the 6-d culture of telencephalic neuroepithelial cells from gp130-deficient mice, in which all IL-6 family cytokine signals were missing *(27)*. These results suggested that both IL-6 family cytokines and BMPs are indispensable for effective induction of astrocyte differentiation of neuroepithelial cells in vivo as well as in vitro.

Mechanisms of BMP2-Mediated Cell-Fate Alteration of Neural Precursors from Neurogenesis to Astrocytogenesis

As described earlier, BMPs act in synergy with IL-6 family cytokines on neuroepithelial cells to induce astrocytes. We have also shown that BMPs alter the neurogenic fate of neuroepithelial cells so that they develop into astrocytic cells *(28)*.

BMPs-Induced Suppression of Neurogenesis

When E14 mouse neuroepithelial cells were cultured with BMP2 for 2 d, the number of neurons markedly reduced. This was not due to the specific elimination of neurons but to inhibiting proliferating neural precursors from developing into neurons *(28)*. In addition to BMP2, the anti-neurogenic effect was observed with BMP4 and BMP7 *(25)*.

BMP2-Induced Fate Switch to Astrocyte Differentiation of Neural Precursors

While reducing the number of neurons, BMP2 has been shown to also reduce the number of cells expressing a marker for undifferentiated neural precursors, nestin, in the 2-d culture of neuroepithelial cells *(28)*. However, BMP2 increased the number of cells expressing S100-β, an early astrocytic marker, suggesting that BMP2 did not keep the neural precursors in an undifferentiated state but rather changed their fate from neuronal to astrocytic cells. Consistent with the finding described in the previous section, GFAP-positive astrocytes were not detected following 2 d of BMP2 stimulation. This because BMP2-signaling must cooperate with gp130-signaling to enable neural precursors to terminally differentiate into GFAP-positive astrocytes. The possible cooperative involvement of IL-6 family cytokines in the anti-neurogenic effect of BMP2 was clearly excluded as neuronal differentiation was effectively suppressed by BMP2, even in neuroepithelial cells prepared from gp130 knockout mice *(28)*. That posits the question, what then is the mechanism underlying BMP2-mediated suppression of the neurogenesis of neural precursors?

Involvement of Negative HLH Proteins in BMP2-Induced Suppression of Neurogenesis

In the nervous system, neurogenesis is promoted by proneural basic helix-loop-helix (bHLH) transcription factors such as Mash1 (mammalian achaete-scute homologue), Neurogenin and NeuroD. These tissue-specific bHLH factors form heterodimers with ubiquitously expressed bHLH proteins such as *E2A* gene products, E12 and E47, and activate transcription of genes that have a CANNTG sequence (E box) in the promoter region *(29–31)*. In contrast, functions of the bHLH

proteins are negatively regulated by another set of HLH factors, Hes-1 and Hes-5 (where Hes is a homologue of hairy and Enhancer of Split). These mammalian bHLH proteins are distant relatives of the product of *Drosophila* pair-rule gene *hairy (32–34).* Hes-1 and Hes-5 are induced by Notch-signaling, and are known to inhibit transcriptional activity of neurogenic bHLH proteins by competitive binding to their heterodimer partners, E12 and E47 *(32–34).* Another type of negative regulator of bHLH proteins is the Id (for inhibitor of differentiation) family of proteins, which have an HLH domain but lack a basic region *(35–37).* Id proteins inhibit the function of tissue specific bHLH factors, by a mechanism analogous to that used by Hes proteins *(35–38).*

In light of these findings, the involvement of negative HLH factors in BMP2-mediated suppression of neurogenesis was examined. BMP2 upregulated the expression of the negative HLH genes *Hes-5, Id1,* and *Id3* but not *Hes-1 (28).* The upregulated expression is dependent on the BMP2-downstream transcription factors, Smads, and the forced expression of these negative HLH factors in neuroepithelial cells inhibited the transcriptional activity of neurogenic bHLH proteins such as Mash1 and Neurogenin, leading to the suppression of neurogenesis in cultured neuroepithelial cells *(28).* A schematic model of the role of negative HLH factors in BMP2-induced inhibition of neurogenesis is depicted in Fig. 4.

In the four Id proteins so far identified, the expression patterns of *Id1* and *Id3* in developing brain are quite similar and both are found in proliferating neural precursors in the ventricular zone *(39–42).* BMP receptors are also expressed in cells located in the ventricular zone during a developmental window when *Id* expression is observed *(24).* Thus, the mechanism suggested by the aforementioned in vitro experiments, by which BMP2 inhibits neurogenesis, may also operate under physiological conditions. In support of this, enhanced neurogenesis has been observed in fetal brain of Id1-Id3 double-deficient mice *(42).* Transient Notch activation has been reported to initiate an irreversible switch from neurogenesis to gliogenesis in adult hippocampus-derived multipotent progenitors *(43).* The Notch downstream target gene products Hes-1 and Hes-5 (the latter is also induced by BMP2) have been suggested to inhibit neurogenesis and induce Müller glial differentiation in retina *(44,45).* These results indicate the existence of cross-talk between Notch and BMP signaling.

Mechanism of BMP2-Promoted Neurogenesis via the Neurogenic bHLH Transcription Factor, Neurogenin

Contrary to the previous observations, BMPs have been shown to promote neurogenesis of neural precursors at a certain stage of development. In cultures of cortical of cells at late gestation, BMPs suppressed neurogenesis *(14),* whereas in those at midgestation, BMP-stimulation resulted in neuronal differentiation *(16,46).* These completely opposite biological effects of BMPs may be attributed, at least in part, to an intriguing function, recently found, of a neurogenic bHLH transcription factor, Neurogenin *(47).* Neurogenin expression is abundant in cortical cells in midgestation, but is very low in late gestation when BMPs suppress neurogenesis and induce astrocyte differentiation, most likely in cooperation with IL-6 family cytokines *(14,18,19).* Smad proteins phosphorylated and activated following BMP-stimulation then bind to p300. The resultant Smads / p300 complex, which has previously been shown to from a complex with STAT3 to effectively induce astrocyte differentiation *(18),* was also indicated to associate with Neurogenin during early cortical development when Neurogenin expression is abundant *(47).* In situations where Neurogenin is in excess, this mechanism leads to the prevention of Smad / p300 complex binding to STAT3 (Fig. 5) *(47).* It was also demonstrated that Neurogenin with the Smads / p300 complex formation enhanced the transcriptional activity and neurogenic potential of Neurogenin, implying

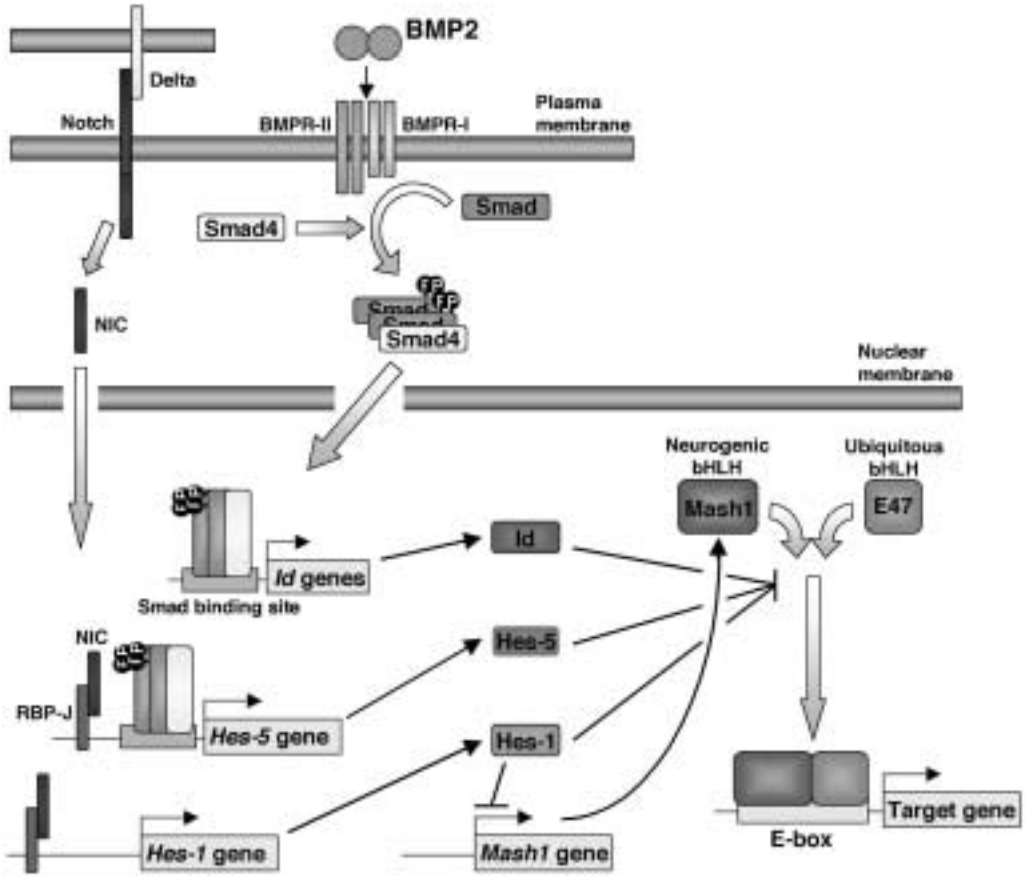

Fig. 4. Mechanism of the anti-neurogenic effect of BMP2 involving negative HLH proteins. BMP2 upregulates expression of negative HLH factors via Smad-activation. These negative HLH factors inhibit function of such neurogenic bHLH transcription factors as Mash1 and Neurogenin by competitively binding to their heterodimeric bHLH partners, E12 and E47, leading to suppression of neurogenesis of neural precursors. When the transmembrane protein Notch is activated by its ligand Delta, proteolysis occurs and the intracellular domain of Notch (NIC) is translocated into nucleus and complexes with RBP-J. The complex activates transcription of *Hes-1* and *Hes-5,* the latter of which is activated by BMP2-stimulation as well, implying cross-talk between Notch and BMP2 signalings.

that the Smads / p300 complex acts as a coactivator for Neurogenin (Fig. 5) *(47)*. In this report, Neurogenin was suggested to inhibit the STAT3 phosphorylation and activation critical for astrocytogenesis by an undetermined mechanism. Neurogenesis precedes gliogenesis during the development of the nervous system *(48)*, and as the expression level of Neurogenin is high when cortical neurogenesis is dominant and low when gliogenesis occurs, these Neurogenin-involved mechanisms may explain why neurogenesis precedes and dominates gliogenesis during cortical development even in the presence of such gliogenic factors as IL-6 family cytokines and BMPs *(47)*.

Involvement of Epigenetic Modification of DNA in Cytokine-Induced Cell Fate Determination

Methylation of genomic DNA at CpG dinucleotides is a major epigenetic modification of

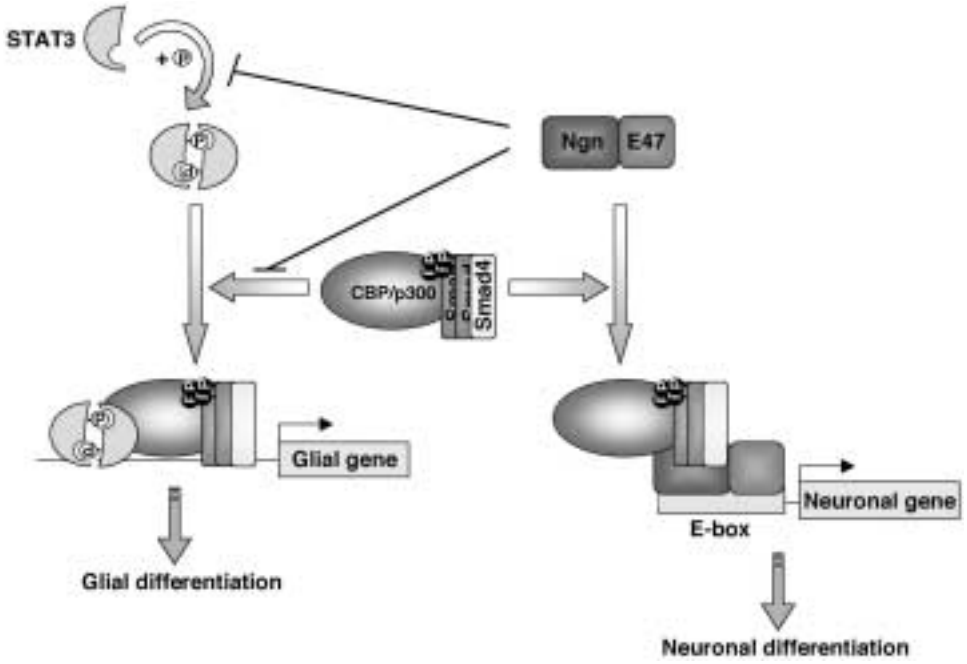

Fig. 5. Mechanism of Neurogenin-mediated suppression of astrocytogenesis and promotion of neurogenesis. Smads/p300 complex, which has previously been shown to from a complex with STAT3 to effectively induce astrocyte differentiation, was also indicated to associate with Neurogenin during early cortical development when Neurogenin expression is abundant. In situations where Neurogenin is in excess, this mechanism leads to the prevention of Smad/p300 complex binding to STAT3. It was also demonstrated that Neurogenin with the Smads/p300 complex formation enhanced the transcriptional activity and neurogenic potential of Neurogenin, implying that the Smads/p300 complex acts as a coactivator for Neurogenin. Neurogenin was also suggested to inhibit the STAT3 phosphorylation and activation critical for astrocytogenesis by an undetermined mechanism. Ngn, Neurogenin.

mammalian genomes and is implicated in the regulation of tissue-specific gene expression *(48)*, and also functions in some aspects of gene expression such as genomic imprinting, X chromosome inactivation, aging, and tumorigenesis *(48–50)*. In these events, CpG-methylation contributes in a general way to transcriptional suppression by favoring the formation of inactive chromatin, or preventing transcriptional regulators from binding to their target DNA elements. It has become increasingly evident that methylation of DNA also participates in the cell-fate regulation of neural precursors by cytokines.

In contrast to the culture of proliferating neural precursors, GFAP expression was not induced by LIF-stimulation in a culture of postmitotic neurons prepared from E14 mouse telencephalon, even though STA3 activation was clearly detected (T. Takizawa, K.N., T. T., et al., unpublished results). Further, the CpG site was found in the STAT3-binding element (TTCCGAGAA) within the GFAP gene promoter *(13,18)*. In consideration of these findings, the methylation status of this particular STAT3 binding site in the GFAP promoter was examined. It was revealed that the STAT3-binding site was highly methylated in postmitotic neurons, but barely methylated in neural precursors from E14 mouse telencephalon and astrocytes, which have a potential to express GFAP *(51)*. Moreover, it has been suggested that STAT3 does not bind to the methylated-form of its target sequence, resulting in the gene promoter harboring methylated STAT3-binding

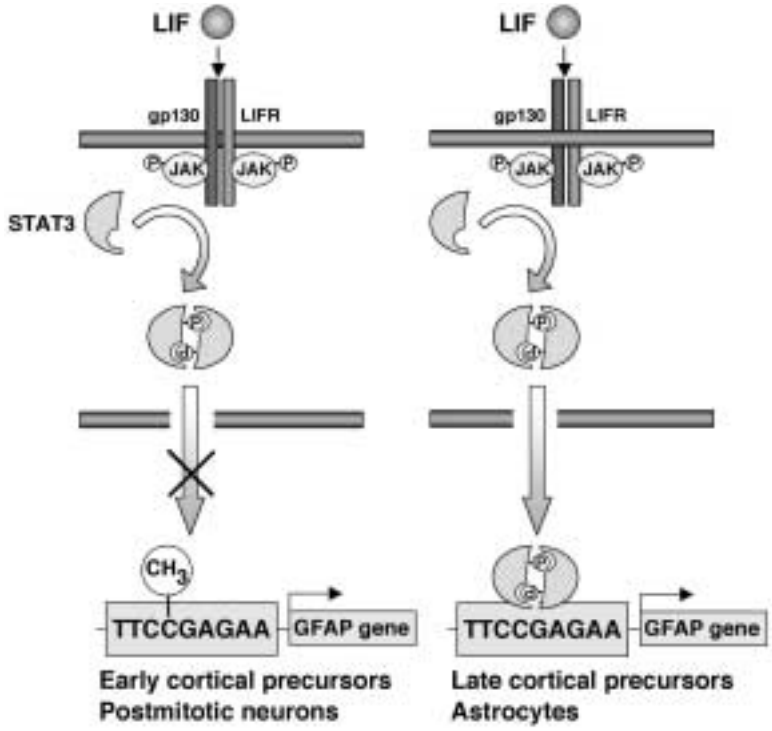

Fig. 6. Methylation-mediated transcriptional inhibition of the glial gene. The STAT3 binding site in the GFAP gene promoter is hypermethylated in early cortical precursors and postmitotic neurons, leading to the suppression of transactivation of the gene by IL-6 family cytokines due to the failure of STAT3-binding to the methylated recognition sequence.

element failing to be activated in response to cytokine-stimulation *(51)*. Intriguingly, neural precursors from E11 mouse telencephalon, where neurogenesis was dominant, were not induced to express GFAP by LIF-stimulation, and the STAT-binding site within the GFAP gene promoter was highly methylated in cells *(51)*. These findings may assist the elucidation of the issue why neurogenesis proceeds gliogenesis during cortical development (Fig. 6).

Conclusions

As mentioned previously, mechanisms by which cytokines regulate cell-fate specification of neural precursors have begun to be elucidated. However, a variety of cytokines are present and thus various kinds of signaling-pathways are possibly activated simultaneously in vivo, so the cross-talk between distinct pathways must be taken into consideration for a more precise understanding of the bona fide mechanisms whereby cytokines exert biological functions in vivo. How cytokine-signaling and DNA methylation are mutually regulated remains an important issue needing verification.

The molecular mechanisms of cytokine-regulated cell-fate determination are not only biologically interesting, they are also clinically significant considering their application for regenerative medicine. Neural stem cells, which are endogenously present in spinal cord in vivo, proliferate in response to injury, yet the vast majority of newly generated cells are GFAP-positive astrocytes *(52)*. This undesirable gliogenesis after nerve injury may be attributable to

the anti-neurogenic function of BMPs, whose upregulated expression has been observed in spinal cord after injury *(53)*. Additionally, adult hippocampus-derived neural stem cells, when implanted into adult brain in regions such as cerebellum or striatum, have been reported to differentiate predominantly into glial cells *(11,54,55)*. Inhibitors of BMP-signaling such as noggin, chordin, and inhibitory Smads may contribute to the promotion of neurogenesis, instead of the naturally occurring gliogenic response, of endogenously present or engrafted neural stem cells. In this respect, it is of note that the ectopic expression of the BMP antagonist noggin in adult mouse striatum, where BMP2 and BMP4 are normally expressed *(14)*, has been shown to promote neuronal differentiation of grafted neural progenitors *(56)*. Thus, strategies that inhibit BMP-signaling could be used to treat spinal-cord injuries and other CNS degenerative diseases; a possibility currently under investigation.

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