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Function and Mechanism of CNTF/LIF Signaling in Retinogenesis

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Abstract

Ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF) exhibit multiple biological effects in the developing vertebrate retina. CNTF/LIF inhibits rod photoreceptor, and promotes bipolar cells and Muller glia differentiation. In addition, CNTF/LIF has been shown to have proliferative and apoptotic effects. Moreover, LIF also inhibits retinal vascular development. CNTF/LIF signaling components CNTFR α , LIFR β , gp130, and a number of STAT proteins are expressed in the retina. CNTF/LIF activates Jak-STAT, ERK, and Notch pathways during retinal development. Perturbation of CNTF induced signal transduction reveals that different combinations of CNTF/LIF signaling pathways regulate differentiation of retinal neurons and glia. Gene expression studies show that CNTF/LIF affects retinogenesis by regulating various genes involved in transcription, signal transduction, protein modification, apoptosis, protein localization, and cell ion homeostasis. Most past studies have deployed ectopic expression or addition of exogenous CNTF/LIF, thus further analysis of mice with conditional mutations in CNTF/LIF signaling components will allow better understanding of in-vivo functions of CNTF/LIF associated signaling events in retinogenesis.

74.1 Introduction

The mature vertebrate retina consists of seven major neuronal cell types generated from a common progenitor pool. Lineage tracing analyses using retrovirus infection and fluorescent dye single cell injection have shown that retinal progenitor cells are multipotent and cell fates are specified during or after their terminal mitosis (Wetts and Fraser 1988; Turner et al. 1990). Molecular genetic studies have further demonstrated that retinal cell fates are regulated by both cell intrinsic and extrinsic mechanisms. A proposed model suggests that progenitors pass through a series of intrinsically determined competence states, and under each state, progenitors are capable of giving rise to a limited subset of cell types under the influence of extrinsic signals (Livesey and Cepko 2001). Various signal molecules have been shown to influence retinal cell differentiation either positively or negatively (Levine et al. 2000; Yang 2004). Among them, ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF), members of the interleukin (IL)-6 family of cytokines, exhibit multiple biological effects in the developing vertebrate retina.

CNTF/LIF family of cytokines bind to a tripartite receptor complex consisting of two single membrane spanning beta-receptor subunits and an additional alpha receptor specific for certain members of the cytokine family (Ip, 1998). CNTF/LIF triggers multiple intracellular signaling events, including activation of the Janus kinases (Jak)-Signal Transducer and Activator of Transcription (STAT), the extracellular signal-regulated kinases (ERK) (Boulton et al. 1994) and the phosphatidylinositol 3 kinase (PI3K)-Akt pathways (Oh et al. 1998).

74.2 Effects of CNTF/LIF on Photoreceptor and Bipolar Neuron Differentiation

CNTF/LIF has been shown to strongly inhibit differentiation of rod photoreceptors in both mouse and rat retinas (Ezzeddine et al. 1997; Neophytou et al. 1997; Kirsch et al. 1998; Schulz-Key et al. 2002; Sherry et al. 2005; Elliott et al. 2006). Both CNTFR α and LIFR β knockout retinas display increased proportions of rod photoreceptors when cultured as explants for 10 days in-vitro starting from post-natal day 0 (Ezzeddine et al. 1997). In contrast, in a chicken retinal culture, CNTF appears to promote the differentiation of a subclass of cone photoreceptors (Fuhrmann et al. 1995; Xie and Adler 2000). Consistent with the observed influence of CNTF/LIF on photoreceptor development in-vitro, retinas of transgenic mice that misexpress LIF from the alpha crystallin promoter during embryonic development show dose-dependent disruption of photoreceptor differentiation (Graham et al. 2005).

In addition to their effects on photoreceptor development, CNTF/LIF also regulates other late born retinal cell types. Concomitant to suppression of rod differentiation, CNTF/LIF promotes the expression of several bipolar cell markers in the postnatal retina (Ezzeddine et al. 1997; Schulz-Key et al. 2002; Bhattacharya et al. 2004; Zahir et al. 2005). By tracking cell birthdates and analyzing proportions of different cell markers, Ezzeddine et al. (1997) concluded that the bipolar marker-positive cells are derived from cells that would normally give rise to rod photoreceptors. This result indicates that CNTF/LIF affects the differentiation process of immature postmitotic neuronal precursors. They also observed that once rod cells begin to express rhodopsin protein, they become increasingly resistant to the inhibitory effects of CNTF. Consistent with this observation, Schulz-Key et al. (2002) showed that retinal explants treated with CNTF contain bipolar marker-positive cells in the outer nuclear layer and greatly reduced opsin-positive cells. Yet, the suppression of rhodopsin and upregulation of bipolar markers are reversible (Schulz-Key et al. 2002). This effect is interesting as rod and bipolar cells are derived from common postnatal progenitor cells that share key transcription factors including Otx2 and Crx (Furukawa et al. 1997; Koike et al. 2007). Nonetheless, transgenic mice that overexpress LIF during embryogenesis do not overproduce bipolar cells in-vivo (Sherry et al. 2005), suggesting that timing and/or duration of signaling may play a role in the cellular effects.

In contrast to in-vitro studies where rod photoreceptor differentiation is blocked, Elliott et al. (2006) reports that misexpressing CNTF during development in-vivo causes reduction of photoreceptors resulting from the cell death of postmitotic rod precursors. In this study, mouse retinas were infected at P2 with an adenovirus encoding a secretable CNTF. When analyzed at P32, the outer nuclear layer (ONL) was considerably thinner than the control and TUNEL positive apoptotic cells were observed in ONL. The authors argue that the enhanced cell death was due to activation of cytokine signaling, which positively regulates nitric oxide production to influence developmental programmed cell death. Unlike the previous studies, there was no change in the number of bipolar cells. Interestingly, embryonic misexpression of LIF results in broad defects of multiple retinal cell types and abnormal synaptogenesis, including disruption of bipolar cells and ganglion cells (Sherry et al. 2005).

74.3 Effects of CNTF/LIF on Muller Glia Genesis and Late Progenitor Proliferation

CNTF promotes astrocyte development in the central nervous system (Bonni et al. 1993; Rajan and McKay 1998). In the mouse retina, CNTF enhances the production of Muller glia in-vitro up to 3-folds (Goureau et al. 2004). As Muller glial cells are the last born cells in the vertebrate retina, this Muller glia promotion effect maybe linked to the proliferative effects of CNTF detected during the late neurogenic phase. Goureau et al. (2004) observe a 2- to 3-fold increase in BrdU-positive cells only when retinal explants were labeled with BrdU from P5-P6, but not from P0 to P3, despite a continuous exposure to CNTF from P0. Furthermore, BrdU-positive cells were found to co-label with Muller glial markers GFAP and CRALBP. Therefore, CNTF/LIF may promote cell proliferation of the residual progenitors before the completion of retinogenesis, leading to an increased number of progenitor cells adopting Muller glial fate.

74.4 Effects of LIF Misexpression on Retinal Vasculature Development

The development of retinal vasculature involves multiple cell types including endothelial cells, astrocytes, pericytes, and Muller glia. Developing retinal ganglion cells are known to express vascular endothelial growth factor (VEGF) (Yang and Cepko 1996; Hashimoto et al. 2003) among other growth factors. In transgenic mice overexpressing LIF from the embryonic lens, the retinal vascular development is severely retarded and the expression of VEGF is aberrantly elevated (Ash et al. 2005). The potential normal functions of LIF-like cytokines in retinal vessel development remain to be explored.

74.5 Expression of CNTF/LIF Signaling Components in the Developing Retina

Multiple CNTF/LIF signaling components are detected in the developing vertebrate retina (Kirsch and Hofmann 1994; Kirsch et al. 1997; Rhee and Yang 2003; Zhang et al. 2003; Hertle et al. 2008). In the postnatal mouse retina, immunolabeling has detected the expression of gp130, CNTFR α , Jak kinases, STAT1, and STAT3 proteins at low levels in the proliferative zone and at higher levels in the plexiform layers as well as in the ganglion cell layer and horizontal cells (Rhee and Yang 2003). In addition to STAT1 and STAT3, STAT2, 4, 5, 6 are also expressed in the developing retina (Zhang et al. 2003). Thus, the necessary components required for cytokine signaling are present in the retina.

CNTFR α has been defined as the specific receptor required for CNTF binding and signaling. However, its location in the retina remains controversial. In the mouse retina, CNTFR α protein was detected at low levels in the proliferative zone and localized to the inner and outer segments of mature photoreceptor cells (Rhee and Yang 2003). Hertle et al. (2008) analyzed presumptive and differentiating ONL using laser-capture and RT-PCR, and showed that both LIFR β and CNTFR α are expressed in the immature rat photoreceptors. Using immunocytochemistry and several anti-CNTFR α antibodies, Beltran et al. (2005) detected CNTFR α localization to the photoreceptors of adult non-rodent mammalian retinas but not to the rodent retinas. Using laser capture and single cell RT-PCR, Wahlin et al. (2004) detected CNTFR α expression in the mouse Muller cells but not in photoreceptors.

74.6 Signaling Events Triggered by CNTF/LIF During Retinogenesis

Analysis of CNTF/LIF signal transduction involved in retinogenesis has revealed activation of different signaling pathways, including Jak-STAT, ERK, and Notch pathways. Both in-vitro and in-vivo results demonstrate that only the Jak-STAT pathway is involved in the

inhibition of rod photoreceptor differentiation by CNTF/LIF (Ozawa et al. 2004; Rhee et al. 2004; Zhang et al. 2004). Using antibodies that recognizing phosphorylated STAT proteins (pSTAT) and serine/threonine phosphorylated ERK1/2 (pERK1/2), we provide evidence that both STAT and ERK are rapidly activated by CNTF in the neonatal mouse retina (Rhee et al. 2004). Interestingly, the pSTAT3 and pERK1/2 are distributed differently in the retina, with pERK1/2 predominantly activated in progenitor cells and pSTAT3 primarily activated in postmitotic ganglion cells and amacrine cells. In addition, STAT3 is activated by CNTF in newly postmitotic Crx-positive rod precursors located near the ventricular surface. Disruption of the two signaling pathways in-vitro using pharmacological inhibitors and dominant negative STAT mutant showed that CNTF/LIF inhibition of rod differentiation requires activation of the Jak-STAT, but not the ERK pathway (Rhee et al. 2004; Zhang et al. 2004). Consistent with the in-vitro findings, in mice that carry conditional deletion of STAT3 in the peripheral retina, CNTF inhibition of photoreceptor differentiation is blocked (Ozawa et al. 2004). In contrast, in the gp130^{F759} knockin mouse, which encodes a SHP2 binding site mutant (gp130^{F759}) that blocks the PI3K and ERK pathways without interfering with the Jak-STAT pathway, CNTF inhibition of rod differentiation is not affected (Ozawa et al. 2004).

In contrast to the inhibitory effect on rod differentiation, promotion of Muller glial development by CNTF/LIF appears to involve several signaling pathways. Blocking signaling by pathway specific inhibitors or STAT mutant indicates that activation of both Jak-STAT and ERK are involved in gliogenesis (Goureau et al. 2004). Recently, Bhattacharya et al. (2008) reports that CNTF/LIF regulates neuronal versus glial differentiation of progenitors in the neural sphere culture via ERK, Jak-STAT, and Notch signaling pathways. Their results show that low concentrations of CNTF activates ERK pathway to promote bipolar cell differentiation, whereas high concentrations activates Jak-STAT pathway to promote Muller glia differentiation. In addition, dominant negative MAPK (dnMAPK) abolished bipolar cell differentiation and dominant negative STAT3 (dnSTAT3) abolished Muller glia differentiation. Furthermore, Notch pathway activation plus CNTF treatment inhibited bipolar differentiation, but promoted Muller glia differentiation. Moreover, disruption of Notch and Jak-STAT pathways promoted differentiation of rod photoreceptors. These results are consistent with previously reported functions of CNTF and Notch effectors in Muller glia genesis (Furukawa et al. 2000; Hojo et al. 2000; Satow et al. 2001). In addition, Zhang et al. (2005) showed that Hes1 was upregulated with STAT3 overexpression, and perturbation of Jak-STAT pathway using dnSTAT3 eliminated co-labeling of pSTAT3 with progenitor marker Hes1. These results are further supported by the recent finding that STAT3 co-oscillates with Hes1 (Yoshiura et al. 2007).

74.7 CNTF/LIF Regulate Numerous Genes Involved in Retinogenesis

CNTF/LIF regulates broad categories of genes during retinal development. Real-time RT-PCR shows that CNTF/LIF inhibits phototransduction genes, Crx, Nrl and Nr2e3 (Ozawa et al. 2004; Graham et al. 2005). Low levels of CNTF promote bipolar differentiation, and upregulate transcription factors Ath3, Chx10 and mGluR6, while down regulate both Hes1 and Hes5 (Bhattacharya et al. 2004, 2008). As expected, high levels of CNTF promote Muller glia genesis and upregulate vimentin, Notch1, GFAP, GS, and Hes5. When perturbation of both Jak-STAT and Notch pathways increased rod photoreceptor differentiation, both Nrl and Otx transcription factors were elevated.

Subtractive hybridization has identified pleiotrophin (Ptn), which is upregulated when P0 retinal explants are exposed to CNTF/LIF (Roger et al. 2006). Overexpression of Ptn inhibits rod differentiation and promotes bipolar cell differentiation mimicking CNTF/LIF

effects in retinogenesis. Further analysis revealed 7 genes upregulated and 17 genes downregulated after 6 days of CNTF treatment and these were classified into six categories involving in transcription regulation, signal transduction, protein modification, apoptosis, protein localization and cell ion homeostasis (Roger et al. 2007). These studies show that CNTF/LIF potentially influences retinogenesis via regulation of numerous genes.

74.8 Perspective

Despite studies showing effects of CNTF/LIF in retinogenesis, the majority of the studies involves misexpression of genes or addition of exogenous CNTF/LIF in-vitro or in-vivo. Thus, endogenous functions of CNTF/LIF signaling events in retinogenesis remain to be examined by using advanced molecular genetic approaches. Nonetheless, current evidence establishes CNTF/LIF as potent growth factor agents that can affect retinal neuronal differentiation and survival.

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