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Roles of Transforming Growth Factor-α and Related Molecules in the Nervous System

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Abstract

The epidermal growth factor (EGF) family of polypeptides is regulators for tissue development and repair, and is characterized by the fact that their mature forms are proteolytically derived from their integral membrane precursors. This article reviews roles of the prominent members of the EGF family (EGF, transforming growth factor-alpha [TGF-α] and heparin-binding EGF [HB-EGF]) and the related neuregulin family in the nerve system. These polypeptides, produced by neurons and glial cells, play an important role in the development of the nervous system, stimulating proliferation, migration, and differentiation of neuronal, glial, and Schwann precursor cells. These peptides are also neurotrophic, enhancing survival and inhibiting apoptosis of post-mitotic neurons, probably acting directly through receptors on neurons, or indirectly via stimulating glial proliferation and glial synthesis of other molecules such as neurotrophic factors. TGF-α, EGF, and neuregulins are involved in mediating glial-neuronal and axonal-glial interactions, regulating nerve injury responses, and participating in injury-associated astrocytic gliosis, brain tumors, and other disorders of the nerve system. Although the collective roles of the EGF family (as well as those of the neuregulins) are shown to be essential for the nervous system, redundancy may exist among members of the EGF family.

Index Entries: EGF; TGF-α; neuregulin; ErbB; neurons; glia; proliferation; differentiation; survival; neurotrophic factors

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Superfamily of ErbB Receptors and Their Ligands

EGF Family and ErbB-1 Receptor

The epidermal growth factor (EGF) family of growth factors, including EGF, transforming growth factor-alpha (TGF-α), amphiregulin (AR), heparin-binding EGF (HB-EGF), betacellulin (BTC), and epiregulin (EPR), are a family of polypeptides that are distinguished by the fact that their soluble forms are proteolytically derived from their integral membrane precursors (Lee et al., 1995a). These membrane-integral peptides contain one or more conserved 3-disulfide loop compact motif (EGF-like domain), providing the proper folding of the extracellular binding domains. These ligands are 26–50% homologous in their EGF-like sequences, and bind to their common receptor, EGF receptor (EGF-R) or ErbB1 molecule. EGF-R is an integral membrane tyrosine kinase receptor (Prigent and Lemoine, 1992); binding of a ligand extracellularly induces dimerization of the receptor, with concomitant activation of the intrinsic tyrosine kinase in the cytoplasmic domain, leading to proliferation, differentiation, survival, or migration of various cells of epithelial and mesenchymal origins.

Neuregulin Family and the ErbB Receptors

EGF-R and its ligands are components of an extended superfamily of ErbB1-related receptors and EGF-like ligands. The ErbB family consists of four distinct members, including the ErbB-1 (EGF receptor or EGF-R, or HER1) (Prigent and Lemoine, 1992), ErbB2 (HER2, or neu) (Bargmann et al., 1986), ErbB3 (HER3) (Kraus et al., 1989), and ErbB4 (HER4) (Plowman et al., 1993), which have all been found expressed in the developing and adult brains (Kornblum et al., 2000; Kornblum et al., 1997; Steiner et al., 1999). The EGF-related neuregulins (NRG, neuregulin-1) or heregulins (HRG) (Homes et al., 1992), are a family of structurally diverse glycoproteins sharing a common EGF-like domain, and are also referred to neu differentiation factors (NDF) (Dong et al., 1995), glial growth factors (GGF) (Marchionni et al., 1993), and acetylcholine-receptor-inducing activity (ARIA) (Fails et al., 1993). Recently, sets of neuregulin-like growth factors termed neuregulin-2 (Carraway et al., 1997; Chang et al., 1997), neuregulin-3 (Zhang et al., 1997), and neuregulin-4 (Haravi et al., 1999) have been discovered, which were found to have activities that are either similar to or distinct from those of neuregulin-1. Neuregulins, expressed transiently during development by several cell types, including migrating cranial neural crest cells and embryonic neurons, are shown to affect the in vitro proliferation and cell fate of pluripotent neural crest cells and to be a survival factor and differentiation factor for Schwann cells (Lemke et al., 1996; Mirsky et al., 1996; Gassman and Lemke, 1997; Mirsky and Jessen, 1999) and oligodendrocytes (Vartanian et al., 1999). The ErbB receptors are activated by binding the ligands of the EGF family or the neuregulin family, and can signal either through homodimerization or through heterodimerization with other ErbB molecules, followed by the receptor auto- or trans-phosphorylation on specific tyrosine residues in their cytoplasmic tails. Activation of ErbB receptors plays an important role in the regulation of cell proliferation, differentiation, and survival in several different tissues.

Cross-Interactions Within the EGF- and ErbB1-Related Superfamilies

There is increasing evidence of cross-talk among the ErbB receptor members, which may reflect heterodimerization and/or transactivation (Riese and Stern, 1998). Evidence has indicated that, despite the complexity of the ErbB molecules and their ligands, there is some specificity of receptor-ligand interaction and some tissue specificity of receptor/ligand expression within the superfamily. It has been shown that binding of a specific ligand to one of the ErbB receptors triggers the formation of specific receptor homo- and hetero-dimers,

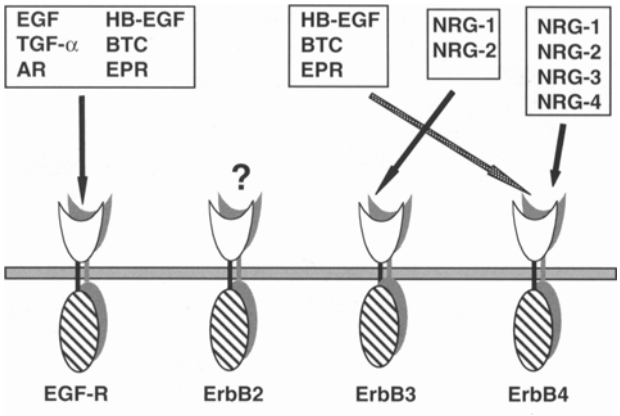

Fig. 1. Specificity and complexity of ligand-receptor interactions between EGF and neuregulin families of ligands and ErbB receptors.

with ErbB2 being the preferred heterodimerization partner. Although ErbB1 is the preferred receptor for EGF, TGF-α, AR, HB-EGF, betacellulin, and epiregulin, ErbB4 can also bind HB-EGF, betacellulin, and epiregulin; ErbB-3 and/or ErbB-4 bind neuregulins (Carraway et al., 1997; Chang et al., 1997; Zhang et al., 1997; Harari et al., 1999; Graus Porta et al., 1997) (Fig. 1). An in vitro study (Sundaresan et al., 1998) with nontransformed cell lines derived from a variety of tissues including the nervous system demonstrated that although all cell lines examined expressed detectable levels of ErbB2, the other types of ErbB receptors expressed depended on the cellular responsiveness to particular ligand treatment. While the high levels of expression of ErbB3 were correlated with responsiveness to neuregulins, the expression of ErbB-1 or EGF-R was correlated with responsiveness to EGF and betacellulin. Conversely, the sensitivity of a cell line to ErbB ligands was also correlated with the levels of expression of the appropriate ErbB receptors in the cell line. This study suggests that appropriate biological responsiveness to ErbB ligands is determined by the levels of expression of specific ErbB receptor combinations within a given tissue (Sundaresan et al., 1998). The superior ability of ErbB2 to form heterodimers with other ErbB receptors and its high basal tyrosine kinase activity may in part explain why ErbB2 is the most oncogenic family member and is often associated with tumors (Tzahar et al., 1996).

Although there are excellent reviews on different aspects of the EGF family of growth factors (Lee et al., 1995; Derynck, 1988, 1992; Barnard et al., 1995; Gangarosa et al., 1996), there is lack of a proper review focusing on roles of the EGF family in the nervous system. The purpose of this review is to discuss and summarize the current knowledge on the expression and the mitogenic and neurotrophic functions of EGF family, as well as the related neuregulin family of growth factors in the normal nervous system (Table 1), and in injuries or disorders of the brain, spinal cord, and peripheral nerves (Table 2). The possible action mechanisms of the EGF family of ligands, including their interaction with other neurotrophic factors and their role as a messenger mediating the glial-neural or axonal-glial cross-talk, will be briefly described, and the possible redundancy of the EGF-related ligands will be discussed. Because EGF receptor (EGF-R, ErbB1, or HER1) and its ligands EGF and TGF-α are the most studied molecules of the EGF- and ErbB1-related superfamily in the nervous system, these molecules will be the focus of discussion in this review, although the other ligands of EGF-R and ligands of other ErbB receptors (neuregulins) will be described briefly.

TGF-α, EGF, HB-EGF, and EGF-R Expression in the Nervous System

Previous studies have demonstrated that TGF-α and EGF-R are expressed at high levels in the central nervous system (CNS). Due to its much higher abundance and wider distribution compared to other ligands, TGF-α has been considered to be the prominent ligand for EGF-R in the CNS (Lee et al., 1985; Seroogy et al., 1991; 1994, 1995; Lazar and Blum, 1992).

Table 1
Roles of TGF-α, EGF, HB-EGF, and Related Molecules in the Nervous System

Functions	Target cells
(A) TGF-α, EGF, and HB-EGF support proliferation, survival, and differentiation of brain multipotent neural progenitors and neuronal and glial precursor cells during development	Embryonic neural and glial precursor cells (Kornblum et al., 1999, 1998; 1997; Svendsen et al., 1997; Threadgill et al., 1995)
(B) TGF-α/EGF modulates neuronal/glial differentiation:	
In vivo cell fate regulation of substantial nigra progenitor cells	Dopaminergic neuronal precursors (Blum, 1998)
TGF-α/EGF supports neural differentiation in vitro	Cultured dopaminergic neurons (Casper et al., 1991)
Promotion of progenitor cell maturation by extra EGF-Rs introduced by retrovirus in vivo	Ventricular cortical progenitor cells (Burrows, 1997)
In vitro cell fate modification of cerebral cortical neuronal differentiation	Sensorimotor neurons showing expression of limbic phenotype in the presence of EGF or TGF-α (Ferri and Levitt 1995)
Inhibition of cholinergic differentiation	Cultured basal forebrain cholinergic neuron precursors (Jonakait et al., 1998; Mazzoni and Kenigsberg, 1996)
Promotion of neuritogenesis in vitro	Embryonic chick CNS neuron precursors; PC12 cells (Rosenberg and Noble, 1989; Wu and Howard, 1995; Zhang et al., 1990)
Promotion of Muller glial cell differentiation after in vivo introduction of extra EGF-Rs by retrovirus	Retinal progenitor cells (Lillien, 1995)
(C) TGF-α/EGF neurotrophic effects, promoting survival, and inhibiting apoptosis	Dopaminergic and GABAergic neurons, some sensory neurons (Ferrair et al., 1991; Chalazonitis et al., 1992)
(D) TGF-α/EGF stimulation of glial proliferation and differentiation	Astrocytes (Seroogy et al., 1995; Welkert and Blum, 1995; Sibilia et al., 1998; Zhang et al., 1990; Leutz and Schachner, 1981)

(E) Mediation of glial-neuronal or axonal-glial interactions:	
Guidance of cortical neuronal migration by radial glial fibers during cortical development	Neuregulin (GGF) produced by migrating neurons interacts with ErbB receptors on glial fibers (Anton et al., 1997)
TGF-α/neuregulins indirectly involved in glial-neuronal interaction in female sexual maturation	TGF-α/neuregulins induce hypothalamic glial activation, and secretion of prostaglandin E2, which stimulates neuronal LHRH synthesis (Ma et al., 1997; Ojeda and Ma, 1998; Ma et al., 1992)
Neuregulins in glial-neuronal interaction in sympathetic neuronal development	Sympathoneuroblast-derived neuregulins stimulate non-neuronal cells to produce neurotrophin NT-3 (Verdi et al., 1996)
Regulation of axonal-glial interactions for Schwann cell survival and axonal development	Sensory or motor neuron axon or neurite-derived neuregulins interact with ErbB3 on Schwann cells (Vartanian et al., 1997; Grinspan et al., 1994; Syroid et al., 1996)
(F) TGF-α, EGF, and neuregulin induction of neurotrophic factors: NGF, NT-3, TGF-β1, IGF-I, CNTF, etc.	Astrocytes, PC-12 cells, fibroblasts, smooth muscle cells, etc. (Lindholm et al., 1992; Spranger et al., 1990; Kamiguchi et al., 1995; Han et al., 1992; Yoshida and Gaga, 1992; Creedon and Tultle, 1997)
(G) Neuregulins in development of peripheral nerve axons of sensory and motor neurons	Schwann cell proliferation, differentiation, survival (Dong et al., 1995; Marchionni et al., 1993; Meyer and Birchmeier, 1995; Zorick and Lemke, 1996; Baek and Kim, 1998)
(H) Neuregulins in survival of sensory and motor neurons	Sensory neurons in DRG and motor neurons (Beithmacher et al, 1997)
(I) Neuregulins in axonal guidance in CNS, development and differentiation in cerebellum	Proliferation, survival, and differentiation of oligodendrocytes (Erikson et al., 1997; Gassmann et al., 1995)
(J) Neuregulins in development of sympathetic ganglia	Sympathetic neurons (Britsch et al., 1998)

Table 2
Roles of TGF-α and Related Ligands in Disorders or Injuries of the Nerve System

Functions	Targets
(A) TGF-α mediation of hypothalamic lesion-induced precocious female puberty	Injury-induced reactive astrocytes (Junier et al., 1993, 1991)
(B) Neurotrophic effects after brain injury:	
TGF-α and EGF neurotrophic to injured dopaminergic neurons in the nigro-striatal pathway	Dopaminergic neurons (Ventrella, 1993)
EGF possible modulation of endogenous forebrain precursor cells after brain injury	Differentiation of precursor cells into neuronal/glial cells and promote cell migration in adult brain (Craig et al., 1996)
HB-EGF possibly an endogenous neuroprotective agent after seizure-induced brain injury	Hippocampal neurons (Opanashuk et al., 1999)
(C) Exogenous TGF-α and EGF neuroprotective against ischemic injury	Hippocampal neurons (Peng et al., 1998, Justieia and Planas, 1999)
(D) Upregulated TGF-α in reactive glia is involved in injury-induced astrocytic gliosis in CNS	Astrocytes (Nieto-Sampedro, 1998, Topp et al., 1989; Junier et al., 1994; Rabchevsky et al., 1998)
(E) Induction of TGF-α in motoneurons is associated in degeneration of these neurons	Motoneurons (Lisovski et al., 1997; Junier et al., 1998)
(F) Overexpression of EGF-R, TGF-α, and EGF in astrocytes is associated with astrocytic gliomas: proliferation, migration, invasion	Astrocytes (von Diemling et al., 1995; Kleihues et al., 1995; Ohgaki et al., 1995; Leon et al., 1994; Yamazaki et al., 1990; Saxena and Ali, 1992; Oude Weernink et al., 1996; Goumnerova, 1996; Lund Johanson et al., 1990; Chicoine et al., 1995; Tang et al., 1997)
(G) Involved in peripheral nerve injury responses:	
EGF-R upregulation at peripheral nerve injury site	Schwann cells, fibroblasts (Toma et al., 1992)
Upregulation of neuregulins, ErbB2 and ErbB3 in Schwann cells after nerve injury	Schwann cells (Carroll et al., 1997)
TGF-α upregulated in satellite cells while EGF-R in neurons in dorsal root ganglia after peripheral nerve injury	Satellite cells and sensory neurons in DRG (Xian and Zhon, 1999)

While the expression of EGF mRNA in the brain is limited to smaller discrete areas (mainly in olfactory bulb, basal hypothalamus, and cerebellum) (Lazar and Blum, 1992), TGF-α gene expression is almost ubiquitous, widely distributed in the brain (Seroogy et al., 1991; Wilcox and Derynck, 1988). A quantitative mRNA analysis (Lazar and Blum, 1992) has shown that the levels of TGF-α mRNA in the brain, equal in both sexes, are about 15–170 times greater than the regional levels of EGF mRNA. While highest in the striatum, a relatively high abundance of TGF-α mRNA is also noted in the olfactory tubercle and olfactory bulb, followed in descending order by brainstem, hippocampus, thalamus, hypothalamus, cerebral cortex, and cerebellum. Recent *in situ* hybridization studies have demonstrated the presence of HB-EGF mRNA within the brainstem as early as E14 and subsequently in the cortical plate, hippocampus, cerebellar Purkinje cells, and ventrobasal thalamus in the developing brain (Kornblum et al., 1999).

EGF-R has also been shown to be expressed in the brain with a similar pattern of regional differential expression to that of TGF-α mRNA (Kaser et al., 1992). In the brain, TGF-α is expressed in neurons (Kornblum et al., 1997; Wilcox and Derynck, 1998; Kudlow et al., 1989; Ferrer et al., 1995, 1996) and astrocytes (Ferrer et al., 1995, 1996; Fallon et al., 1990; Seroogy et al., 1993), while EGF-R is expressed in neurons (Kornblum et al., 1997; Ferrer et al., 1996; Werner et al., 1998; Tucker et al., 1993), astrocytes, and oligodendrocyes (Mazzoni and Kenigsberg, 1994). In the embryonic midbrain, EGF-R mRNA overlapped extensively with that of tyrosine hydroxylase mRNA, suggesting that fetal dopaminergic neurons express EGF-R (Kornblum et al., 1997). These studies suggest that TGF-α may act on both neurons and glia in a paracrine or an autocrine route in the brain. However, studies on ligand binding and receptor activation with brain cell primary cultures in vitro have shown that EGF-R is more abundant in astrocytic than neuronal cells in the neonatal brain (Wang et al., 1989). Furthermore, a recent study has shown that in neuron-enriched cultures derived from the neocortex and the striatum, intermediate filament protein nestin-positive cells (i.e., neural precursor cells) but not neurons express EGF-R mRNA, indicating that functions of EGF-R ligands on neurons may be a result of indirect stimulation mediated by non-neuronal cells (Kornblum et al., 1999).

Proteins and mRNAs of TGF-α and its receptor are also expressed in the peripheral nervous system (PNS) (Werner et al., 1988; Birecree et al., 1991; Yasui et al., 1992; Huerta et al., 1996; Vega et al., 1994). For example, EGF-R immunoreactivity is expressed in human cutaneous nerves and sensory corpuscles, suggesting a role for EGF or TGF-α in the PNS (Vega et al., 1994). In the human dorsal root ganglia (DRG), EGF-R has been found in most (86%) primary sensory neurons with immunoreactivity stronger in small- and intermediate-sized neurons, satellite glial cells, as well as in the intraganglionic and dorsal root Schwann cells (Huerta et al., 1996). Similarly, in normal adult rat DRGs, it has been demonstrated that TGF-α immunoreactivity is also mainly localized to sub-populations of small and medium-sized neurons (Xian and Zhou, 1999). While most of the large or medium neurons were devoid of TGF-α immunoreactivity in their cell bodies, satellite cells surrounding these negative neurons displayed positive immunostaining (Xian and Zhou, 1999). Furthermore, in the normal rat DRG, EGF-R shares a similar distribution pattern with TGF-α, localizing in a subpopulation of small neurons and in satellite cells ensheathing some large or medium-sized neurons (Xian and Zhou, 1999). Therefore, there seems to be a complementary trend in TGF-α or EGF-R expression in the normal DRG, i.e., when the small neurons are immunopositive for TGF-α or EGF-R, there are no surrounding immunopositive glial cells, and conversely, some large- or medium-diameter neurons displaying the immunopositivities in their surrounding satellite cells do not express TGF-α and EGF-R in their somata. The biological implications for this reciprocal expression remains to be determined.

Roles of TGF-α, EGF, HB-EGF, and Their Receptor in the Nervous System

Functions in CNS Stem Cells and Brain Development

The early expression and widespread distribution of TGF-α and EGF-R in the developing brain suggest that TGF-α may play a role in brain development, possibly involved in the genesis, differentiation, migration, or survival of numerous cell populations in the embryonic brain (Kornblum, 1997). Consistent with this, various in vivo and in vitro studies have demonstrated that TGF-α stimulated proliferation and differentiation of both neuronal and glial precursor cells (Seroogy et al., 1995; Tucker et al., 1993; Ferri and Lavitt, 1995; Weickert and Blum, 1995; Lu et al., 1996) and improved survival of cultured astrocyte precursor cells (Yoshida et al., 1993). Similarly, expression of HB-EGF in developing brain in vivo and the proliferative responsiveness of CNS astrocytes and the neuronal survival response in vitro to HB-EGF indicate that HB-EGF may be an important trophic factor in the developing CNS (Kornbium et al., 1999).

Recent studies have suggested that multipotent neural progenitor cells (stem cells) play an important role in neural lineage elaboration during neurogenesis and gliogenesis after migration from paramedian generative zones (Rao, 1999; Mehler and Gokhan, 1999). EGF and/or basic fibroblast growth factor (bFGF) induce(s) in vitro proliferation, self-renewal, and expansion of neural stem cells isolated from specific regions of the embryonic and adult CNS including lateral ventricle subventricular zone, forebrain subependymal compartment, telencephalic germinal zone, and spinal cord; these cells exhibit multipotent properties and can differentiate into both neurons and glia when exposed to a substrate (Rao, 1999; Mehler and Gokhan, 1999; Santa-Olalla and Covarrubias, 1995; Weiss et al., 1996a, 1996b; Chiasson et al., 1999; Tropepe et al., 1999). For example, in the embryonic mouse telencephalic germinal zone, there are two separate types of neural stem cells that are respectively responsive to EGF and bFGF (Tropepe et al., 1999). Apart from the proliferative effect, EGF also mediates the survival of some brain-derived neural stem cells in cell culture; withdrawal of EGF from cultural medium induced apoptosis within 24h through a Bcl-2-related common mechanism as for other growth factor-related apoptotic systems (Loo et al., 1998). Similarly, a multipotent clonal cell line (MEB5) from embryonic mouse forebrain relies on EGF for survival and suppression of apoptosis (Nakagaito et al., 1998).

The lineage relationship between the EGF- and bFGF-responsive cells has been elucidated recently. The bFGF-responsive neural precursors have been found to be the source of EGF-responsive neural precursors (Ciccolini and Svendsen, 1998; Santa-Olalla and Covarrubias, 1999). The differentiation effect of bFGF leads to the appearance of EGF-R mRNA, resulting in the acquisition of EGF responsiveness of the bFGF-responsive precursor cells (Santa-Olalla and Covarrubias, 1999). Among the EGF family of growth factors, effects of other ligands on neural stem cells isolated from different regions of the CNS are less well-studied. In contrast to EGF, subventricular zone stem cells can not be expanded by HB-EGF (Whittemore et al., 1999), although one other recent study showed that some multipotent progenitor cells were responsive to the HB-EGF in vitro (Kornblum et al., 1999). TGF-α, like EGF and bFGF, also induced cell proliferation of the cultured neural precursor cells of mouse embryonic mesencephalon and formation of colonies; however, the number of colonies formed did not increase significantly when TGF-α was used in combination with bFGF in contrast to the combined use of EGF and bFGF (Santa-Olalla and Covarrubias, 1995). In adult mammalian forebrain subependyma, where the neural stem cells and their progeny constitutively proliferate, the endogenous expression of TGF-α has been shown to be necessary for the full proliferation of the progenitor cells,

since in TGF-α null mice there was a decreased proliferation of these progenitor cells (Tropepe et al., 1997).

An in vitro study showed that EGF-responsive cells derived from different CNS regions of postnatal mice can differentiate into region-specific phenotypic neurons when stimulated with an appropriate neurotrophic factor (e.g., brain-derived neurotrophic factor [BDNF]) (Shetty and Turner, 1998). Proliferative progenitor cells isolated from the developing human CNS, when expanded using a combination of EGF and bFGF and then transplanted into the striatum of adult rats, migrated into other regions of the brain and differentiated into both astrocytes and neurons. This in vivo study indicates that these cells as such may have potential for development as an alternative source of tissue for neural transplantation in degenerative diseases (Svendsen et al., 1997). However, in vivo and in vitro studies have demonstrated that neural stem cells responsive to EGF or bFGF may have different differentiative potentials. In the adult rat brain, intracerebroventricular administration of EGF expanded the progenitor cell population in the subventricular zone, but reduced the total number of newborn neurons reaching the olfactory bulb, the normal destination for neuronal progenitors migrating from the subventricular zone (Kuhn et al., 1997). Consistent to this study, EGF-responsive neural progenitor cells from mouse brain, after *in utero* xenotransplantation into the embryonic rat brain, displayed widespread incorporation into distinct forebrain and midbrain structures, but were found to differentiate predominantly into glial cells (Winkler et al., 1998). Similarly, an in vitro study showed that although neurons, oligodendrocytes, and astrocytes were differentiated from cultures of neural progenitor cells isolated form the adult rat subventricular zone, EGF-generated neural precursor cells were more restricted to an astrocytic lineage, whereas bFGF-generated precursor cells had a greater capacity for neuronal differentiation (Whittemore et al., 1999).

EGF-R knockout studies have shown that EGF-R expression is critical for the development and maintenance of the brain. Although the brain from EGF-R null mice at birth was cytoarchitecturally normal, it was shown smaller particularly at the cortical regions (Threadgill et al., 1995; Kornblum et al., 1998), and there was massive neural degeneration and atrophy particularly in the anterior areas of the cerebral cortex, which was in part due to apoptosis (Threadgill et al., 1995; Kornblum et al., 1998). Apart from the lower numbers of neuronal cells both in the cerebellum and cerebral cortex, there was a retarded cellular migration (Threadgill et al., 1995). Furthermore, there were also delays in glial fibriliary acidic protein (GFAP) expression within the glia limitans and within structures outside the germinal zones in early postnatal ages, and a lower number of GFAP positive astrocytes in the cerebral cortices, which also displayed reduced proliferation in vitro (Threadgill et al., 1995; Kornblum et al., 1998; Sibilia et al., 1998). These observations indicate a critical role of the EGF-R in mediating the functions of its ligands in the development and maintenance of the CNS.

However, in the EGF-R null mice study, mice were generally quite ill and died at very young ages, which raises the possibility that many of CNS effects of EGF-R knockout could be secondary to systemic abnormalities. Of note, some of the neuronal populations that degenerated do not normally express EGF-R; and some neuronal populations including striatal GABAergic and midbrain dopaminergic neurons, which have previously been shown to express EGF-R, appeared to be intact at birth (Kornblum et al., 1998). Furthermore, some multipotent progenitors do not need EGF-R activation (Tropepe et al., 1999); and the multipotent precursor cells and astrocytes derived from EGF-R knockout mice were capable of proliferation in response to bFGF (Kornblum et al., 1998). Therefore, loss of some neuronal populations in EGF-R null mice may be a result of indirect mechanisms of neuronal death.

Neuronal Differentiation

Increasing evidence indicates that specific genes and signaling pathways are involved in controlling the formation of discrete areas in the cerebral cortex and the neuronal differentiation. Studies conducted both in vitro and in vivo have suggested that TGF-α and EGF can regulate cell fate of progenitor cells and neuronal or glial differentiation. In TGF-α null mice, there were 50% fewer dopaminergic neurons and a 20% reduction in the overall volume of the dorsal striatum, indicating that TGF-α is required for the normal proliferation or differentiation of a selected population of dopaminergic neurons within the substantial nigra (Blum, 1998). This in vivo finding is consistent with an in vitro observation showing that EGF was neurotrophic to the dopaminergic neurons, enhancing the survival of these neurons in rat embryonic mesencephalon primary cell culture (Casper et al., 1991).

In vivo and in vitro studies have suggested that exogenous TGF-α or EGF or higher levels of EGF-R can control the timing of progenitor cell maturation, and alter the fate of neuronal or glial differentiation. For example, EGF-R expressed by progenitor cells in the cortex has been shown to contribute to the timing of their maturation (Burrows et al., 1997). Introduction of extra EGF-Rs into the early cortical progenitor cells of the ventricular zone with a retroviral delivery system in vivo and in vitro resulted in premature expression of traits characteristic of the late progenitor cells of the subventricular zone, including migration patterns, differentiation into astrocytes, and proliferation of multipotential cells to form spheres (Burrows et al., 1997). Similarly, introduction of extra EGF-Rs into the progenitor cells in the retina, which normally will differentiate into rod photoreceptor cells, neurons, and Muller glial cells, promoted the differentiation of the progenitor cells into Muller glial cells (Lillien, 1995). Furthermore, TGF-α and EGF have been shown to be able to modify the differentiation of cerebral cortical neuron precursor cells in culture (Ferri and Levitt, 1995). In cultured sensorimotor neuron precursors harvested from the sensorimotor (nonlimbic) zone of E12 rat brain, in combination with some collagen matrix components in the culture, EGF and TGF-α increased the expression of limbic system-associated membrane protein (*LAMP*), a neuron-specific marker of limbic cortical areas. However, in vitro studies have shown that while TGF-α or EGF expands the progenitor cell pool of the cultured embryonic rat forebrain, they inhibit cholinergic differentiation, as measured by the number of neurons that express choline acetyltransferase (Jonakait et al., 1998) or choline acetyltransferase/acetylcholine esterase activities (Mazzoni and Kenigsberg, 1996).

Moreover, in vitro studies have indicated that TGF-α or EGF can induce neuritogenesis in some neurons. For example, in cultured embryonic chick CNS neuronal precursors, EGF appeared to be a primary neurite-inducing growth factor, since an EGF neutralizing antibody not only inhibited biosynthesis of plasmic membrane-associated gangliosides but also neuritogenesis (Rosenberg and Noble, 1989). In PC12 pheochromocytoma cells, both TGF-α and EGF can induce neurite outgrowth (Wu and Howard, 1995; Zhang et al., 1990). In calretinin-immunoreactive neuronal culture derived from E14 rat embryo at the thalamic eminence, however, it was demonstrated that, while BDNF increased the length of the primary and secondary neurites and the numbers of secondary neurites, TGF-α did not affect the number and length of the primary or secondary neurites (Iwasaki et al., 1998), indicating that maturation and development of some neurites may be controlled by specific growth factors.

Neurotrophic Effects

Evidence has shown that EGF is a potent trophic factor for a variety of neurons in CNS, including dopaminergic and GABAergic neurons (Ferrair et al., 1991), neocortical neurons (Kornblum et al., 1990), and other neurons isolated from neonatal rat brain (Morrison et al.,

1987). Mice lacking EGF-R exhibit brain defects after birth and develop a progressive neurodegeneration in the brain, characterized by massive apoptosis and upregulation of c-fos (Sibilia et al., 1998). These studies indicate the important roles of TGF-α and possibly other ligands of EGF-R in survival of post-mitotic neurons. Several in vitro studies have demonstrated that TGF-α can promote the survival and inhibit apoptosis of differentiated neurons (Abe and Saito, 1992; Boniece and Wagner, 1993; Yamada et al., 1995; Alexi et al., 1997), suggesting that in the normal adult brain, TGF-α may act as a neurotrophic factor in the maintenance and modulation of functions of the differentiated neurons. EGF, as well as other neurotrophic factors such as nerve growth factor (NGF) and FGF, blocked the apoptosis of cultured rat embryonic neuronal cells and PC12 cells induced by exposure to a high oxygen atmosphere (Satoh et al., 1998). In the PNS, although TGF-α has been shown to be able to promote survival of some sensory neurons in DRG in vitro, it did not affect the survival of neurons from nodose, trigeminal, and sympathetic ganglia (Chalazonitis et al., 1992).

Mitogen and Differentiation Agent for Astrocytes

TGF-α and EGF function as both mitogen and differentiation agents for astrocytes in vivo and in vitro, stimulating their proliferation, process extension, and GFAP expression during neurogenesis or regeneration of the nervous system (Seroogy et al., 1995; Weickert and Blum, 1995; Zhang et al., 1990; Leutz and Schachner, 1981). It has been shown that in TGF-α deficient mice (wa-1 mice), less GFAP and fewer proliferating astrocytes were found in the brain (Weickert and Blum, 1995). Furthermore, cerebral cortices from mutant mice lacking EGF-R contain lower numbers of GFAP-positive astrocytes, which display reduced proliferation in vitro (Sibilia et al., 1998). These studies indicate the important roles of TGF-α and possibly other ligands of EGF-R in proliferation and differentiation of astrocytes.

Regulation of Glial-Neuronal Interactions

Neurons and glial cells have a close anatomical and functional relationship. Astrocytes in the CNS express receptors for a variety of growth factors and neurotransmitters; in turn, neuronal cells can respond to astrocyte-derived growth factors and control astrocyte function via a common set of signaling molecules and intracellular transducing pathways. In CNS, these neurotransmitter- or growth factor-mediated glial-neuronal interactions play a crucial role during development of neural pathways and in the maintenance of normal functions and neuroprotection in adult brain (Lauder and Liu, 1994; Marchetti; 1996). For example, neuregulin glial growth factor (GGF) functions as a mediator of interactions between migrating neurons and radial glial cells in the developing cerebral cortex (Anton et al., 1997). GGF was shown to be expressed by migrating cortical neurons and promotes their migration along radial glial fibers, which express GGF receptor ErbB molecules. Concurrently, GGF also promoted the maintenance and elongation of radial glial cells, which are essential for guiding neuronal migration to the cortex in the developing brain.

Recent studies have shown that the neuroendocrine mechanisms controlling the development of mammalian puberty involve glial cell activation, glial-neuronal interaction, and neuronal secretion of the neuropeptide luteinizing hormone-releasing hormone (LHRH). LHRH governs sexual development by stimulating the secretion of pituitary gonadotropins. TGF-α and its related peptide neuregulin (NRG) have been identified to be indirect messengers of the glial-neuronal interaction controlling the initiation of female puberty (Ma et al., 1997; Ojeda and Ma, 1998). TGF-α and NRG are produced by the hypothalamus astrocytes and activate the EGF-R and/or ErbB2/ErbB4 receptor complex, not on LHRH neurons,

which are lacking in the ErbB receptors, but on astrocytes. The activation of the receptors leads to glial release of prostaglandin (PG) E2, which then acts directly on LRHR neurons to stimulate LHRH release. Furthermore, a central blockade of TGF-α or NRG action delays puberty, and focal overexpression of TGF-α advances it (Ojeda and Ma, 1998; Ma et al., 1992), indicating that both TGF-α and NRG are important components of the central mechanisms controlling the development of female puberty.

During the development of sympathetic neurons, sympathoneuroblasts synthesize several forms of neuregulins, which stimulate the nearby non-neuronal cells to synthesize neurotrophin 3 (NT-3). In turn, NT-3 can support the survival of some embryonic sympathetic neuroblasts (Verdi et al., 1996). This study suggests a reciprocal cell-cell interaction between sympathoneuroblasts and non-neuronal cells, in which neuroblast-derived neuregulins promote NT-3 production by neighboring non-neuronal cells, which in turn promotes neuroblast survival and further differentiation.

Regulation of Axonal-Glial Interactions

Signaling between axons and glia play an important role in axonal growth, extension, myelination, maintenance, and regeneration. It has been demonstrated that neuregulins and their ErbB receptors are implicated in this axonal-glial interaction process (Vartanian et al., 1997). While oligodendrocytes in the CNS express ErbB2 and ErbB4 but no ErbB3, Schwann cells in the PNS express mainly ErbB2 and ErbB3 but little ErbB4 (Vartanian et al., 1997). A gene knockout study has shown that ErbB3, strongly expressed in Schwann cells (Meyer and Birchmeier, 1995), is essential for development of Schwann cells that accompany peripheral nerves (Riethmacher et al., 1997). Furthermore, neuregulins have been demonstrated to play important roles in the axonal-Schwann cell interactions regulating Schwann cell apoptosis and the number of Schwann cell in developing peripheral nerve (Grinspan et al., 1996; Syroid et al., 1996). ErbB3 mediates a signal given by sensory and motor neurons, which express the specific ligand neuregulin-1 (Marchionni et al., 1993; Meyer and Birchmeier, 1994). Neuregulin was found present on neurites of cultured dorsal root ganglion cells and it was released into the culture medium in a form that promoted ErbB receptor tyrosine phosphorylation (Vartanian et al., 1997). An in vitro study showed that cultured Schwann cells isolated from sciatic nerve of the rat at postnatal d 3 underwent apoptosis upon serum withdrawal, and that this cell death could be prevented by neuregulin-beta (Syroid et al., 1996). Similarly, after losing contact with axons as a result of axotomy, Schwann cells in the rat sciatic nerve (expressing ErbB2 and ErbB3) underwent apoptosis owing to the loss of appropriate survival factors. However, apoptosis of Schwann cells resulting from normal development or from axotomy can be inhibited markedly by exogenous neuregulins. These data suggest that Schwann cell survival in developing peripheral nerve is regulated by apoptosis through competition for axonally derived neuregulin (Drinspan et al., 1996).

EGF and TGF-α in Disorders or Injuries of the Nerve System

Recent evidence has indicated that an alteration in the levels of neurotrophic factors and/or their receptors may be involved in the pathophysiology of human neurodegenerative disorders. Since TGF-α and its related molecules are synthesized by neurons and glial cells, and are involved in controlling the glial and neuronal functions, they can serve as a mechanism for coupling glial or neuronal cells to its environment. Altered expression patterns of TGF-α, EGF, and EGF-R have been implicated in the neuronal-glial interactions in a number of diseases of the CNS and are involved in the host response to injuries of the nerve system.

Hypothalamic Lesion-Induced Precocious Female Puberty

Evidence has shown that TGF-α is an important contributor in the neuropathophysiology of hypothalamic lesion-induced precocious female puberty by indirectly acting on LHRH neurons (Junier et al., 1991). Previous studies have shown that hypothalamic injury resulted in a marked increase in TGF-α production as well as EGF-R expression in reactive astrocytes near the site of injury (Junier et al., 1991; Junier et al., 1993). It has also been shown that, under sex steroid control, TGF-α expression is involved in the developmental process that leads to normal sexual maturation (Ma et al., 1992), and the increase in TGF-α expression by reactive glial cells after hypothalamic injury contributes to the hypothalamic lesion-induced precocious female puberty (Junier et al., 1991).

Neurotrophic Effects and Modulation of Endogenous Precursor Cells After Brain Injury

The poor ability of axons to regenerate in mammalian CNS has been attributed in part to the limited ability of the supportive astrocytes to migrate and thus repopulate the injury site. One in vitro study has shown that EGF and TGF-α in combination with IGF-I can stimulate the migration of cultured astrocytes to the wounding site (Faber Elman et al., 1996). Craig et al. (1996) have demonstrated that intraventricular-administrated exogenous EGF can act as a proliferation, survival, and migration factor for the subependymal multipotent stem cells to expand these populations, differentiate into astrocytes and new neurons, and promote the movement of these cells into normal brain parenchyma (Craig et al., 1996). This study suggests that it may be possible to use EGF for *in situ* modulation of endogenous forebrain precursor cells and to achieve adult replacement of neurons and glia lost to disease or trauma.

In rat hemiparkinsonism made by unilateral mechanical transection of the nigrostriatal pathway, it has been shown that infusion of EGF can increase the number of surviving substantia nigra neurons and the ipsilateral striatal tyrosine hydroxylase-positive fiber staining, as well as enhance a behavioral recovery, indicating EGF neurotrophic effects on dopaminergic neurons in the nigro-striatal pathways (Ventrella, 1993). In addition, HB-EGF may function as an endogenous neuroprotective agent after seizure-induced neural injury. HB-EGF mRNA is expressed in various regions of the brain and the expression was significantly increased in kainate-induced excitotoxic seizures in rat hippocampus (Opanashuk et al., 1999). Furthermore, pretreatment of embryonic hippocampal cell cultures with HB-EGF protected neurons against kainate toxicity (Opanashuk et al., 1999).

Cerebral Ischemia and Excitotoxic Lesioning

Transient cerebral ischemia induces selective neuronal degeneration in the brain. Because of the survival effects of EGF or TGF-α, the potential beneficial effects of EGF and TGF-α in promoting neuronal survival after brain ischemia have been attracting attention. An in vivo study has shown that cerebroventricular infusion of EGF has a neuroprotective effect on hippocampal neurons against transient forebrain ischemia and can effectively prevent ischemia-induced neuronal apoptotic death (Peng et al., 1998). Similarly, in a rat model of permanent middle cerebral artery occlusion, administration of TGF-α to the ipsilateral lateral ventricle was effective in causing a significant reduction in infarct volume compared to vehicle treatment in the ischemic rats, indicating that TGF-α can protect neurons from ischemic damage and prolong their survival after ischemic injury (Justicia and Planas, 1999). In a rat model of Huntington's disease induced by a single unilateral intrastriatal injection of excitotoxin quinolinic acid, intrastriatal administration of TGF-α or neu-

rotrophin-4/5 can partially protect the calretinin-immunopositive striatal neurons against the phenotypic degeneration (Alexi et al., 1997). Consistent with the in vivo effects, in primary hippocampal cultures, EGF extended neuronal survival, facilitated neurite outgrowth, prevented neuronal damage caused by the hydroxyl radical-producing agent $FeSO_4$ and by the peroxynitrite-producing agent 3-morpholinosydnonimine, and attenuated $FeSO_4$-induced lipid peroxidation of the cultured neurons (Peng et al., 1998). These in vitro studies suggest that the neuroprotective effects of EGF on ischemic hippocampal neurons might possibly result from its function of inhibiting free-radical neurotoxicity and lipid peroxidation (Peng et al., 1998).

However, in a rat model of global brain ischemia (Kawahara et al., 1999), low or no EGF mRNA was detected in the postischemic brain as in the normal control brain. Although TGF-α mRNA was widely expressed in the normal brain, its expression did not change appreciably following the ischemia. In contrast, HB-EGF mRNA was rapidly increased in the CA3 sector and the dentate gyrus of the hippocampus, cortex, thalamus, and cerebellar granule and Purkinjie cell layers (Kawahara et al., 1999). This study suggests that HB-EGF, rather than EGF and TGF-α, may be a stress-inducible endogenous neuroprotective factor in cerebral ischemia.

CNS Injury-Associated Astrocytic Gliosis

In response to brain trauma, some astrocytes in the brain expressed a higher level of EGF-R and had a higher proliferation rate (Nieto, 1988; Topp et al., 1989), suggesting that the ligands of EGF-R are involved in glial reaction in the brain in response to trauma. Similarly, owing to the mitogenic effects, overexpression of TGF-α and EGF-R in astrocytes has been implicated in spinal cord injury-associated astrocytic gliosis. Upregulated TGF-α expression has been demonstrated in reactive astrocytes following spinal-cord injury, with its level correlated with motor neuronal degeneration (Junier et al., 1994). Furthermore, TGF-α receptor is localized in a subset of reactive glial cells, with its level following closely with the astrogliosis (Junier et al., 1994). In a transgenic study, overexpression of TGF-α in the transgenic mice induced enhanced expression of GFAP in CNS, induced characteristic morphological features of reactive astrocytes, and increased proliferation among the GFAP-immunoreactive astrocytes in striatum, hippocampus, and cervical spinal cord, the three CNS areas monitored (Rabchevsky et al., 1998). Furthermore, overexpression of the transgene also induced a two-fold increase in EGF-R phosphorylation among the GFAP-positive astrocytes. These results indicate that enhanced synthesis of TGF-α in astrocytes is sufficient to trigger astrogliosis throughout the CNS through a direct action on the upregulated EGF-R on the astrocytes (Rabchevsky et al., 1998).

Motor Neuron Degeneration

It has been found that TGF-α expression in motor neurons is upregulated in response to axonal injury and to mutation-induced degeneration (Lisovoski et al., 1997; Junier et al., 1998). Normally the murine hypoglossal motor neurons were devoid of pro-TGF-α immunoreactivity. In the lumbar spinal cord of a murine wobbler mutant as a model of spinal atrophy, pro-TGF-α immunoreactivity in motor neurons appeared as soon as the disease developed and the expression persisted until the latest stages of degeneration. Following hypoglossal nerve crush and cut, pro-TGF-α expression in motoneurons was precocious and transient, visible at 1 d post-injury and lasting for only 3 d, during which time astrocyte-like cells immunoreactive for both TGF-α and EGF-R appeared within the injured nucleus (Lisovoski et al., 1997). It has been suggested that during the course of their degeneration, motoneurons can initiate expression of TGF-α, which is endowed with trophic and/or differentiative properties for the neurons themselves and their glial environment (Junier et al., 1998).

CNS Tumors

Also owing to the mitogenic property, overexpression and action of TGF-α, EGF, and their receptor EGF-R in astrocytes are associated with brain tumor pathogenesis. Numerous studies (von Diemling et al., 1995; Kleihues et al., 1995; Ohgaki et al., 1995; Leon et al., 1994; Yamazaki et al., 1990) have demonstrated the EGF-R gene amplification and its up-regulated expression in astrocytic gliomas, the most common forms of primary tumors in the CNS. EGF and TGF-α have also been shown to be overexpressed in gliomas (Leon et al., 1994; Yamuzaki et al., 1990; Saxena and Ali, 1992).

In vitro studies with glioma cell cultures and tumor biopsies have shown that EGF stimulates proliferation of glioma cells (Engebraaten et al., 1993; Oude Weernink et al., 1996; Goumnerova, 1996), enhances glioma cell migration (Engebraaten et al., 1993; Lund Johansen et al., 1990), and increases invasion of brain tumor cells (Engebraaten et al., 1993; Lund Johansen et al., 1990). Some studies have also shown that EGF or TGF-α and their receptor are required or responsible for tumor cell proliferation, migration, and invasion, since neutralization antibodies against EGF or TGF-α, and tyrosine kinase inhibitors can inhibit these events in cell culture (Oude Weernink et al., 1996; Goumnerova, 1996; Lund Johansen et al., 1990; Chicoine et al., 1995). Downregulation of the expression of TGF-α by antisense RNA constructs was shown to inhibit tumor cell growth (Tang et al., 1997). Furthermore, Schwannoma-derived growth factor (SDGF), also a EGF-related molecule, is mitogenic on astrocytes, and its expression in gliomas is upregulated and is co-localized with EGF receptor. Moreover, since SDGF antisense RNA can suppress the in vitro growth as well as in vivo tumorigenicity of glioma cells, it has been suggested to be an autocrine growth factor in the development and growth of gliomas (Mishima et al., 1996).

In hemangliobblastoma, co-expression of TGF-α and EGF-R has been observed, indicating that an autocrine or justacrine growth stimulation via the EGF-R may contribute to tumor growth in capillary hemangliobblastomas (Reifenberger et al., 1995). An in vitro study (Goldman et al., 1993) has also shown that in cells from glioblastoma, the most common and malignant of human brain tumors, the activation of EGF-R by EGF led to enhanced secretion of vascular endothelial growth factor (VEGF). VEGF released by glioma cell *in situ* was proposed to account for histopathological and clinical features of glioblastoma tumors, including striking tumor angiogenesis, increased vascular permeability, cellular proliferation, and increased cerebral edema (Goldman et al., 1993). Like platelet-derived growth factor (PDGF), FGF, and transforming growth factor-beta (TGF-β), EGF is implicated in the angiogenesis of a number of tumors including those of glial origin, probably by inducing the secretion of the neovascularization and tumor progression factor VEGF (Jensen, 1998).

It has been found that, among the genetic alterations in gliomas, whereas p53 inactivation and PDGF/PDGF-R activation represent early events, the loss of chromosome 10 and gene amplification and rearrangement of EGF-R represent late events (Tang et al., 1997). Although EGF, TGF-α, and EGF-R have been shown to play important role in brain tumors, much work is needed to understand the details of the signaling pathway of these molecules during the development of gliomas. Understanding the molecular detail of glioma malignant transformation and progression is crucial for developing effective therapeutic strategies.

TGF-α and Neuregulins in Response to Peripheral Nerve Lesion

In response to an axonal injury in the sciatic nerve (Toma et al., 1992), EGF-R is upregulated in Schwann cells and fibroblasts in the nerve particularly close to the site of lesion, indicating that TGF-α or other members of EGF family may play a role in the peripheral nerve. Similarly, in response to axotomy, expression of EGF-related neuregulins and their receptors, ErbB2 and ErbB3, is induced in the Schwann cells, indicating that neuregulins produced by

Schwann cells themselves may be partially responsible for Schwann cell proliferation during Wallerian degeneration, probably acting via autocrine or paracrine mechanisms (Carroll et al., 1997).

In DRGs where somata of sensory neurons are located, differential changes in TGF-α and EGF-R expression have been recently demonstrated following sciatic nerve lesion (Xian and Zhou, 1999). A marked increase in TGF-α immunoreactivity has been observed in satellite cells surrounding many large neurons, forming ring-like structures, and to a lesser extent in the cell bodies of most small and some medium-sized neurons within DRGs. For EGF-R, a rapid upregulation in neuronal immunoreactivity was seen in the DRGs, resulting in intense staining in expanded populations of neurons (from staining in small neuron somata and glial rings around large neurons in normal DRGs to staining in nearly all neuron somata by d 3 after the sciatic nerve lesion). Following a nerve injury, satellite cells around sensory neurons in the injured DRG are known to proliferate (Lu and Richardson, 1991; Stephenson and Byers, 1995), and become activated, expressing a higher level of GFAP (Zhou et al., 1996). These changes in immunoreactivities of TGF-α (mainly glial) and its receptor (mainly neuronal) and their localization may suggest a possible role for the up-regulated TGF-α in satellite proliferation and activation as well as in neuronal survival after nerve lesion. This may also suggest that glial-derived TGF-α may act on large- or medium-sized neurons via a paracrine fashion, with TGF-α probably acting as a means for mediating glial-neuronal interaction for neuronal survival in the degenerating DRG. Consistent with the idea of TGF-α acting as a messenger for the interaction between reactive glia and affected neurons, in an animal model of infantile spinal muscular atrophy, it has been shown that TGF-α synthesized by degenerating motoneurons acts on astrocytes to stimulate their differentiation and astrogliosis in the spinal cord (Junier et al., 1994).

Action Mechanisms of TGF-α, EGF, and HB-EGF

Although there is increasingly accumulating evidence for the functions of EGF and TGF-α on neurons in the CNS, the mechanisms of action remain largely unknown. However, signaling of TGF-α or EGF in mitotic cells is better understood than that in postmitotic neurons. Studies with rat pheochromocytoma PC12 cells and cultured cerebral cortical neurons have indicated that while the mitogenic effects of EGF, TGF-α, and HB-EGF are via the activation of EGF-R leading to activation of mitogen-activated protein kinase (MAPK) in normal transient fashion, the neuronal differentiation effects of EGF on a subclone of PC12 cells (PC12h-R cells) are mediated by sustained activation of EGF-R and the MAPK in response to EGF (Kornblum et al., 1999; Yamada et al., 1997; 1996a, 1996b), which is correlated with a decreased rate of EGF-R intracellular degradation (Yamada et al., 1997).

Although some in vitro studies have demonstrated that EGF or TGF-α can act directly on some cultured cerebral cortical and cerebellar neurons to enhance neurite outgrowth and survival, EGF can also affect functions of other types of neurons, including septal cholinergic and mesencephalic dopaminergic neurons, indirectly through glial cells (Yamada et al., 1997). In primary cultures derived from the fetal-rat medial septal area, the effect of EGF on inhibiting choline acetyltransferase activity in forebrain cholinergic neurons was found to be mediated via the proliferation and action of astroglia (Kenigsberg and Mazzoni, 1995). Similarly, the neurotrophic effects of TGF-α on dopaminergic neuronal culture has been found to be accompanied by cell proliferation and mediated by astroglial cells. Two highly specific inhibitors of EGF-R signal transduction pathway, 4,5-dianilinophthalimide and tyrphostin B56, selectively blocked mitogenic effects of EGF and TGF-α and their neurotrophic capacity (Krieglstein and Unsicker, 1997). Furthermore, in dissociated mouse mes-

encephalic cell cultures, TGF-α induced c-fos expression predominantly in glia but not in dopaminergic neurons, consistent with the proposed glial-mediated indirect mode of action of TGF-α in supporting the survival and differentiation of embryonic dompaminergic neurons (Engele and Schilling, 1996).

Interaction Between TGF-α or EGF and Other Neurotrophic Factors

It has been shown that, following an injury of the nervous system, a number of upregulated neurotrophins, growth factors, and cytokines interact and co-operate, and have been implicated as regulators of injury responses (Logan et al., 1994). For example, TGF-β1 can modulate nerve regeneration after brain injury by upregulating NGF synthesis and by controlling the extent of astrocyte proliferation and scar formation (Lindholm et al., 1992). Interleukin (IL)-1 can regulate synthesis of NGF in non-neuronal cells of rat sciatic nerve (Lindholm et al., 1987).

In vivo and in vitro studies have shown that TGF-α and EGF can induce in both astrocytes and retinal Muller cells the expression of c-fos (Condorelli et al., 1989; Sagar et al., 1991), one of the immediate early genes coding for transcription factors, which regulate expression of their target genes, indicating that TGF-α or EGF can induce the expression of other factors. In support of this, TGF-α significantly increased NGF mRNA and protein levels in rat astrocyte cultures (Spranger et al., 1990); and EGF increased the secretion of ciliary neurotrophic factor (CNTF) (Kamiguchi et al., 1995) and the production of TGF-β1 (Lindholm et al., 1992) in cultured astrocytes. An interaction between EGF and IGF-I exists in gliogenesis of neonatal rat brain, since the mitogenic activity of EGF on astroglia was in part due to the local synthesis of IGF-I by astroglial cells (Han et al., 1992). Similarly, both TGF-α and EGF upregulate the synthesis and secretion of NGF in skin fibroblasts (Yoshida and Gage, 1992); and EGF has been shown to increase the release of NGF from smooth muscle cells (Creedon and Tuttle, 1997), one of the primary targets of sympathetic nerves.

Furthermore, the interaction between the EGF family ligands and neurotrophins may play a role in regulating neuronal mitogenesis or differentiation. In PC12 neuronal cell culture, EGF, that induces cell growth, as well as NGF and FGF, which cause morphological differentiation, produce a significant increase in TGF-β1 transcripts (Cosgaya and Arauda, 1996). On the other hand, in PC12 cells, NGF treatment decreased the sensitiveness of the differentiating cells to mitogens by downregulating the synthesis and binding capacity of the EGF-R (Lazarovici et al., 1987). Similarly, BDNF induced downmodulation of EGF-R in Swiss mouse 3T3 fibroblasts by upregulating the receptor's internalization (Haang et al., 1988). All the aforementioned evidence suggests that TGF-α or EGF can interact with other neurotrophic factors in both neuronal and non-neuronal cells, and it is possible that TGF-α or EGF may initiate the upregulation of other neurotrophic factors during neural development and in response to nerve lesion.

Essential Roles of and Possible Redundancy Within the EGF Family

EGF-R knockout studies (Threadgill et al., 1995; Kornblum et al., 1998; Sibilia et al., 1998) and other studies have indicated that EGF-R expression and the collective role of the EGF family is critical for the development and maintenance of the nervous system. Although TGF-α has been found to be the prominent ligand of EGF-R in the nervous system, it does not necessarily mean that TGF-α is essential for the overall development and normal function of the nervous system. Knockout or disruption of TGF-α gene does not dramatically affect the development and normal functions of the ner-

vous system (Luetteke et al., 1993; Mann et al., 1993). In the TGF-α null mice, although there was a reduction in midbrain dopaminergic neurons in the substantia nigra in the developing brain (Blum, 1998) and a decrease in neural progenitor cell proliferation shown in the adult forebrain subependyma, the number of neural stem cells is maintained throughout life (Tropepe et al., 1997), and there appears to be no impairment of brain functions. Furthermore, triple inactivation of three EGF-R ligands together (EGF, amphiregulin, and TGF-α), half of the known EGF family ligands, did not affect the viability, fertility, and longevity of the triple null mice, nor did it seem to influence the brain functions (Luetteke et al., 1999), indicating a possible redundancy among the EGF-R ligands in the nervous system. Apart from TGF-α, HB-EGF mRNA and protein have also been found to be widely expressed in CNS, with the expression anatomically and temporally correlated to the proliferating neuroblasts, suggesting its mitogenic nature for neuroblasts. Furthermore, expression of HB-EGF was also found in post-mitogenic cells, also suggesting its nonmitogenic function (Nakagawa, 1998). Recently, we have tested whether TGF-α is essential for the regeneration of sensory and motor neurons in the sciatic nerve in TGF-α null mutant mice. We found that there is no statistical difference between TGF-α mutant and wild type mice in the number of neurons regenerating into their peripheral target after the sciatic nerve is crushed (Xian, Deng, Zhao and Zhou, unpublished data). All these studies suggest an essential collective role of, but probably a redundancy within, the EGF family of growth factors in the nervous system.

Essential Roles of Neuregulins and Their Receptors in the Nervous System

Neuregulins have been shown to be essential for nerve development, since in mice with neuregulin gene knock out, Schwann cell precursors and cranial ganglia fail to develop normally (Meyer et al., 1995). Neuregulins are products of neurons and mediate the proliferation, differentiation, survival and gene expression of Schwann cells and their precursors (Dong et al., 1995; Maschionni et al., 1993; Zoricle and Lemke, 1996; Baek and Kim, 1998). Recent experiments suggest that neuregulins are responsible for at least part of the mitogenic effect of axons on Schwann cells which express ErbB2 and ErbB3 (Vartanian et al., 1997).

The ErbB2 and ErbB3 receptors and their ligand neuregulin-1 have been recently shown to be essential for development of the sympathetic nervous system (Britsch et al., 1998). Targeted null mutations of either of these molecules markedly decreased the synthesis of catecholamines in embryonic sympathetic ganglia and caused a severe hypoplasia of the primary sympathetic ganglion chain due to a lack of the crest precursor cells in the anlage of the primary sympathetic ganglion chain (Britsch et al., 1998). Targeted inactivation of ErbB3 receptor resulted in severe neuropathies because of the lack of Schwann-cell precursors and Schwann cells that accompany peripheral axons of sensory and motor neurons, and the severe apoptosis of most motor and sensory neurons in DRGs in the mutant embryos (Riethmacher et al., 1997), as well as an aborted development of the cerebellum and the heart (Erikson et al., 1997). By comparison, the degeneration of the peripheral nervous system in ErbB3 mutant pups has been shown to be much more severe than the cell death in mice with neurotrophin or neurotrophin-receptor gene knockouts (Lewin and Barde, 1996; Snider, 1994). Similarly, mice lacking ErbB4 die during mid-embryogenesis from the aborted development of heart ventricle trabeculae and mis-innervation of the hindbrain in the CNS (Gassmann et al., 1995). Mice with null mutation of ErbB2 die before E11, also probably as a result of dysfunctions associated with a lack of cardiac trabeculae, retarded development of the cranial neural-crest derived sensory ganglia as well as the motor nerves (Erikson et al., 1997;

Lee et al., 1995b). All these studies suggest that, like EGF-R (ErbB1), all other ErbB receptors and their ligands are essential for the development and functions of the nerve system.

Conclusions

TGF-α, EGF and other members of the EGF family as well as their common receptor (EGF-R) are locally produced in nervous tissues, with TGF-α being the prominent ligand particularly in the brain. Various studies have shown that TGF-α, EGF, and probably HB-EGF play an important role in the development of the nervous system, stimulating proliferation of the multipotent neural progenitor cells, and migration and differentiation of the precursor neuronal cells and glial cells. TGF-α and EGF have also been shown to be neurotrophic, enhancing survival and inhibiting apoptosis of differentiated neurons, probably via two modes of action, either directly through interacting with EGF-R on neurons or indirectly via stimulating glial synthesis of other molecules such as neurotrophic factors, which then act on neurons. Owing to these mitogenic and/or neurotrophic effects, EGF, TGF-α, and related neuregulins have been shown to be involved in mediating glial-neuronal and axonal-glial interactions and to participate in regulating injury responses of the nerve system. Moreover, overexpression of TGF-α, EGF, and EGF-R by the astrocytes is implicated in the pathogenesis of injury-associated astrocytic gliosis in the CNS and astrocytic brain tumors.

The functions and action mechanisms of TGF-α, EGF, and other members of the EGF family as well as neuregulins in the nervous system are far from well-characterized. Issues particularly requiring more effort to resolve include the regulation of gene expression; roles of these growth factors in injury and regeneration of the nerve system; roles in the pathophysiology of some neurological disorders such as gliosis and brain tumors; identification of the signaling mechanisms that are unique to the nervous system; involvement in mediating glial-neuronal or axonal-glial cross-talk; and their possible synergistic or antagonistic interactions with neurotrophins and other trophic factors in modulating development, damage, and repair of the system. Furthermore, a more detailed comparison of the expression patterns of EGF family ligands and neuregulins as well as the ErbB receptor family in the nervous system is needed. Moreover, due to the interactions of the ligands with selective recruitment of homo- and heterodimers of ErbB receptors, a better appreciation of the extent and physiological significance of the diverse, overlapping or the distinct roles of the EGF-R ligands and of neuregulins and their ErbB receptors in the nervous system is needed. In addition, it is also important to investigate and to discover any new EGF-like growth factors, especially those having a localization and function exclusive to the nervous system.

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