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EGF FAMILY OF GROWTH FACTORS: ESSENTIAL ROLES AND FUNCTIONAL REDUNDANCY IN THE NERVE SYSTEM

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1. ABSTRACT

Evidence has suggested that the epidermal growth factor receptor (EGF-R) and its ligands are important regulators for development and maintenance of various tissues including the nerve system. Gene knockout, transgenic or other *in vivo* and *in vitro* studies have demonstrated that these molecules, produced by neurons and glial cells, play important roles in regulating neural/glial precursor cell or stem cell proliferation, migration, differentiation and survival, in maintaining tissue homeostasis, and in regulating CNS gliosis and peripheral nerve injury responses. These studies have revealed that, despite a possible functional redundancy among the individual EGF family ligands, collectively EGF-R ligands are required for development and maintenance of the nerve system.

2. INTRODUCTION

The epidermal growth factor (EGF) family comprises 6 known structurally related ligands, namely EGF, transforming growth factor-alpha (TGF-alpha), amphiregulin (AR), heparin binding EGF (HB-EGF), betacellulin (BTC), and epiregulin (EPR) (1). This family of polypeptides is distinguished by the fact that their soluble forms are proteolytically derived from their integral membrane precursors and that they all contain a conserved three-loop compact structure, known as the EGF-like domain (1, 2). These ligands bind and activate the common membrane integral tyrosine kinase receptor, EGF receptor (EGF-R or ErbB-1) (3). EGF-R functions as part of a network, forming heterodimers with 3 related proteins, ErbB2/c-neu, ErbB3, and ErbB4, and 3 of the EGF-R ligands HB-EGF, BTC and EPR also directly bind erbB4 (1). 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.

The EGF family of growth factors has been implicated in growth, differentiation, maintenance and repair of various tissues including the nerve system (4) and the gastrointestinal tract (2). These growth factors, produced by various cell types such as neurons, glial cells and gut epithelial cells, play important roles in regulating cellular proliferation, differentiation, migration, and survival, in maintaining tissue homeostasis, and in regulating tissue injury responses.

Although there are excellent reviews on different aspects of the EGF family of growth factors (1, 2, 4, 5), there is a lack of a more comprehensive review on genetic studies (gene knockout or transgenic investigations) examining physiological roles of the endogenous EGF family in the nerve system. Important information on the functions of EGF family *in vivo* has been gained from loss of function mutants of various ligands or the receptor as well as from transgenic mice that overexpress some ligands. The purpose of this review is to discuss and summarize the current knowledge on the physiological roles of EGF family of growth factors and their receptor EGF-R in the nervous system.

3. EXPRESSION OF EGF-R AND LIGANDS IN THE NERVOUS SYSTEM

TGF-alpha is expressed at high levels in the central nervous system (CNS). Due to its much higher abundance and

wider distribution compared to other ligands, TGF-alpha has been considered to be the prominent ligand for EGF-R in the developing and adult CNS particularly in striatum, olfactory bulb, hippocampus and brain stem (6, 7). While the expression of EGF mRNA in the brain is limited to smaller discrete areas (7), TGF-alpha gene expression is widely distributed in the brain (7, 8), with its transcripts in both neurons and glia and mRNA levels about 15 - 170 times greater than the regional levels of EGF mRNA (7). HB-EGF mRNA has also been found within the brainstem as early as embryonic day 14 (E14) and subsequently in the developing rat brain particularly in the cortical plate, hippocampus, cerebellar Purkinje cells and ventrobasal thalamus, with the expression level peaking in the early postnatal period (9). Similarly, it has been found that both neurons and glia express HB-EGF in the prenatal and early-postnatal rat CNS (10). Recently, amphiregulin was found to be expressed in the choroid plexus of the ventricular system and in the hippocampus in the adult brain, the regions where adult stem cells are produced (11). EGF-R has also been shown to be expressed in the brain with a similar pattern of regional differential expression to that of TGF-alpha mRNA (12) and a similar temporal expression pattern to that of HB-EGF (9). Similarly, EGF-R transcripts have been found in neurons (13, 14), astrocytes and oligodendrocytes (15). TGF-alpha and EGF-R transcripts were also expressed in the ventral part of the cervical spinal cord of mice from E12 until adult ages, with both transcripts localised to embryonic motoneurons, suggesting an autocrine-paracrine role on developing motoneurons (16).

TGF-alpha and its receptor are also expressed in the peripheral nervous system (17-22), with TGF-alpha localized to sub-populations of small and medium-sized sensory neurons as well as satellite cells surrounding large neurons in the dorsal root ganglia (DRG) (22) and EGF-R immunoreactivity expressed in cutaneous nerves, sensory corpuscles (21), DRG primary sensory neurons, satellite glial cells, and the intraganglionic and dorsal root Schwann cells (20, 22).

4. ROLES IN THE CENTRAL NERNOUS SYSTEM

4.1. Proliferation of CNS precursor cells or stem cells and glial reaction in gliosis or gliomas

Multipotent neural progenitor cells (stem cells) play an important role in neural lineage elaboration during neurogenesis and gliogenesis after migration from paramedian generative zones (23, 24). The early expression and widespread distribution of EGF-R and ligands in the developing brain suggest that EGF family may play an important role in brain development, possibly involved in the genesis, differentiation, migration, or survival of numerous cell populations in the embryonic brain (13). Consistent with this, various *in vivo* and *in vitro* studies have demonstrated that TGF-alpha stimulated proliferation and differentiation of both neuronal and glial precursor cells (9, 25-28) and improved survival of cultured astrocyte precursor cells (29). Similarly, expression of HB-EGF in developing brain *in vivo* and the proliferative responsiveness of CNS astrocytes and multipotent progenitors to HB-EGF *in vitro* indicate that HB-EGF may be an important trophic factor in the developing CNS (9). These proliferative effects to HB-EGF and TGF-alpha require the expression of EGF-R, as no such effects are observed in cells derived from EGF-R-/- mice (9).

Neurons are continuously generated from stem cells in the hippocampus and along the lateral ventricles in the adult brain. Neural stem cells can be propagated *in vitro* in the presence of EGF or fibroblast growth factor-2 (FGF-2) (23). EGF and/or FGF-2 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 (23, 24, 30-33). 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 FGF-2 (33). Recently, it has been demonstrated that, contrary to the commonly accepted hypothesis that EGF-responsive cells derive from a relatively quiescent population, the majority of EGF-responsive cells in the adult subventricular zone correspond to the rapidly dividing transit-amplifying C cells. These transit C cells express high levels of EGF-R and constitute the majority of neurosphere-forming activity in the subventricular zone (34). Apart from the proliferative effect, some brain-derived neural stem cells are also dependent on EGF for survival in the cell culture, as the withdrawal of EGF induced apoptosis within 24 hours through a Bcl-2 related common mechanism as for other growth factor-related apoptotic systems (35). Similarly, a multipotent clonal cell line (MEB5) from embryonic mouse forebrain relies on EGF for survival and suppression of apoptosis (36).

TGF-alpha, like EGF, 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-alpha was used in combination with FGF-2 in contrast to the combined use of EGF and FGF-2 (30). In adult mammalian forebrain subependyma where the neural stem cells and their progeny constitutively proliferate, the endogenous expression of TGF-alpha has been shown to be necessary for the full proliferation of the progenitor cells, since in TGF-alpha null mice there was a decreased proliferation of these progenitor cells (37).

Similarly, some multipotent progenitor cells were responsive to HB-EGF *in vitro* (9), and neural stem cell cultures can be initiated and the cells propagated as efficiently in the presence of amphiregulin as with EGF (11). Amphiregulin is expressed in the choroid plexus of the ventricular system and in the hippocampus in the adult brain, suggesting that endogenous amphiregulin may participate in the regulation of neural stem cell proliferation and neurogenesis in the adult brain (11).

Studies have shown that TGF-alpha and its receptor are involved in the glial reaction in response to brain or spinal cord injury. In response to brain trauma, some astrocytes in the brain expressed a higher level of EGF-R and had a higher proliferation rate (38), suggesting that the ligands of EGF-R are involved in glial reaction in the brain in response to trauma. Similarly, due to the mitogenic effects, over-expression of TGF-alpha and EGF-R in astrocytes has been implicated in spinal cord injury-associated astrocytic gliosis. Up-regulated

TGF-alpha expression has been demonstrated in reactive astrocytes following spinal cord injury, with its level correlated with motor neuronal degeneration (39). Furthermore, EGF-R is localized in a subset of reactive glial cells, with its level following closely with the astrogliosis (39). In a transgenic study, overexpression of TGF-alpha in the transgenic mice induced enhanced expression of glial fibrillary acidic protein (GFAP) in CNS, induced morphological features characteristic of reactive astrocytes, and increased proliferation among the GFAP-immunoreactive astrocytes in striatum, hippocampus, and cervical spinal cord, the three CNS areas monitored (40). Furthermore, overexpression of the transgene also induced a 2-fold increase in EGF-R phosphorylation among the GFAP-positive astrocytes. These results indicate that enhanced synthesis of TGF-alpha in astrocytes is sufficient to trigger astrogliosis throughout the CNS through a direct action on the up-regulated EGF-R on the astrocytes (40).

Also due to the mitogenic property, over-expression and action of TGF-alpha, EGF, and their receptor EGF-R in astrocytes are associated with brain tumour pathogenesis. Numerous studies (41-44) 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-alpha have also been shown to be over-expressed in gliomas (43-45).

4.2. Cellular migration during CNS development

Increasing evidence indicates that specific genes and signaling pathways are involved in controlling the formation of discrete areas of CNS and in the neuronal differentiation. EGF-R has been shown to regulate migration of a variety of cells through direct and indirect mechanisms (for references, see reference 46), and EGF-R and its ligands have been implicated in the control of cellular migration from the proliferative zones to specific sites of the cerebral wall within the CNS. Various studies have shown that at mid-embryonic development, a high level of EGF-R expression is found in a small heterogeneous population of cells including astrocytes, neurons and multipotent stem cells. Many of these cells that express high levels of EGF-R are located in migration pathways, including the tangential pathway to the olfactory bulb via the rostral migratory stream (RMS), the lateral cortical stream (LCS) leading to ventrolateral cortex and the radial pathway from proliferative zones to cortical plate (46, 47). The targets of these pathways express the ligands HB-EGF and/or TGF-alpha. Burrows *et al* (1997) have demonstrated that the level of EGF-R expression affects the timing of migration and the settling patterns of cells (48). The study by Caric *et al* (2001) has demonstrated that EGF-R regulates cell migration in the developing brain via a chemoattractive mechanism. Expression of a threshold high level of EGF-R (characteristic of late embryonic cells) confers chemotactic competence in stem cells, neurons and astrocytes in cortical explants *in vitro*. Expansion of the population of cells expressing threshold levels of EGF-R *in vivo* by viral transduction resulted in an increase in migration radially to the cortical plate and ventrolaterally in the LCS, but not tangentially in the RMS, supporting the role of EGF-R in mediating migration in these pathways. Within the bulb, however, EGF-R also mediates radial migration. These data suggest that developmental changes in EGF-R expression, together with changes in ligand expression regulate the migration of specific populations of cells in the telencephalon by a chemoattractive mechanism (46).

Consistent to the above study, targeted disruption of EGF-R resulted in not only a lower number of neuronal cells both in the cerebellum and cerebral cortex, but a retarded cellular migration in several regions particularly in the cerebral cortex (49). In the null mice, although the overall structure of the E18.5 cerebral cortex was comparable to that of wild type, the migration of neurblasts and formation of cerebral cortical plate was compromised, with the ventricular zone being abnormally thick and the intermediate zone substantially thin in some animals, suggesting that progenitor cells appearing to accumulate in proliferative zones of the late emryonic telecephalon rather than migrating to the cortical plate or olfactory bulb (49). Cellular migration defect was also seen in another EGF-R knockout study where ectopic neurons were observed in the white matter of hippocampus in neonatal EGF-R null mice (50).

4.3. Neuronal and glial differentiation

Studies conducted both *in vitro* and *in vivo* have suggested that TGF-alpha and EGF can regulate cell fate of progenitor cells and neuronal or glial differentiation. In TGF-alpha null mice, there were 50% fewer dopaminergic neurons and a 20% reduction in the overall volume of the dorsal striatum, indicating that TGF-alpha is required for the normal proliferation or differentiation of a selected population of dopaminergic neurons within the substantia nigra (51). Furthermore, TGF-alpha and EGF have been shown to be able to modify the differentiation of cerebral cortical neuron precursor cells in culture (52). 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-alpha 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-alpha 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 (53) or choline acetyltransferase/acetylcholine esterase activities (54).

In vivo and *in vitro* studies have suggested that exogenous TGF-alpha 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 (48). 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 multipotent cells to form spheres (48). Similarly, introduction of extra EGF-Rs into the progenitor cells in the retina, which normally differentiate into rod photoreceptor cells, neurons, and Muller glial cells, promoted the differentiation of the progenitor cells into Muller glial cells (55).

TGF-alpha 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 (25, 27, 56). In TGF-alpha deficient mice (*wa-1* mice), less GFAP and fewer proliferating astrocytes were found in the brain (27). Furthermore, cerebral cortices from mutant mice lacking EGF-R contain lower numbers of GFAP-positive astrocytes, which display reduced proliferation *in vitro* (50). These studies indicate the important roles of EGF-R ligands in proliferation and differentiation of astrocytes.

Craig *et al* (1996) have demonstrated that intraventricular administrated exogenous EGF can act as a proliferation, differentiation, survival, and migration factor for the subependymal multipotent stem cells to expand these populations, to differentiate into astrocytes and new neurons, and to promote the movement of these cells into normal brain parenchyma (57). This study suggests that it may be possible to use EGF for *in situ* modulation of endogenous forebrain precursor cells. Similarly, endogenous stem cells and progenitors around the fourth ventricle and central canal of the spinal cord proliferate in response to exogenously applied EGF and FGF-2 in combination, but unlike in the hippocampus and along the lateral ventricle where they generate some new neurons, they only produce new astrocytes and oligodendrocytes at 7 weeks post-infusion (58).

4.4. Neurotrophic function

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 (50). These studies suggest important roles of EGF-R ligands in survival of post-mitotic neurons.

In vitro studies have shown that EGF is a potent trophic factor for a variety of neurons in CNS, including dopaminergic (59) and GABAergic neurons (60), neocortical neurons (61) and other neurons isolated from neonatal rat brain (62). Similarly, several *in vitro* studies have demonstrated that TGF-alpha can promote the survival and inhibit apoptosis of differentiated neurons (63-65), suggesting that in the normal adult brain, TGF-alpha may act as a neurotrophic factor in the maintenance and modulation of functions of the differentiated neurons. An *in vitro* study has shown that HB-EGF, which is expressed in close proximity of developing mesencephalic dopaminergic neurons, promotes the survival of tyrosine hydroxylase (TH)-positive neurons *in vitro*, indicating that HB-EGF may be an important molecule for developing dopaminergic neurons of the ventral midbrain (66). Consistently, animal studies have shown that EGF may have neurotrophic effects on dopaminergic neurons in the nigro-striatal pathways. 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 TH-positive fiber staining, as well as enhance a behavioural recovery (67).

In vivo studies have also demonstrated that EGF family ligands can protect neurons from ischemia- or some chemical- induced cell death. Transient cerebral ischemia induces selective neuronal degeneration in the brain. Cerebroventricular infusion of EGF has been shown to have a neuroprotective effect on hippocampal neurons against transient forebrain ischemia and can effectively prevent ischemia-induced neuronal apoptotic death (68). Similarly, in a rat model of permanent middle cerebral artery occlusion, administration of TGF-alpha 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-alpha can protect neurons from ischemic damage and prolong their survival after ischemic injury (69). In a rat model of Huntington's disease induced by a single unilateral intrastriatal injection of excitotoxin quinolinic acid, intrastriatal administration of TGF-alpha can partially protect the calretinin-immunopositive striatal neurons, against the phenotypic degeneration (65).

However, some studies have implicated that HB-EGF, rather than EGF and TGF-alpha, may be a stress-inducible endogenous neuroprotective factor in cerebral ischemia. In a rat model of global brain ischemia, low or no EGF mRNA was detected in the postischemic brain as in the normal control brain. Although TGF-alpha mRNA is 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 Purkinje cell layers (70). HB-EGF is found in cerebral neurons, and its expression is increased after hypoxic or ischemic injury. Furthermore, intracerebroventricular administration of HB-EGF in adult rats increased BrdU labeling in the subventricular zone and in the subgranular zone of dentate gyrus, where EGF-R was also expressed and where ischemia-induced neurogenesis is observed (71). This evidence suggests that HB-EGF stimulates neurogenesis in proliferative zones of the adult brain that are also affected in ischemia and that it does so by interacting with EGF-R. It is possible that HB-EGF may help to trigger proliferation of neuronal precursors in brain after hypoxic or ischemic injury (71). Similarly, 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 (72).

Studies have shown that TGF-alpha is also neurotrophic to motorneurons. TGF-alpha promoted the survival of embryonic E15 motoneurons *in vitro* in a dose-dependent manner, with a potency similar to that of brain-derived neurotrophic factor (BDNF) (16). In TGF-alpha transgenic mice, axotomy of the facial nerve provoked significantly less degeneration in the relevant motor pool of 1-week-old mice than in wild-type animals. Since TGF-alpha as well as its receptor EGF-R are expressed by the embryonic motoneurons, these results suggest that TGF-alpha may be a physiological neurotrophic factor for developing motoneurons, and that this motoneuron-survival promoting function of TGF-alpha is achieved through an autocrine-paracrine manner (16).

5. ROLES IN THE PERIPHERAL NERVOUS SYSTEM

Although roles of TGF-alpha and other ligands in the peripheral nervous system remain largely unknown, it has been shown that TGF-alpha can support survival of some DRG

sensory neurons *in vitro* (73), and they are involved in nerve injury-induced responses (74). After a nerve injury, EGF-R is up-regulated in Schwann cells and fibroblasts in the nerve particularly close to the site of lesion (74). In DRGs, differential changes in TGF-alpha and EGF-R expression have been demonstrated following sciatic nerve lesion, with a significant increase in TGF-alpha immunoreactivity observed mainly in satellite cells surrounding many large neurons, and a marked neuronal upregulation in EGF-R immunoreactivity seen in the DRGs 3 days after the sciatic nerve lesion (22). These studies indicate that TGF-alpha or other members of EGF family may play a role in the injury-induced responses in the peripheral nerve.

In the peripheral nerve system, we have tested whether TGF-alpha is essential for the regeneration of sensory and motor neurons in the sciatic nerve in TGF-alpha null mutant mice. Compared to wild type mice, TGF-alpha null mice exhibited normal axonal extension of both primary sensory neurons in the DRG and the motor neurons in the spinal cord after a sciatic nerve crush, as well as an unaltered gross morphology of the regenerated nerve fibres in the distal nerve (75). This study suggests that TGF-alpha may not be an essential EGF-R ligand in the peripheral nerve axonal regeneration.

6. ESSENTIAL ROLES OF AND POSSIBLE FUNCTIONAL REDUNDANCY WITHIN THE EGF FAMILY IN THE NERVE SYSTEM

EGF-R knockout studies have shown that EGF-R expression and collective role of the EGF family are critical for the development and maintenance of the brain (49, 50, 76, 77). However, although TGF-alpha has been found to be a prominent ligand of EGF-R in the nervous system, knockout of TGF-alpha gene does not dramatically affect the development and normal functions of the nervous system (78, 79). In TGF-alpha null mice, although there was a reduction in the number of midbrain dopaminergic neurons in the substantia nigra in the developing brain (51) and a decrease in neural progenitor cell proliferation shown in the dorsolateral corner of adult forebrain subependyma, the number of neural stem cells is maintained throughout life (37) and there appears to be no impairment of brain functions. Furthermore, triple inactivation of three EGF-R ligands together (EGF, amphiregulin, and TGF-alpha), 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 gross morphology and overall functions (80). These studies suggest possible functional redundancy among the EGF family ligands in the development and functions of the nerve system.

Apart from TGF-alpha, HB-EGF has also been found to express widely in CNS, with the expression anatomically and temporally correlated to the neuroblast proliferation and gliogenesis, suggesting its mitogenic nature. Furthermore, expression of HB-EGF was also found in post-mitogenic cells, also suggesting its nonmitogenic function (10). 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 (9). Recent unpublished data show that the majority of HB-EGF knockouts died in the first week of life, with survivors dying prematurely, having a modestly but consistently smaller brain, and showing signs of congestive heart failure most likely as a result of defective heart valves (DC Lee, personal communication). From this HB-EGF knockout study, while it is tempting to speculate that HB-EGF might be more important than TGF-alpha for brain development, detailed analysis of the effects of HB-EGF gene knockout on CNS development is in progress (DC Lee, personal communication). Similarly, recent unpublished data show that quadruple null mice lacking all four ligands (HB-EGF, TGF-alpha, EGF, and AR) invariably die *in utero* (DC Lee, personal communication).

In the peripheral nerve system, we have demonstrated that TGF-alpha is not essential for axonal regeneration of DRG sensory neurons and spinal cord motor neurons after a sciatic nerve injury (75). Our gene analysis reveals that, as a possible explanation for the functional redundancy among TGF-alpha-related ligands in response to a nerve injury, prior to and 1 day after nerve lesion, there was a higher level of EGF-R mRNA in DRGs and in nerve in TGF-alpha ?null mice compared to wild types, and there was an induction of ligand amphiregulin mRNA in DRGs in mutant mice in place of the TGF-alpha upregulation present in wild types (75). After a nerve lesion, while TGF-alpha expression was not induced in the degenerating sciatic nerve, HB-EGF and betacellulin were upregulated, suggesting that HB-EGF and betacellulin might be more involved in the nerve injury-induced responses than TGF-alpha in the degenerating or regenerating nerve. In DRGs, while both TGF-alpha and HB-EGF were transiently upregulated after a nerve lesion in the wild type animals, increased expression of a different ligand amphiregulin as well as the HB-EGF was apparent in the TGF-alpha null mice, compensating for the loss of TGF-alpha upregulation in the mutant mice. These results indicate that TGF-alpha gene knockout does not affect peripheral nerve regeneration, probably due to a functional redundancy within the EGF family through a compensatory expression mechanism at both the receptor and ligand levels in TGF-alpha knockout mice. However, despite this redundancy, upregulation of, TGF-alpha, HB-EGF and EGF-R in the DRG, and HB-EGF and betacellulin in the degenerating nerve, after the nerve injury indicates that collectively, EGF family plays a role in the nerve-injury responses in the peripheral nerve system.

7. CONCLUSIONS AND PERSPECTIVE

EGF-R and its ligands (particularly TGF-alpha and HB-EGF), expressed in the developing and adult brains, appear to have numerous functions on central nervous system cells during CNS development, maintenance and injury response or disorders. Gene knockout, transgenic, and other *in vivo* or *in vitro* studies have indicated that EGF family of ligands stimulates proliferation and migration of astrocytes and multipotent progenitor cells, promotes differentiation and survival of postmitotic neurons during development, and is involved in brain tumor pathogenesis, injury-induced CNS gliosis, and peripheral nerve lesion-induced injury responses. These genetic and other studies have revealed an important essential collective role of, but probably a functional redundancy within, the EGF family of growth factors in the nervous system.

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