Inhibition of Brain EGFR Activation: A Novel Target in Neurodegenerative Diseases and Brain Injuries

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EGFR Inhibitors for Neurodegeneration and Brain Injuries

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Abbreviations:
5-HT, 5-hydroxytryptamine; 6-OHDA, 6-Hydroxydopamine; AD, Alzheimer’s Disease; ALS, Amyotrophic Lateral Sclerosis; AREG, Amphiregulin; BBB, Blood-Brain-Barrier; BTC, Betacellulin; COX-2, Cyclooxygenase-2; CRMP2, Collapsin Response Mediator Protein-2; CSPG Chondroitin Sulfate Proteoglycan; EGF, Epidermal Growth Factor; EGFR, Epidermal Growth Factor Receptor; EPGN, Epigen; EREG, Epiregulin; GAP-43, Growth-Associated Proteins-43; GFAP, Glial Fibrillary Acidic Protein; GPC, Glial Progenitor Cell; GSK3β, Synthase Kinase-3-beta; HB-EGF, Heparin-Binding EGF-like Growth Factor; HD, Huntington’s Disease; iNOS, Inducible Nitric Oxide Synthase; KRAS, Kirsten Rat Sarcoma Viral Oncogene Homolog; MBP, Myelin Basic Protein; MCAO, Middle Cerebral Artery Occlusion; mTOR, Mammalian Target of Rapamycin; NDDs, neurodegenerative diseases; NPC, Neural Progenitor Cell; NSC, Neural Stem Cell; NSCLC, Non-Small Cell Lung Cancer; OGD, Oxygen/Glucose Deprivation; OLs, Oligodendrocytes; PD, Parkinson’s Disease; PK/PD, Pharmacokinetics/Pharmacodynamics; SCI, Spinal Cord Injuries; SVZ, Subventricular Zone; TGFA, Transforming Growth Factor-Alfa; TH, Tyrosine Hydroxylase; TKI, Tyrosine Kinase Inhibitor; TNF-α, Tumor Necrosis Factor; UPS, Ubiquitin/Proteasome System; ZNRF1, Zinc and Ring Finger-1
Abstract
Several reports have been published recently demonstrating a beneficial effect of epidermal growth factor receptor (EGFR) inhibitors in improving pathological and behavioral conditions in neurodegenerative diseases (NDDs) such as Alzheimer’s disease (AD) and Amyotrophic Lateral Sclerosis (ALS) as well as the brain and spinal cord injuries (SCI). Despite successful therapeutics effects of EGFR inhibition in these pathological conditions, there is still no report of proof-of-concept studies in well-characterized animal models using recently developed Blood-Brain-Barrier (BBB) penetrating EGFR inhibitors which is due to previous conflicting reports concerning the level of EGFR or activated EGFR in normal and pathological conditions which caused target engagement a concern in any future EGFR inhibition therapy. In this review, the level of EGFR expression and activation in developing CNS compared to adult CNS will be explained as well as how neuronal injury or pathological conditions especially inflammation and amyloid fibrils induce reactive astrocytes leading to increase in the expression and activation of EGFR and finally neurodegeneration. Furthermore, in this review, we will discuss two main molecular mechanisms that can be proposed as neuroprotective effects of EGFR inhibition in these pathological conditions. We will also review the recent advances in the development of BBB- penetrating EGFR inhibitors in cancer therapy, which may eventually be repositioned for NDDs and SCI therapy in the future.

Keywords:
Autophagy, Brain and Spinal Cord Injuries, EGFR, EGFR inhibitor, Neurodegeneration, Reactive Astrocytes
Significance Statement:

Based on lessons from applications of EGFR inhibitor in oncology, it is concluded that EGFR inhibitors can be beneficial in treatment of neurodegenerative diseases and spinal cord injuries. They carry their therapeutic potentials through induction of autophagy and attenuation of reactive astrocytes.
Introduction

Several reports have been published in the last 10-years demonstrating beneficial effect of epidermal growth factor receptor (EGFR) inhibitors in improving pathological and behavioral conditions in neurodegenerative diseases (NDDs) such as Alzheimer’s Disease (AD) (Wang, Chiang et al. 2012, Wang, Liang et al. 2013, Wang, Her et al. 2017) and Amyotrophic Lateral Sclerosis (ALS) (Le Pichon, Dominguez et al. 2013) as well as brain and Spinal Cord Injuries (SCI) (Erschbamer, Pernold et al. 2007, Li, Tang et al. 2011, Yang, Wang et al. 2011, Qu, Tian et al. 2012, Kjell, Pernold et al. 2014, Li, Li et al. 2014, Zhang, Ju et al. 2016). EGFR inhibitors are mainly designed to inhibit mutants of EGFR or excess EGFR activation in cancer and tumor tissues (Sigismund, Avanzato et al. 2018, Roskoski 2019). In cancer, it has been known that oncogenic EGFR mutation, amplification, or overexpression leads to the excessive activation of EGFR protein, causing uncontrolled proliferation. Mutations in the EGFR gene are mutually exclusive with the other driver genetic alterations such as Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS) mutations. In non-small cell lung cancer (NSCLC), to be positive for EGFR mutation determinates if a patient is a candidate for treatment with the EGFR inhibitors (Schilsky 2010, Tavassoly, Hu et al. 2019). Of note, in addition to the fact that EGFR plays an oncogenic role in many cancers, EGFR signaling is also associated with aberrant cellular differentiation in cancer (Kolev, Mandinova et al. 2008, Sato, Yoo et al. 2019), which may be targeted by EGFR inhibitors similarly as that in NDDs and SCI. Although there are polymorphisms in EGFR genes that are associated with AD (Chen, Wang et al. 2018), there is no report of an association between known cancer-related EGFR mutations and the development of AD and other neurodegenerative diseases. Furthermore, there have been conflicting reports concerning the level of EGFR in NDDs. It would appear that, in some cases, total EGFR level is reported to be reduced (Iwakura, Piao et al. 2005, Wang, Liang et al. 2013), and that, in other cases, it is reported to be increased (Birecree, Whetsell et al. 1988, Styren, DeKosky et al. 1993, Liu, Chen et al. 2006) or unchanged (Wang, Her et al. 2017). Therefore, repositioning of EGFR inhibitor drugs for the treatment of NDDs and SCI requires a better understanding of their molecular mechanism in NDDs and SCI as well as proof of target engagement in both in vitro and preclinical animal models. Here in this paper, we will emphasize that the fraction of phosphorylated receptor (p-EGFR/EGFR), we call it activated EGFR in this paper, is essential to be considered as a biological marker of pathology as it has been shown in cancer studies as well as Alzheimer's disease (Noro, Gemma et al. 2006, Hammoud, Burger et al. 2009, Wang, Chiang et al. 2012, Wang, Liang et al. 2013, Dhaunsi, Alsaed
et al. 2016, Overmiller, McGuinn et al. 2016, Wang, Liu et al. 2016). Furthermore, a common pathological feature in both NDDs and SCI is reactive astrocytes, which are characterized as high-expressed EGFR astrocytes with high levels of activated EGFR (Liu and Neufeld 2004, Zhang and Neufeld 2005, Liu, Chen et al. 2006, Liu and Neufeld 2007, Chen, He et al. 2017). Stimulations such as injuries, genetic mutations in familial NDDs (Booth, Hirst et al. 2017, Frost and Li 2017, González-Reyes, Nava-Mesa et al. 2017, Ceyzériat, Ben Haim et al. 2018, Joe, Choi et al. 2018, Perez-Nievas and Serrano-Pozo 2018), and amyloid fibrils (Forman, Lal et al. 2005, Kahlson and Colodner 2015, Chavarría, Rodríguez-Bottero et al. 2018, Diniz, Matias et al. 2019, Lohmann, Bernis et al. 2019, Tremblay, Cookson et al. 2019) can induce reactive astrocytes condition, which finally leads to neurodegeneration. Considering the beneficial effect of EGFR inhibition in neurodegenerative diseases and treatment of reactive astrocytes pathology in SCI, there is an essential need to clarify the role of EGFR activation in non-cancer brain pathologies as well as molecular pharmacology of EGFR inhibition as a novel therapy for both NDDs and SCI. In this paper, we review the literature and discuss possible molecular mechanisms for the therapeutic effect of EGFR inhibition in NDDs and SCI in comparison to its role in cancer, including; (1) Autophagy Induction and (2) Attenuation of Reactive Astrocytes Pathology. One of the main modes of action of EGFR inhibitors in cancer therapy is to induce autophagy [14-16], which also is reported in AD [1]. Moreover, EGFR expression is up-regulated in reactive astrocytes, and its hyper-activation leads to astrogliosis [6, 9, 17]. Another issue regarding the use of EGFR inhibitors as a therapy in NDDs and SCI is that most of the previous reports involve two of the classical EGFR inhibitors (gefitinib and erlotinib) [1-10, 17]. These drugs were designed for peripheral tumors and don’t display sufficient brain penetration [11]. Thus, they are not suitable for proof-of-concept studies in NDDs and SCI. In this paper, we will also review the recent advances in the development of BBB penetrating EGFR inhibitors in cancer therapy, which may eventually be repositioned for NDD and SCI therapy in the future.

**EGFR Structure and Function**

EGFR or HER1 or EebB-1 is a member of HER or ErbB family of tyrosine kinase receptors. It has a single transmembrane domain, an extracellular domain that binds to specific ligands as well as the intracellular domain, which undergoes autophosphorylation at tyrosine residues upon activation (Carpenter and Cohen 1990, Yarden 2001, Herbst 2004, Avraham and Yarden 2011).
Binding of EGFR ligands to the extracellular domain leads to homodimerization or heterodimerization with the other ErbB family members, and autophosphorylation of the C-terminal domain, which activates a cascade of downstream phosphorylation to control cellular differentiation and proliferation. EGFR has seven ligands, including EGF, transforming growth factor-alpha (TGFA), heparin-binding EGF-like growth factor (HB-EGF), betacellulin (BTC), amphiregulin (AREG), epiregulin (ERE), and epigen (EPGN) (Schreiber, Winkler et al. 1986, Toyoda, Komurasaki et al. 1995, Raab and Klagsbrun 1997, Dunbar and Goddard 2000, Vecchione, Jacobs et al. 2011, Berasain and Avila 2014, Schneider and Yarden 2014).

**EGFR Expression Level and Its Role Developing and Adult Brain**

EGFR has an essential role in cellular differentiation in developing brain, which includes differentiation of both neurons (neurogenesis) and glial cells (gliogenesis; generation of glial cells including astrocytes, oligodendrocytes, Schwann cells and microglia during development) from neural stem cells (NSC), neural progenitor cells (NPC) and glial progenitor cells (GPC). There is an increase in the expression and activation of EGFR during the neurogenesis and gliogenesis, especially in NSCs, NPCs, and GPCs of germinal zones (Enwere, Shingo et al. 2004, Galvez-Contreras, Quinones-Hinojosa et al. 2013, Lupo, Gioia et al. 2019). During embryonic development, EGFR expression starts as early as E11-E13 (11-13 days-old rat embryo) in the midbrain (germinal zone, rostral, and pretectum) in the rat. The EGFR expression starts in the diencephalon (ventral thalamus, dorsal hypothalamus, and hypothalamic germinal zone) and cerebellum (external granule cell layer and fourth ventricular germinal zone) at E15 of embryonic development. Forebrain EGFR expression starts at E17 in basal ganglia germinal zone and neostriatum (caudate-putamen). During the perinatal period, the level of EGFR expression increases progressively, and at P0 (one day after birth), almost all brain regions express EGFR. In the substantia nigra of midbrain, tyrosine hydroxylase expressing dopaminergic neurons also express EGFR during the perinatal period (Kaser, Lakshmanan et al. 1992, Seroogy, Gall et al. 1995, Kornblum, Hussain et al. 1997). The EGFR expression and activation are essential for the development of astrocytes and neurons in the developing CNS, but upon differentiation in the adult CNS, EGFR expression in these cells will be reduced, and its activation is absent in adult brain (Enwere, Shingo et al. 2004, Galvez-Contreras, Quinones-Hinojosa et al. 2013, Lupo, Gioia et al. 2019). Astrocytes in the adult brain will stay as quiescent...
astrocytes, but following neuronal injuries or pathological conditions, EGFR up-regulates and quiescent astrocytes are transformed to reactive astrocytes (Enwere, Shingo et al. 2004, Liu, Chen et al. 2006, Liu and Neufeld 2007, Galvez-Contreras, Quinones-Hinojosa et al. 2013, Chen, He et al. 2017, Lupo, Gioia et al. 2019). In the adult brain, the high levels of EGFR expression are limited to NSCs and NPCs of subventricular zone (SVZ), but EGFR expression is detected in neurons at basal levels through life. The expression level of EGFR in astrocytes becomes barely detectable or absent after 2-3 months after birth (Enwere, Shingo et al. 2004, Galvez-Contreras, Quinones-Hinojosa et al. 2013, Liu and Neufeld 2007, Lupo, Gioia et al. 2019).


EGFR Activation in The Pathological Adult Brain Leads to Induction of Reactive Astrocytes

oxygen/glucose deprivation (OGD) (Wang, Zhang et al. 2012, Liu, Zhao et al. 2019) and ischemic injury (Morizawa, Hirayama et al. 2017, Laug, Huang et al. 2019). In all these conditions, there is an increase in the level of EGFR expression and activated EGFR (p-EGFR/EGFR) in the brain and spinal cord associated with an increase in the level of Glial Fibrillary Acidic Protein (GFAP) which is a marker of reactive astrocytes (Liu and Neufeld 2004, Zhang and Neufeld 2005, Liu, Chen et al. 2006, Erschbamer, Pernold et al. 2007, Li, Tang et al. 2011, Yang, Wang et al. 2011, Qu, Tian et al. 2012, Kjell, Pernold et al. 2014, Li, Li et al. 2014, Zhang, Ju et al. 2016, Chen, He et al. 2017). Under normal conditions, with no injury or pathology, astrocytes exist as quiescent astrocytes with barely detectable levels of EGFR. Quiescent astrocytes are required for neuronal survival, and the loss of normal astrocyte function in reactive astrocytes is associated with the pathology of neurodegenerative diseases (Liu and Neufeld 2004, Zhang and Neufeld 2005, Liu, Chen et al. 2006, Erschbamer, Pernold et al. 2007, Li, Tang et al. 2011, Yang, Wang et al. 2011, Qu, Tian et al. 2012, Kjell, Pernold et al. 2014, Li, Li et al. 2014, Zhang, Ju et al. 2016, Chen, He et al. 2017). (Figure 1) Besides the indirect effect of EGFR activation in reactive astrocytes on neuronal degeneration, it has been reported that oxidative stress can directly activate EGFR in neuronal cells and finally lead to degeneration and apoptosis in neurons (Wakatsuki, Furuno et al. 2015, Wakatsuki and Araki 2016). Oxidative stress induced by treatment of neuronal cells with 6-hydroxydopamine (6-OHDA) or H$_2$O$_2$ as well as in vivo by traumatic injury induction in animals, which causes the generation of NADPH oxidases in axons, stimulates neuronal EGFR tyrosine kinase activity. Activated EGFR, phosphorylates Zinc and Ring Finger-1 (ZNRF1) at Y103 and subsequently activates the E3 ubiquitin ligase activity of ZNRF1. Activated ZNRF1 ubiquitinates AKT to be degraded by Ubiquitin/Proteasome System (UPS). Reduction in the level of active AKT rescues the inhibitory phosphorylation of Glycogen Synthase Kinase-3-beta (GSK3β) by AKT and subsequently activated GSK3β, phosphorylates Collapsin Response Mediator Protein-2 (CRMP2). CRMP2 is required for microtubule stabilization in axons, but phosphorylation of CRMP2 by GSK3β inactivates CRMP2 and finally causes microtubule disassembly and axonal degeneration (Wakatsuki, Furuno et al. 2015, Wakatsuki and Araki 2016). The same pathway in soma activates EGFR and downstream ZNRF1 signaling pathway, which leads to an increase in caspase 3 cleavage, annexin V–positive staining, lactate dehydrogenase release, and neuronal apoptosis. Knocking down EGFR or
treatment with EGFR inhibitors prevents neuronal degeneration induced by oxidative stress (Wakatsuki, Furuno et al. 2015, Wakatsuki and Araki 2016). This activation of EGFR in both astrocytes and neurons due to pathological conditions indicates an important role of EGFR and its downstream signaling pathway in neuronal degeneration and brain injuries.

**EGFR Inhibition in Neurodegenerative Disease**

There are few reports showing the neuroprotective effect of EGFR inhibition in neurodegenerative diseases such as AD and ALS (Chiang, Wang et al. 2010, Wang, Chiang et al. 2012, Le Pichon, Dominguez et al. 2013, Wang, Liang et al. 2013, Wang, Her et al. 2017, Chen, Wang et al. 2018). There are several main points arising from these reports that can be considered for any future proof-of-concept animal studies using EGFR inhibitors as therapeutics in NDDs.

Chen et al., for the first time, showed an association of polymorphism in three EGFR Single Nucleotide Polymorphisms (SNPs) with the risk of AD (Chen, Wang et al. 2018). In this study, the genetic association of three known cancer-related EGFR SNP sites (rs730437, rs3752651, and rs1468727) was studied in a Chinese Han population (139 patients with AD and 152 healthy control individuals). Polymorphism in two of these sites (rs730437 and rs1468727) showed a significant association with the development of AD (Chen, Wang et al. 2018). Frequencies of CC genotype and C allele of rs730437 compared with the controls (AA genotype and A allele) were significantly lower in the patients with AD showing that polymorphism of rs730437 is protective against the risk of AD. In the case of other SNP (rs1468727), the presence of the TT genotype (TT vs. CC) is associated with a reduced risk of AD, but the frequency of T allele (T vs. C) is significantly higher in AD patients. Furthermore, linkage disequilibrium analysis of EGFR polymorphisms showed that the A-C-C haplotype (Site1-rs730437; site2-rs3752651; site3-rs1468727) was significantly correlated with an increased risk of AD compared with the A-T-T haplotype in control (Chen, Wang et al. 2018). Beside this recent genetics study, the first clues of involvement of the EGFR pathway in the development of AD were reported by Yi Zhong et al (Chiang, Wang et al. 2010). They have shown that fruit flies overexpressing Aβ42 have severe memory loss and represent hyperactivity of the Phosphoinositide 3-kinase (PI3K) pathway. The Aβ42-induced memory loss in flies was prevented by knocking-down of PI3K pathway regulatory subunit, P60, or pharmacological inhibition of PI3K functions using its specific
inhibitor, wortmannin (Chiang, Wang et al. 2010). Then, the authors investigated the upstream receptor responsible for this neuroprotective pathway among the receptors associated with this PI3-kinase effect, insulin receptor and EGFR, by evaluating behavioral changes of receptor overexpression in memory loss of flies. The results showed that co-overexpression of Aβ42 and EGFR leads to a high level of memory loss in flies and suggests the role of EGFR in the pathology of AD (Chiang, Wang et al. 2010). Following this study, the first report that characterized EGFR as a target for AD therapy was also published by Yi Zhong et al. (Wang, Chiang et al. 2012). The novel findings in this study include: (a) Activation of EGFR by oligomeric Aβ42 in cell cultures (b) Level of activated EGFR (p-EGFR/EGFR) is increased in APP/PS1 double transgenic mice (c) EGFR inhibition rescues memory loss in APP/PS1 double transgenic mice and Aβ42 over-expression transgenic fruit flies (Wang, Chiang et al. 2012). Cell culture studies using COS-7 cells overexpressing human WT-EGFR confirmed that treatment with synthetic oligomers of Aβ42 (25 µg/ml) generated in vitro leads to an elevation in the level of p-EGFR in the Western Blot (WB) analysis. Furthermore, treatment of cells with EGFR inhibitors (gefitinib and erlotinib) rescues Aβ42-induced EGFR activation. The authors also investigated the direct binding of Aβ42 oligomers to EGFR using coimmunoprecipitation in COS-7 cells, expressing a secretory form of Aβ42 and human WT-EGFR. The results indicated that endogenously produced Aβ42 species were pulled down with EGFR, which suggests the direct binding of these amyloid structures with EGFR (Wang, Chiang et al. 2012). Activation of EGFR by Aβ42 was also further investigated in a double transgenic mouse model that expresses a mutant chimeric mouse/human APPswe and a mutant human presenilin 1 (Delta E9), both driven by the prion protein promoter (APP/PS1 double transgenic mice). WB analysis of hippocampus lysates (n=4 mice/group) showed an elevation in the level of activated EGFR (p-EGFR/EGFR) compared to the wild-type mice. This activation was suppressed by administration of an EGFR inhibitor, gefitinib, for 18 days at 10 mg/kg/day. After this pharmacokinetic evaluation of gefitinib in the mice brain, the efficacy of gefitinib and erlotinib to improve Aβ42-induced memory loss was tested in fruit flies overexpressing a secretory form of human Aβ42 in the brain. Male flies at 3-dayes old were treated with different doses of these EGFR inhibitors (0.01, 0.1, 1, and 10 µg/mL) for 7 days. In almost all doses of both drug regimens, the memory loss was rescued significantly. This improvement was concentration-dependent for gefitinib, whereas in the case of erlotinib, the improvement was not concentration-dependent (Wang,
Chiang et al. 2012). The efficacy of gefitinib in rescuing memory loss was also investigated in APP/PS1 double transgenic mice. This mouse model shows high levels of amyloid plaques as well as a memory-loss phenotype at 6-9 months of age. Thus, in this study, short-term treatment for 18 days starting at 8-months of age was used to evaluate any improvements in Morris water maze. Results demonstrated that gefitinib, at a minimum dose of 0.01 mg/kg/day, is efficient in rescuing memory loss in this model. Biochemical studies of mice brains in gefitinib-treated and control groups using WB of hippocampus lysates and Thioflavin-S staining (n=3 mice/group) shows a reduction in the level of oligomer/monomer and plaques area at 10 mg/kg/day dose, but the results were not statistically significant (Wang, Chiang et al. 2012). We suggest that this non-statistically significant reduction might be due to insufficient power of the study as well as using a general amyloid staining method (i.e., Thioflavin-S staining) to detect amyloid plaques instead of immunohistochemical analysis of brain section using marker-specific antibodies. The same authors in another paper showed that the role of EGFR in AD pathology depends on age (Wang, Liang et al. 2013). In young flies (10-day old) expressing human pan-Aβ42 (showing early stages of AD phenotypes), the level of EGFR is raised and at this stage Aβ42 activates EGFR/PI3K, disrupts the normal synaptic plasticity and at the end leads to memory loss phenotype. But in aged flies (35-day old) showing the late stages of AD pathology and memory loss, Aβ42 causes a significant reduction in the level of EGFR which ends in neuronal degeneration. Moreover, despite reduction in the level of total EGFR and p-EGFR (normalized to actin) in APP/PS1 double transgenic mice at 8 months of age, the level of activated EGFR (p-EGFR/EGFR) is significantly increased compared to wild-type mice (Wang, Liang et al. 2013). In another study, an irreversible EGFR inhibitor, CL-387,785, was used to study the neuroprotective effect of EGFR inhibition in cell cultures, zebrafish, and a mouse model of AD (APP/PS1 double transgenic mice) (Wang, Her et al. 2017). This inhibitor causes a significant reduction in p62 and a concurrent increase in the LC3-II/LC3-I ratio and subsequently leads to the autophagy activation, which finally facilitates degradation and clearance of Aβ40 and Aβ42. Comparing the efficacy of gefitinib and CL-387,785 in APP/PS1 double transgenic mice, shows that both inhibitor rescue memory loss in this model but CL-387,785 even at lower doses (5 mg/kg/day) ameliorates pathological markers by induction of autophagy (Wang, Her et al. 2017). This might be due to the IC₅₀ value of CL-387,785, which is ~10 fold more efficient in EGFR inhibition compared with gefitinib or erlotinib (Greulich, Chen et al. 2005, Kobayashi, Ji et al.
In another study, the therapeutic benefit of EGFR inhibition using erlotinib was studied in a SOD1 mouse model of ALS, *i.e.* SOD1$^{G93A}$ mice that express a G93A mutant form of human SOD1 (Le Pichon, Dominguez et al. 2013). In this study, two separate experimental designs were used to evaluate the effect of erlotinib therapy in behavioral tests, body weight and survival (first experimental design) as well as histology (second experimental design). While in both experimental designs, treatment started at 5 weeks of age, dosage and duration of drug administration were different in these designs. In the first experimental design, mice were treated with 75 mg/kg/day for more than 4 months, but in the second experimental design a lower dose (60 mg/kg/day) of erlotinib was administrated for a shorter time (1 month). The results of the first experimental design showed that erlotinib therapy ameliorated weight loss and resulted in improvement of behavioral tests including wire hang and balance beam, but this therapy has no effect on survival rate. The second experimental design failed in protecting motor synapses and also did not modulate the number of GFAP- or Iba1-positive cells as markers for astrocytes and microglia (Le Pichon, Dominguez et al. 2013). This unsuccessful pathology results might be due to using different dosing regimens in the second experimental design which is less than the dose in the first experimental design and the shorter administration time.

**EGFR Inhibition in The Brain and Spinal Cord Injuries**

Brain and spinal cord injuries up-regulate EGFR level and its kinase activity in astrocytes - leading to transformation of quiescent astrocytes to reactive astrocytes which are high proliferative cells expressing GFAP marker of astrogliosis as well as secrete inhibitory molecules and proinflammatory cytokines/mediators such as Chondroitin Sulfate Proteoglycans (CSPGs), Tumor Necrosis Factor Alpha (TNF-α), Inducible Nitric Oxide Synthase (iNOS), Cyclooxygenase-2 (COX-2), and IL-1β. These changes finally lead to glial scar formation, demyelination, and loss of oligodendrocytes (OLs) and neurons. Administration of EGFR inhibitors ameliorates pathological markers associated with these injuries (Liu and Neufeld 2004, Zhang and Neufeld 2005, Liu, Chen et al. 2006, Erschbamer, Pernold et al. 2007, Li, Tang et al. 2011, Yang, Wang et al. 2011, Qu, Tian et al. 2012, Kjell, Pernold et al. 2014, Li, Li et al. 2014, Zhang, Ju et al. 2016, Chen, He et al. 2017). In a traumatic brain injury rat model induced by weight-drop technique, AG1478 administration rescues EGFR activation and GFAP production...
to the level of non-injured samples (Li, Tang et al. 2011). Furthermore, EGFR inhibition by AG1478 alleviates spinal cord myelin loss and reduces the level of CSPGs. Moreover, Growth-Associated Proteins-43 (GAP-43), a marker of regenerating neurons, was increased by EGFR suppression. These beneficial effects also correlate with the prevention of body weight loss as well as improvement in behavioral measurements of open-field locomotor testing (Li, Tang et al. 2011). In another study, Zhang et al. showed that in a mouse model of SCI generated by laminectomy, treatment with an EGFR inhibitor, PD168393, results in an increase in the expression of Myelin Basic Protein (MBP) and remyelination of axons in injured spinal cord as well as reduction in the number of GFAP expressing astrocytes (Zhang, Ju et al. 2016). Furthermore, EGFR inhibition increased the number of oligodendrocyte precursor cells (OPCs), which subsequently differentiate to form mature oligodendrocytes (OLs) (Zhang, Ju et al. 2016). EGFR inhibition using PD168393 was also studies in the astrocyte scratch injury model in vitro and the weight-drop SCI rat model in vivo (Li, Li et al. 2014). Astrocytes in cultures were scratched with the sterile plastic pipette tips and treated with PD168393 (20 µM and 40 µM). EGFR inhibition in this in vitro scratch wound model reduces p-EGFR, GFAP, and secretion of astroglial cell proinflammatory cytokine (TNF-α, iNOS, COX-2, and IL-1β) (Li, Li et al. 2014). Administration of PD168393 to SCI mouse model also leads to a significant diminish in p-EGFR, GFAP, demyelination and production of CSPGs. Furthermore, PD168393 therapy improves locomotion and bladder function recovery in SCI rat assessed by Basso, Bresnahan, and Beattie (BBB) hindlimb locomotion rating scale and recording residual urine volume of rats every day (Li, Li et al. 2014). Erschbamer et al. also applied PD168393 in the same animal model (weight-drop SCI rat model) and have shown the same therapeutic benefits of EGFR inhibition in this model as well as an increase in the level of tyrosine hydroxylase (TH) and 5-hydroxytryptamine (5-HT) immunoreactive axons (Erschbamer, Pernold et al. 2007). In another study, erlotinib was administrated at 5 mg/kg/day to the weight-drop SCI rat model for 5 days post-injury (Kjell, Pernold et al. 2014). The results confirmed an improvement in both locomotion and bladder function recovery (Kjell, Pernold et al. 2014). Not just only small molecule inhibitors of EGFR were used as astrogliosis therapy in SCI, but also monoclonal antibody therapy was successfully used in both in vitro and animal studies to inhibit EGFR activation and downstream pathways (Yang, Wang et al. 2011, Qu, Tian et al. 2012). A human-mouse chimeric anti-EGFR monoclonal neutralizing antibody, C225, which is currently in the
clinic for treatment of cancers associated with EGFR over-expression, was tested to target EGFR up-regulation in SCIs (Yang, Wang et al. 2011, Qu, Tian et al. 2012). Yang et al. generated reactive astrocytes by oxygen-glucose deprivation/reoxygenation (OGD/R) induction. Pre-treatment of these cells before OGD/R induction with C225 (10 lg/mL) resulted in significant reduction in p-EGFR, GFAP expression, and astrocytes proliferation. Administration of C225 (106 lg/day) for a minimum of 3 days in a transient (1h) middle cerebral artery occlusion (MCAO) rat model rescues p-EGFR and GFAP positive astrocytes phenotypes (Yang, Wang et al. 2011). Qu et al. also tested the efficacy of C225 in ameliorating EGFR activation in injury-induced activated microglia in vitro and in vivo. Microglia cells were treated with C225 (20 nM) and 30 minutes later, cells were activated by adding lipopolysaccharide (1 µg/ml) (Qu, Tian et al. 2012). The traumatic SCI rat model was generated by the weight-drop technique. Results demonstrated a significant reduction in the p-EGFR and production of cytokines such as IL-1β and TNFα in both cell culture and rat model treated with C225 (Qu, Tian et al. 2012).

**Molecular Mechanisms for the Neuroprotective Effect of EGFR Inhibition**

Two possible and main molecular mechanisms are proposed for the neuroprotective effect of EGFR inhibition in NDDs and SCI. The first one is to rescue reactive astrocyte pathology, which is discussed above (Erschbamer, Pernold et al. 2007, Li, Tang et al. 2011, Yang, Wang et al. 2011, Qu, Tian et al. 2012, Kjell, Pernold et al. 2014, Li, Li et al. 2014, Zhang, Ju et al. 2016). The second one is the induction of autophagy, leading to degradation of toxic amyloid structures such as amyloid-β aggregates. Generally, in mammalian cells, autophagy is a stress response that is triggered to keep the cellular homeostasis and survival (Tavassoly 2015). Autophagy in cancer is a mechanism to stop cell death and is a significant component of resistance to therapies (Tavassoly, Parmar et al. 2015). EGFR inhibits autophagy through blocking Belin-1 (Wei, Zou et al. 2013). The complexity of crosstalk between autophagy and EGFR is controlled by the mammalian target of rapamycin (mTOR). Since suppression of mTOR induces autophagy and suppresses cell growth, feedback loops between cellular growth pathways and autophagy are organized in a way that inhibition of cellular growth pathways (such as EGFR inhibition) is associated with increased autophagy (El-Rayes and LoRusso 2004, Dorvash, Farahmandnia et al. 2019, Dorvash, Farahmandnia et al. 2019). (Figure 2 and 3)
Although in the contexts of neoplasia and cancer, autophagy is a characteristic of malignant cells (Hanahan and Weinberg 2011), in neurodegeneration, autophagy is a clean-up process that keeps the neurons safe against aggregation of damaged proteins and organelles (Wong and Cuervo 2010). In other words, autophagy is a protective mechanism against aging and neurodegeneration in neurons. In brain injuries, since oxidative stress and cell death happens, activating the autophagy is a good intervention to help overcome the injury and keep the neuronal homeostasis (Lipinski, Wu et al. 2015). (Figure 2) When all these pathways are studied on a systems-level scale, it becomes clear that the network dynamics of cellular growth, autophagy, and cell death indicates the inhibition of EGFR to be beneficial as a therapeutic intervention in NDDs and SCI while in cancer EGFR antagonists can increase the autophagy and cause resistance in particular condition. The inhibition of EGFR will induce the activation of autophagy in all these cases. In cancer, EGFR inhibition stops cell proliferation, and at the same time, causes increased autophagy. Autophagy has a dual role in cancer; if it is activated beyond the cellular capacity, it turns on the cell death pathways, it is when the autophagy level surpasses its rheostat capacity (Tavassoly, Parmar et al. 2015). Rheostat capacity of autophagy is the level in which autophagy is cytoprotective, and in cancer, activation of autophagy at this level is a part of cancer hallmarks and can eventually lead to resistance to therapy. In NDDs and SCI, EGFR inhibition induces autophagy as a repair and quality-control mechanism and can be a therapeutic intervention. In cancer cells, while higher doses of EGFR antagonists are needed to trigger cell death, ineffective doses only increase autophagy and can cause therapeutic resistance and increase tumor progression. This can be concluded from the dynamical models of autophagy and apoptosis in cancer (Tyson, Baumann et al. 2011, Tavassoly, Parmar et al. 2015, Dorvash, Farahmandnia et al. 2019). In a recently published paper, the connection between EGFR inhibition and induction of autophagy in AD was reported (Wang, Her et al. 2017). Administration of an EGFR inhibitor (CL-387,785, 5 mg/kg/day) to APP/presenilin-1 (PS1) transgenic mice for three weeks increased the level of autophagy and finally led to a reduction in the level of both βA40 and βA42 and a significant improvement of memory loss phenotype (Wang, Her et al. 2017).

**Recent Advances in the Development of BBB EGFR Inhibitors**

While EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib - can be used in the brain cancer metastasis, but they have not shown significant and persistent effects. Generally,
targeting brain tumors or brain metastasis, which can benefit from EGFR antagonists, is challenging since classic EGFR antagonists are not capable of penetrating the BBB (Ahluwalia, Becker et al. 2018). The doses needed to affect the tumor progression are beyond the safe doses and are not applicable. The new generation of BBB-penetrating EGFR antagonists such as osimertinib and AZD3759 has been shown promising effects in stopping cancer progression in the brain in both preclinical and early-phase clinical trials (Ciardiello and Tortora 2008, Kim, Yang et al. 2015, Ahn, Kim et al. 2016, Yang, Guo et al. 2016). Furthermore, despite the availability of pharmacokinetics/pharmacodynamics (PK/PD) parameters of most of the EGFR inhibitors, these values are measured in cancer-related animal models and might not be suitable to be used for the purpose of NDDs and SCI therapies. There is a need for characterizing PK and PD parameters of BBB-penetrating EGFR inhibitors in NDDs and SCI-related models. Moreover, IC$_{50}$ values of these drugs are characterized in cancer cell lines carrying up-regulated or mutant forms of EGFR, and these concentrations might be toxic for neurons or reactive astrocytes. These values must be assessed in CNS-related cells and correlated with cell viability measurements to find the most effective but non-toxic dose suitable for any future CNS-related cell culture studies. A list of EGFR inhibitor and their characteristics and clinical indications are provided in Table 1 (Muhsin, Graham et al. 2003, Ciardiello, De Vita et al. 2004, Thomas and Grandis 2004, Dowell, Minna et al. 2005, Goldberg and Kirkpatrick 2005, Saltz, Easley et al. 2006, Moy, Kirkpatrick et al. 2007, Ciardiello and Tortora 2008, Messersmith and Ahnen 2008, Bose and Ozer 2009, Dienstmann and Tabernero 2010, Vecchione, Jacobs et al. 2011, Lebouleux, Bastholt et al. 2012, Brzezniak, Carter et al. 2013, Helena and Pao 2013, Tan, Shi et al. 2015, Wei, Li et al. 2016, Tan, Li et al. 2017, Karachaliou, Fernandez-Bruno et al. 2018, Kim, Laramy et al. 2019, Spencer, Riley et al. 2019, Takeda and Nakagawa 2019).

**Discussion and Conclusion**

NDDs and SCI are common CNS diseases affecting many patients world-wide, and every year the number of these patients is increased. There are treatments to control symptoms of these diseases, yet there is no cure to stop or prevent the progression of them. Finding novel therapeutics requires detailed knowledge about molecular mechanisms of these diseases to target the pathological marker or pathways. Systems biology and systems pharmacology methodologies are helpful to understand the pathological complex mechanism causing these diseases and their
vulnerabilities for precision therapeutic targeting (Tavassoly, Goldfarb et al. 2018, Dorvash, Farahmandnia et al. 2019). Even though these diseases have specified molecular mechanisms and markers, they have common pathological features, which might be due to a common molecular mechanism behind all these CNS diseases. Some pieces of evidence suggest EGFR activation as a common pathological pathway in these diseases, which finally causes neuronal apoptosis and degeneration. Targeting EGFR activation might be a novel therapy in both NDDs and SCI. There are several EGFR inhibitors at clinical level for cancer therapy, which have the potential to be repositioned for these diseases. In this review, we summarized and collected all reports on the role of EGFR in the pathological development of these diseases and emphasized the need for considering EGFR as a novel target in NDDs and SCI. There are two possible molecular mechanisms for EGFR inhibition, autophagy induction and rescuing reactive astrocyte, which is known to improve both behavioral and pathological hallmarks of these diseases. Unfortunately, there are few studies showing the therapeutic benefits of EGFR inhibition, especially in NDDs, which might be due to ambiguous information about the role of EGFR in pathology development of these diseases. Our attempt in this paper was to collect all information in this regard and open a new route for future studies targeting EGFR. There are some concerns arising from the previous studies that should be considered. Concentrations and doses of these inhibitors need to be adjusted for the purpose of EGFR targeting in neuronal cell cultures and non-cancer animal models. Also, using novel BBB-penetrating EGFR inhibitors is recommended. Overall, we conclude that inhibition of EGFR activation in NDDs and SCI can be a potential strategy for preventing and stopping neurodegeneration and neuronal apoptosis.

Author Contributions
The manuscript was drafted, revised, and edited by O.T., T. S, and I.T.
Figure Legends:

Figure 1:
Under normal conditions, with no injury or pathology, astrocytes exist as quiescent astrocytes with barely detectable levels of EGFR. EGFR inhibitors will induce the transition from reactive astrocytes (which are seen in pathological conditions like NDD and SCI) to quiescent astrocytes through deactivating the EGFR.

Figure 2:
Autophagy induction is one of the responses induced by EGFR inhibitors and has different functions which is controlled by cellular context and temporal and dynamical characteristics of the drug treatment. Although in the context of neoplasia and cancer, autophagy is a characteristic of malignant cells, in neurodegeneration, autophagy is a clean-up process that keeps the neurons safe against aggregation of damaged proteins and organelles.

Figure 3:
Cell fate decision controlled by EGFR signaling pathway. Activation of EGFR leads to a series of activated pathways which can turn on cellular processes including cell death, autophagy and cell growth. The signaling pathways involved in transducing EGFR activation signal include mTOR, JAK/STAT and RAS.

Table 1:
List of the EGFR inhibitors, their properties and clinical indications.
References:


MOL#119909


Footnotes:

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### Table 1

<table>
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<tr>
<th>Drug Name</th>
<th>Brand Name</th>
<th>Inhibition Site</th>
<th>Clinical Indication</th>
<th>BBB-Penetration</th>
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<td>CSF Penetration Rate (%) in Patients</td>
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*ND: Not Determined.*
Figure 1

Toxic Structures → Apoptosis

Neuron → Autophagy

EGFR Inhibitors → mTOR

EGFR

Cancer Cell → Therapeutic-resistant Cancer Cell
**Figure 2**

- **Physiological Condition**
  - Quiescent Astrocytes
  - EGFR Inactivation
  - EGFR Activator

- **Pathologic Condition**
  - Neurodegeneration
  - Spinal Cord Injuries
  - EGFR Inhibitors
  - Reactive Astrocytes