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Review Article

Oncology Section

The Role of Epidermal Growth Factor Receptor in Cancer and their Application for New Targeted Cancer Therapy

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ABSTRACT

Epidermal Growth Factor Receptor (EGFR) has central role in cancer therapy because it causes tumour progression in many cases. The EGFR has seven ligands. Each factor that can block this binding, inhibits the intracellular signal transduction and prevents progression of the tumours. Immune system response is the most important factor for suppressing the initial stage of tumour growth and destroying some initial malignant cells, daily. On the other hand, tumours have different mechanisms to hide their antigens and escape from immune system responses. In contrary, tumours use some mechanisms to escape from immune system such as: 1) use of TGF-β to initiate angiogenesis and immune suppression; 2) Induces Treg cell activation to modulate other immune cells; 3) secretion of the prostaglandin E2 to convert T cell into Treg. So, if a superantigen fused to one of the EGFR-ligands, causes the induction of immune system responses against the tumour cells. One of the new methods is based on the use of the fused super antigen with a ligand of the EGFR to inhibit ligand attaching to the EGFR and inducing immune system responses. To achieve this goal, we can block binding of EGFR to their ligands in the extracellular domain by fusing ligands with bacterial superantigens, toxins or cytokines of the viruses and plants that can induce immune system responses and kill malignant cells. So, the fused ligand can both block signal transduction and induce immune system response against malignant cells. In addition, with combining traditional drugs, high efficacy of the tumour treatment can be achieved. The aim of this review is to assess the mentioned strategy for targeting tumours.

Keywords: Immune system responses, Ligand targeted therapy, Tyrosine kinase inhibitor

INTRODUCTION

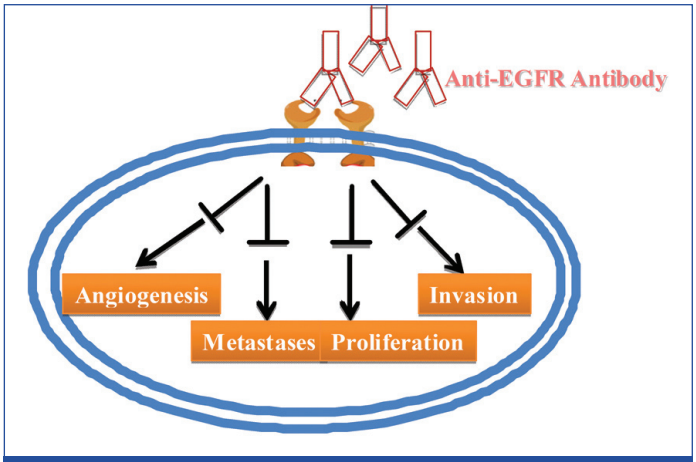
Epidermal growth factor receptor is a member of the ERBB/HER receptor tyrosine kinase superfamily. ERBB/HER super family consists of four members including: (1) EGFR, $ERBB_1/HER_1$, (2) $ERBB_2/HER_2$, (3) $ERBB_3/HER_3$, (4) $ERBB_4/HER_4$. Among these, the role of EGFR has become clearer. EGFR has three parts including, extracellular, transmembrane and intracellular region. The extracellular part has three domains (I, II and III) that ligands bind to domain III. The transmembrane part consists of hydrophobic amino acids and its role is yet unknown. Intracellular part has tyrosine kinase activity and C-terminal tail. At present, both extra and intracellular part of the receptor is targeted with some FDA approved drugs as Epidermal Growth Factor Receptor Inhibitor (EGFRI) and Tyrosine Kinase Inhibitor (TKI) . When EGFR binds to its ligand, EGF at extracellular domain in normal cells induces conformational changes at the allosteric site of the EGFR forming homo or heterodimers which further induces tyrosine phosphorylation in intracellular domain, leading to the cell proliferation and other signal cascades; However, when enhanced in malignant cells, it stimulates uncontrolled proliferation [1]. EGFR is a major cooperator of a complex signaling cascade that modulates growth, migration, signaling, adhesion, differentiation and survival of cancer cells [2]. Some studies have shown that the EGFR can be overexpressed by gene amplification and mutation in regulatory elements or be altered in a variety of malignancies. EGFRs also have a significant role in tumour development and progression, causing cell proliferation, regulating apoptotic cell death, angiogenesis and metastatic spread of the tumour cells [3-6]. In addition, they play an important role in controlling normal cell growth, apoptosis and other cellular functions. Somatic mutations in EGFR can cause abnormal activation of the receptors causing incorrect binding of ligand to the receptor in the extracellular domain that may lead to disturbed signaling and uncontrolled cell division, which can finally cause some type of cancer. Recently, it has been shown that ligand binding to the extracellular domain causes allosteric changes in the intracellular part of the receptor, resulting in the activation of the intracellular tyrosine kinase. Also, the ability of allosteric ligands and their binding affinity varies. Some studies have demonstrated independent activity of EGFR ligand both as homo and heterodimers. These results can be of importance because synthesised antagonists can probably change allosteric conformation of the molecules which can in turn decrease or disturb ligand binding ability and/or tyrosine kinase activity leading to the inhibition of signal transduction in tumour cell and preventing malignant cell growth [7] [Table/Fig-1].

Downregulation of EGFR is partly done by internalisation of the activated EGFR, followed by degradation in the lysosomes and partly by the desensitisation induced by phosphorylation of serine residues in the intracellular domain. A new study has shown that receptor internalisation is more important than the number of receptors in the surface of cell occupied by receptor inhibitor drugs in cancer therapies [8]. On the other hand, EGFR inhibitors accelerate the expression of Major Histocompatibility Complex (MHC) Class I and Class II molecules [9,10] and enhance immunity [11]. Some results demonstrated that if keratinocytes are treated simultaneously with an EGFR, ligand and IFNγ can induce MHC class II [12]. However, EGFRI can increase MHC Class II molecules in normal and malignant keratinocytes through a mechanism that likely involves MHC Class II Transactivator (CIITA) [9] and have adverse effects like dermatologic toxicities [13-15]. Tyrosine kinase inhibitor is another way to control EGFR and inhibit the activity of this pathway [16,17]. EGFRIs have potential role in the treatment of advanced or recurrent cancers [18].

EGFRIs may facilitate antigen presentation in the lung tumour cells contributing to anti-tumour response [19]. Treatment with EGFRIs also prevent interleukin-13 induced mucin production in the rat respiratory epithelium in vivo [20]. Blocking EGF with EGFRIs leads to the inhibition of tumour growth or in certain cases may cause tumour regression in the KRAS-wild type gene however it is not efficient in KRAS mutant gene. New studies have demonstrated

novel strategies to undo KRAS mutant in order to make these drugs efficient [21,22]. Blocking EGFR signaling enhances the inflammation in the skin through upregulation of chemokines, and recruits mononuclear cells including T cells, NK cells, macrophages and dendritic cells [23,24]. EGFR expressed on tumour cells may induce a specific cellular immune response in vivo [25]. Mutated genes are recognised as foreigners by the host immune system, they might cause stronger immune responses and can be an appropriate target for cancer immunotherapy [26]. Several studies have shown that the mutation in tumour-specific EGF-R, such as EGFRvIII [27] in glioblastoma multiform arise from immune responses. EGFR can stimulate hypersecretion of mucin and cause chronic airway disease such as asthma [28,29].

Monoclonal antibody can attach to EGFR and inhibit activation and autophosphorylation of EGFR [30-32]. Monoclonal antibody attaches to the ligand and blocks receptor [32,33]. HER-1 (EGFR) is one of the most extensively studied growth factor receptors. TGF-α is possibly the most potent HER-1 ligand and influences wound healing, epidermal maintenance, gastrointestinal function and lactation [34]. HER-1 is widespread in epithelial cells and also in mesenchymal cells such as fibroblasts, osteogenic and chondrogenic cells. Some tumours differentiated from these cells, express HER-family members and often show TGF-α and/or HER activation. Both TGF-α and EGF have shown to promote the pivotal growth of keratinocytes, helping to cover an epidermal wound. TGF-α is more effective than EGF in stimulating epidermal regeneration after burns [35]. On the other hand, TGF-α like EGF is overexpressed in tumour. TGF-β signaling is one of the major factors in cancer [36]. The TGF-β activation causes metastases and tumour proliferation in cancer tissue. TGF-β have been linked with both experimental and human cancers and can either promote or inhibit tumour development [37]. On the other hand, inhibition of TGF-β signaling have been shown to block hepatocellular carcinoma growth [38]. TGF-β and tumour acting together, first TGF-β equilibrates the components of tumour microenvironment and then tumour cells balance the activity of inflammatory cells or fibroblasts with cancer cells causing tumour growth or progression. Overall, EGFR antibodies suppress tumour development and progression, cell proliferation, angiogenesis and metastatic spread of the tumour cells [Table/Fig-2].



[Table/Fig-2]: Schematic figure of EGFR antibody inhibiting the growth of tumour: EGFRIs have significant role in suppressing tumour development and progression, blocking cell proliferation, angiogenesis and metastatic spread of the tumour cells.

There are two TGF-β signaling responses, the first is called an "early" and the other is termed "late" TGF-β signaling response. The early response pattern is associated with longer response and may reflect the physiological inflammatory response but late response with shorter survival time might associate with long-term TGF-β activation similar to the one previously described in colorectal cancer [39]. TGF-β, plays a dual role in tumour, acting as a tumour suppressor in early stages and as tumour promoter in late stages of tumour progression [40]. TGF-β may be downregulated in cancer cells to promote their growth but their expression increased in some human cancers, including pancreatic, colon, lung, stomach, endometrium, breast, prostate, brain, and bone cancers. Primarily, tumour lose their growth inhibitory response to TGF-β and then produce massive amounts of proteins, the increased expression of TGF-β provides a selective advantage for tumour cell survival. TGF-β are also angiogenic and have potent immunosuppressive effects, including inhibiting NK cells functions exclusively [41]. The increased expression of TGF-β is usually accompanied by a loss in the growth inhibitory response to TGF-β [41]. TGF-β signaling is associated with loss of cell polarity, acquisition of cellular motility, and increased tumour invasion [42,43]. TGF-β, produced by tumour cells and

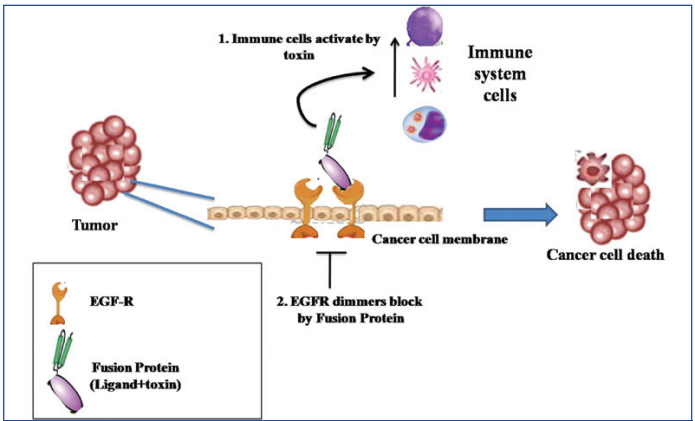
ErbB Family (EGF-R)				
Ligand	**Author-Year**	**Studies**	**Experiment model**	**Role**
EGF	WHJ Ward-[30]	4-(3-chloroanilino) quinazoline (CAQ) which is a potent inhibitor of tyrosine kinase.		stimulates cell growth
TGF-α	Valabrega, Giorgio-[31]	expression of exogenous TGFalpha in breast cancer cells, Trastuzumab-induced HER2 endocytosi was reduced.	Breast cancer	mitogenic polypeptide and signaling pathway for cell proliferation
Betacellulin (BTC)	Huotari, MA-Vallières, Nicolas-[32,33]	BTC overexpression induces Schwann cell proliferation and improves recovery of locomotor function.	nerve repair	Is a protein that synthesised primarily as a transmembrane precursor
Amphiregulin (AR)	McCarthy, FM.-[34]	Amphiregulin is overexpressed by classical monocytes in non-small cell lung cancer	non-small cell lung cancer	Autocrine growth factor
Heparin Binding EGF-like Growth Factor (HB-EGF)	Zhou, Yu [35]	EGF-like growth factor protects the enteric nervous system.	nervous system.	Major role in wound healing, heart function
Epiregulin (EPR)	Bauer, Alison K. [36]	Epiregulin induce lung tumour promotion in murine carcinogenesis model.	Lung cancer	Ligand of EGF receptor
SOS1	Cai, D. [39]	SOS1 mutant, a guanine nucleotide exchange factor, upregulation of MYC and Erb system.	Lung adenocarcinoma	Signal transduction
SRC	Kraus, Sarah [40]	c-Src is activated by the EGFR and gonadotropin-releasing hormone.	COS7 cells	interact with cellular cytosolic and phosphorylation of tyrosine residues

[Table/Fig-1]: Studies in relationship with ErbB family, showing the ligands and their roles.

regulatory T cells support epithelial cells to become mesenchymal stem cells through a process known as Epithelial–Mesenchymal Transition (EMT), thereby promoting metastasis and fibrosis which result in activation of myofibroblasts, causing excessive production of Extracellular Matrix (ECM) and inhibition of ECM degradation [44,45].

Superantigen Activated T Cell

One of the modern methods for attacking tumour cells is the use of a superantigen fused to the TGF-α. This fusion structure can bind to tumour EGF receptor and show inhibitory effects [46,47]. When EGFR block with EGFRIs, not only angiogenesis, metastases and survival of the cancer cells decreases but also the immune system was activated. In other hand EGFRIs can increase immune recognition and recurrent of immune cell like NK cell and T cell to inflammation site [Table/Fig-3]. On the other hand, this fusion can invite the CD4 T cells and CD8 T cells around the tumour [48]. One of the major strategies to cure tumours is to activate immune cells like T cells for recognising tumour antigens [49-55]. Furthermore, exposure of super T cells to the superantigens leads



[Table/Fig-3]: Schematic diagram of EGFRIs and their antitumour effect including recognition and respond to the tumour. EGFRIs can decrease survival, proliferation, angiogenesis and migration of the tumour. On the other hand, EGFRIs can increase immune recognition and the recurrence of immune cell like NK cells and T cells to the inflammation site.

to the activation and upregulation of phosphorylation of tyrosine kinases [55]. Tumour-Targeted Superantigens (TTS) recruits potent T cell activating features of a superantigen like *Staphylococcus aureus* enterotoxin against tumour cells [56] or causing apoptosis [57]. In vitro *Staphylococcus aureus* enterotoxin causes increased secretion of INF-γ from mononuclear cell and leads to the more apoptosis of tumour cells [58]. One problem in this strategy is the secretion of human antibodies, so the use of the superantigens with low antigenicity is more appropriate [59]. After attachment of TGF-α part to the receptor, superantigen part stay outside of fusion structure that cause activation of T cells. For this strategy, first TGF-α binds EGFR that is expressed in the surface of the tumour, in next step, superantigen can attract lymphocytes to the site of action. Superantigen can efficiently induce inflammatory cytokine production [54,60-64].

Another method is to utilise a superantigen fused to the tumour reactive antibody [65-68] or fused to the anticancer drug [69]. In case of antibody, monoclonal antibody is recognised by tumour cell fused to the superantigen. Superantigen with bicomponent can cause apoptosis in tumour cell [70-73].

Tumour

The process of tumour development is obviously an imbalance in the growth homeostasis due to genetic reprogramming and/or damage. The normal growth control mechanisms are overcome by genetic mutations and limiting or completely blocking apoptotic mechanisms [34]. Tumour cells can often take the advantage of genetic programs with the purpose of maintaining homeostasis, e.g., like the angiogenic response to hypoxia or production of growth factors for maintenance [34]. Several studies has shown growth factors and/or their respective receptors overexpressed in tumours or premalignant cells [74,75]. There are many strategies used for tumour immunotherapy including: interleukin-2, antibodies that block Cytotoxic T Lymphocyte-associated Antigen 4 (CTLA4) [76,77], antibodies that block Programmed Death-1 (PD-1) [78,79] and adoptive cell transfer of tumour-infiltrating lymphocytes.

The general troubles in antitumour immune responses are the loss or the mutation of antigens that are recognised by T cells, loss of antigen-presenting machinery components such beta-2-microglobulin and HLA [80,81], tumour cell induced inactivation of T cell signaling [82], resistance to the proapoptotic effects of toxic granules such granzymes and perforin, death receptors, Tumour necrosis factor Related Apoptosis-Inducing Ligand (TRAIL), or interferons [83].

T cells play a major role in tumour escape and in limiting the success of cancer immunotherapy. Unresponsiveness to the specific antigens is early event in tumour progression. Myeloid-Derived Suppressor Cells (MDSC) play an important role in T cell non-responsiveness [84,85]. Immature myeloid cells are able to take up soluble proteins, process them and present antigenic epitopes on their surface and induce Ag-specific T cell anergy. In physiological conditions, APC consists of primarily Dendritic Cells (DCs) and macrophages. In tumour-bearing mice, another group of bone marrow derived cells may be a part of APC population. These cells identified as Gr-1+CD11b+ cells are comprised of precursors of macrophages, granulocytes, DCs, and myeloid cells at earlier stages of differentiation [86-88]. These immature cells are present in bone marrow and under normal conditions and become differentiated into mature myeloid cells [89], however, before this event, like immature myeloid cells, they may cause energy in immune cells. Immature myeloid cells from tumour-bearing mice differentiate into mature cells within five days, adoptive transfer into tumour-free recipient after this period, did not affect peptide specific immune response.

Naturally, $CD4^+CD25^+$ regulatory T cells (T_{reg}) play an important role in induction and maintenance of T cell tolerance [90]. T regulatory cells are produced in the thymus as a functionally mature subpopulation of T cells or induced from T cells in periphery. Tumour can either accumulate Tregs or convert non-Tregs to Tregs around them [90]. The major role of Tregs is their ability to suppress other T cells. However, Tregs can suppress effective cells and tumour can increase this activity by Prostaglandin E2 [90].

DISCUSSION

Many studies provide a clear understanding on how host immunity plays a major role in control of tumour development [91]. Changing in the number and the function of antigen-specific T cells is the main factor responsible for tumour escape. T cell ignorance apparently plays the most critical role at early stages of tumour development [92]. Altered function of antigen-specific T cells is one of the major factors responsible for tumour escape. On the other hand, administration of tumour-derived immature myeloid cells dramatically inhibits $CD8^+$ T cell response to a specific antigen. Activated Tregs in tumours can: 1) inhibit cytokines' secretion; 2) suppress cell function through cytolysis and metabolic disruption. EGFRIs can inhibit both tumour growth and increase MHC Class II in keratinocytes. Antitumour response or antitumour treatment should be used at different stages of disease to prevent

progression and the recurrence of the tumours including, MHC Class II presenting, activated T cell, induce antibody production and regulation of cytokines. To recognise abnormality or tumour, MHC with peptide should be presented to T cells. EGFRIs can stimulate MHC presenting and with control of EGFR that cause vegetates of tumour, strongly may cause surrender of tumour growth. Finally,

Target	Drugs	Antibody type	Cancer	Trial status
EGFR	Cetuximab (Erbitux)	Monoclonal antibody	EGFR inhibitor used for the treatment of metastatic colorectal cancer, metastatic non-small cell lung cancer and head and neck cancer.	Approved
EGFR	Panitumumab	Fully human monoclonal antibody	Metastatic Colorectal Cancer	Approved as a first-line agent in combination with FOLFOX
EGFR	Matuzumab	Humanized monoclonal antibody	Binds to the EGFR with high affinity. Advanced non-small cellular lung carcinoma Advanced adenocarcinomas of stomach and esophagus	Phase II
EGFR	Necitumumab	Recombinant human IgG1 monoclonal antibody	Binds to the EGFR. antineoplastic. non-squamous non-small-cell lung carcinoma.	The US FDA approved necitumumab under the brand name Portrazza.
Anti-EGF receptor	ABX-EGF (Abgenix)	Human anti-EGF receptor monoclonal antibody	Colorectal cancer, metastatic non-small cell lung cancer and head and neck cancer.	Phasel/II
EGFR	h-R3	Humanized	Colorectal cancer, metastatic non-small cell lung cancer and head and neck cancer.	Phasel/II
Tyrosine kinase inhibitor	Erlotinib	-	Non-Small Cell Lung Cancer (NSCLC), pancreatic cancer. It is a receptor tyrosine kinase inhibitor, which acts on the EGFR.	lung cancer in phase III trials. Approved
Tyrosine kinase inhibitor	Gefitinib	-	It works by slowing or stopping the growth of cancer cells. Gefitinib blocks a certain protein (an enzyme called tyrosine kinase).	lung cancer. Approved

[Table/Fig-4]: List of monoclonal antibodies that target EGFR for cancer therapy.
EGFR: Epidermal growth factor receptor

at present, there are some FDA approved HER family inhibitors that their efficacy in patient with various tumours have been proved however, in the patients with KRAS mutation this inhibitor has no efficacy so, studies should be focused on solving this problem. All in all, there are many proposed strategies for solving this problem that are in clinical stages of development and the study for new strategies are ongoing at present [Table/Fig-4].

CONCLUSION

Several studies of recent evidence suggest that the fused ligands can: 1) block signal transduction; 2) induce immune system respond against malignant cells; and 3) mention strategy for treating of tumours. In addition, combining traditional drugs lead to high efficacy of the tumour treatment.

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