Review Article　　　　　　　　　　　　　　　　　　　　**Open Access**

Anti-Epidermal Growth Factor/Epidermal Growth Factor Receptor Therapeutic Anti-cancer Drugs and the Wound Healing Process

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Abstract

Cutaneous wound healing is a complex process involving blood clotting, inflammation, tissue formation, and tissue remodeling. Many experimental and clinical studies have demonstrated varied, but in most cases beneficial, effects of exogenous growth factors on the healing process. The use of targeted anti-cancer agents is increasing. It is common to utilize a multi-modal treatment approach towards solid tumors, often including surgical resection, and it has become apparent that some targeted agents can impair wound healing or cause increasing risk of perioperative complications. There are limited data regarding the wound healing process of anti-cancer target drugs blocking the EGF/EGFR system. The aim of this paper is to review and to comment the effects of anti-EGF/EGFR drugs on the skin wound healing process after programmed or emergency surgical procedures. A review of the current literature, including our own results, was undertaken. We included the monoclonal antibodies cetuximab, panitumumab, nimotuzumab; the small tyrosine kinase molecules erlotinib and gefitinib; and the EGF-based cancer vaccine; CIMAvax and the EGFR-based cancer vaccine; HER-1 vaccine. Apparently, there are no deleterious effects of the anti-EGF/EGFR drugs in the wound healing post-operative process. Taking into account that treatment with anti-EGF/EGFR drugs inhibits tumor cell proliferation, and the lack of deleterious effects of these EGF/EGFR specific inhibitors in the wound healing post-operative process; we suggest that these kinds of drugs could be maintained and their effects tested, with very special surveillance during the post-surgical period.

Introduction

Cutaneous wound healing is a complex process involving blood clotting, inflammation, new tissue formation, and finally tissue remodeling. It is well described at the histological level, but the genes that regulate skin repair have only partially been identified. Many experimental and clinical studies have demonstrated varied, but in most cases beneficial, effects of exogenous growth factors on the healing process. However, the roles played by endogenous growth factors have remained largely unclear. Initial approaches at addressing this question focused on the expression analysis of various growth factors, cytokines, and their receptors in different wound models, with first functional data being obtained by applying neutralizing antibodies to wounds. During the past few years, the availability of genetically modified mice has allowed elucidation of the function of various genes in the healing process, and these studies have shed light onto the role of growth factors, cytokines, and their downstream effectors in wound repair [1].

Epidermal growth factor receptor is normally expressed in the epidermis, sebaceous glands, and hair follicular epithelium, where it plays a number of important roles in the maintenance of normal skin health, including control of differentiation, protection against damage induced by ultraviolet radiation, inhibition of inflammation, and acceleration of wound healing. Although the exact mechanism of skin rash mediated by EGFR-targeted monoclonal antibodies is incompletely understood, inhibition of EGFR is believed to cause follicular occlusion and rupture because of premature epithelial differentiation and an increase in the expression of genes that stimulate inflammation, apoptosis, and cell attachment [2].

Table 1 shows the characteristics and dermatologic reactions associated with anti-epidermal growth factor/epidermal growth factor receptor therapeutic anti-cancer drugs.

The epidermal growth factor (EGF) family of mitogens comprises several members, including EGF, transforming growth factor (TGF), heparin-binding EGF (HB-EGF), amphiregulin, epiregulin, betacellulin, neuregulins, the recently discovered epigen, as well as proteins encoded by Vaccinia virus, and other poxviruses [3,4].

The use of targeted anti-cancer agents is increasing. It is common to utilize a multi-modal treatment approach towards solid tumors, often including surgical resection, and it has become apparent that some targeted agents can impair wound healing or cause an increased risk of perioperative complications. A recent article reviews many of the targeted agents used in solid tumor oncology with an emphasis on clinically relevant details [5].

Up to now, evidences on targeted therapy that might cause surgical complications are limited. The greatest amount of data exists for bevacizumab that causes perioperative complications, possibly due to its mechanism of action, neutralizing the ability of all active vascular endothelial growth factor (VEGF) -isoforms to bind to the VEGF receptor (VEGFR) on the surface of endothelial cells [6].

Agent	Type	Indication	Most common skin toxicities
Cetuximab (Erbitux)	Chimeric (mouse/human) monoclonal antibody	Metastatic colorectal cancer and head and neck cancer	Rash (Monomorphous erythematous maculopapular, follicular, or pustular lesions which may be associated with pruritus/tenderness)

Paronychia and fissuring (Painful periungual granulation-type or friable pyogenic granuloma-like changes, associated with erythema, swelling, and fissuring of lateral nailfolds and/or distal finger tufts)

Hair changes (Alopecia and curlier, finer and more brittle hair on scalp and extremities; trychomegalia and curling of eyebrows and hypertrichosis of the face)

Dry skin (Diffuse fine scaling)

Mucositis (Mucositis, stomatitis, and aphthous ulcers) |
Panitumumab (Vectibix)	Fully human monoclonal antibody	Metastatic colorectal cancer with disease progression despite prior treatment in patients with non-mutated (wild-type) KRAS	
Nimotuzumab (CIMAher)	Humanized monoclonal antibody	High grade glioma, esophagus and head and neck tumor patients	
Erlotinib (Tarceva)	Selective oral inhibitor of EGFR tyrosine kinase	Locally advanced or metastatic non-small cell lung cancer after the failure of prior chemotherapy and in combination with gemcitabine for locally advanced, unresectable or metastatic pancreatic cancer	
Gefitinib (Iressa)	Selective oral inhibitor of EGFR tyrosine kinase	Locally advanced or metastatic non-small cell lung cancer	
EGF vaccine (CIMAvax)	Human recombinant Epidermal Growth Factor (EGF) chemically conjugated to a carrier protein from Neisseria meningitides	Advance patients with non-small cell lung cancer	
Her-1 Vaccine	Extracellular domain of human EGFR/VSSP	Hormone refractory prostatic cancer patients	

Table 1: Characteristics and dermatologic reactions associated with anti-epidermal growth factor/epidermal growth factor receptor therapeutic anti-cancer drugs.

There are limited data regarding the wound healing process and anti-cancer target drugs blocking the EGF/EGFR system as cetuximab, panitumumab, nimotuzumab, erlotinib, and gefitinib and very little for the EGF-based cancer vaccine CIMAvax and the EGFR-based cancer vaccine HER-1 vaccine.

An analysis of their mechanism of actions suggests, a priory, that there should be an important pharmacovigilence with respect to perioperative complications and increased post-surgical support for patients to aid early detection of postoperative complications until definitive data become available [5].

In this Personal View, we provide an overview of the data, including our own results, for an evidence-based recommendation for the medical treatment with anti-EGF/EGFR drugs and their possible effects on the skin wound healing process of cancer patients undergoing programmed or emergency surgical procedures.

In the present article we are not including the effects of these drugs on cornea and tympanic membrane because diverse and inconsistent kinds of responses have been obtained [7-11].

Agents Targeting EGF/EGFR Pathways

Antibody-based therapies

Cetuximab: Cetuximab is a monoclonal antibody targeting EGFR, which is overexpressed in many cancer types. It is licensed by the FDA for its use in patients with metastatic colorectal cancer and locally advanced squamous cell head and neck cancer.

Appropriate management of skin toxicity associated with epidermal growth factor receptor inhibitor therapy is necessary to allow adequate drug administration and to improve quality of life and outcomes.

In a recent study, authors performed a retrospective review of head and neck cancer patients who received salvage neck dissections between 1999 and 2007, at two academic tertiary care centers. Complications from wound healing were compared between radiation and combined therapy groups.

A total of 35 patients received radiation (n=20) or combined radiation and cetuximab therapy (n=15) prior to neck dissection. The treatment groups were similar in regard to demographic and primary tumour-related characteristics. The time between treatment and salvage neck dissection did not differ between the radiation (3.9 months) and combination treatment (3.0 months) groups (p=0.15). Wound healing complications occurred in 13% (2/15) of the patients treated with radiation and cetuximab and there were no complications in patients who received radiation alone (p=0.20).

They concluded that cetuximab did not significantly increase the risk of post-surgical wound complications, although a higher absolute number of wound complications was observed in the group treated with cetuximab and radiation therapy, compared with the group treated with radiation alone [12]. These results should be taken into account with caution since patients had surgery six to eight weeks post-cetuximab treatment, so these patients have been off cetuximab for two months since the cetuximab´s half-life is approximately four days [13].

Panitumumab: Panitumumab is a monoclonal antibody targeting the extracellular domain of the EGFR. It is licensed by the FDA for the treatment of metastatic colorectal cancer. No guidelines have been published regarding the cessation of panitumumab therapy prior to surgery. However, there does not, as yet, appear to be an increase in perioperative complications with this EGFR inhibitor [14].

Small molecule kinase inhibitors

Erlotinib: Erlotinib is an oral EGFR tyrosine kinase inhibitor indicated for the treatment of patients with locally advanced or metastatic non-small cell lung cancer after the failure of prior chemotherapy and in combination with gemcitabine for locally advanced, unresectable or metastatic pancreatic cancer. There are no reports of surgical complications in patients taking erlotinib [5].

Gefitinib: Gefitinib is a selective inhibitor of EGFR tyrosine kinase and is indicated for the treatment of patients with locally advanced or metastatic non-small cell lung cancer. It also represents a potential therapeutic target for head and neck squamous cell carcinoma (HNSCC). *In vitro* and *in vivo* studies have demonstrated that the combination of INF-alpha and gefitinib might have a cooperative antitumor effect of HNSCC-derived cell lines [15-17].

A case series was published describing four patients who underwent different types of surgery (emergency laparotomy, internal fixation for bone metastases and incision, and drainage of labial abscess) concurrently with gefitinib with no adverse effect on wound healing [18].

The Center of Molecular Immunology´s Strategy

The Center of Molecular Immunology (CIM) in Havana, Cuba, has developed its own strategy, blocking the EGFR pathway with monoclonal antibodies (i.e., nimotuzumab) and with cancer vaccines (EGF-vaccine and HER-1 vaccine). The monoclonal antibody nimotuzumab and the EGF-vaccine have been registered in Cuba and other Countries and HER-1 vaccine is in a Phase I Clinical Trial for treating cancer diseases from epithelial origin.

The fact that our registered drugs have demonstrated evidences of clinical benefit with absence of serious toxicities, including during concomitant chemo-radiotherapy, supports the possibility of a long lasting administration period in patients with cancer from epithelial origin.

Several studies are ongoing for demonstrating that our combination of targeting techniques with conventional current treatment protocols may improve the treatment outcome and disease control, without exacerbating the treatment related toxicities.

Nimotuzumab

Nimotuzumab is an anti-EGFR monoclonal antibody registered in 26 markets and in Cuba by the Cuban regulatory Agency, CECMED, for treating high grade glioma, head and neck and esophageal cancer patients.

It was originally generated as a mouse IgG2a antibody and humanized by grafting its complementarity-determining regions (CDRs) to a human IgG1 gene.

In several trials, it has shown activity and been well tolerated when used together with radiation [19]. Preclinical data have suggested that nimotuzumab may enhance the anti-tumor activity of ionizing radiation [20,21]. Clinical experience has demonstrated good tolerability of nimotuzumab at 50–800 mg doses when given as a single agent, [22] or in combination with radiation to head and neck cancer patients [23,24].

The low toxicity profile shown by nimotuzumab has allowed its use in prolonged treatments, lasting several months, and even years in many cases. About two thirds of the 600 patients that have been treated in Cuba since 2002 have received more than six doses of the antibody (200 mg for adult, 150 mg/m² for children), including about 50 patients that have been treated with more than 30, bi-weekly doses during more than one year. In particular, two children with brain stem glioma tumors have received more than 100 doses of nimotuzumab, continuously for more than three years, without showing adverse effects. It is worth noting that the frequency of adverse events (limited to grade 1 or 2) observed in these patients did not increase with drug exposure [25,26].

From this clinical experience we have obtained important evidences on the impact of chronic treatment in disease stabilization and increase of overall survival in advanced cancer patients. In more than 20,000 patients treated world-wide with nimotuzumab, there are no reports of adverse events associated with surgical complications [27-29].

Cancer vaccines

Therapeutic vaccines continue to be one of the most active fields in cancer research. However, despite clear evidence of antitumor effect in laboratory animals, and despite the ability of current vaccine candidates to elicit tumor specific antibodies and T-cells in humans, objective responses in the clinical trials are rare. Actually, only sipuleucel-T (Provenge), a prostatic acid phosphatase–granulocyte/macrophage– colony-stimulating factor (PAP-GM-CSF) fusion protein–loaded autologous blood cell vaccine, has been approved by the US Food and Drug Administration (FDA) in April 2010 for the treatment of asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer patients [28].

The role of therapeutic vaccines in advanced cancer patients, if any, would be to decrease the rate of disease progression and to increase survival and quality of life. Due to the redundant regulatory loops contracting the immune response to antigens that cannot be eliminated, such a role would require chronic vaccination, which is at first sight at odds with the classic experience of vaccinology.

EGF-vaccine

During the last decade, our team has developed a therapeutic vaccine for advanced lung cancer, which consists of human recombinant Epidermal Growth Factor (EGF) chemically conjugated to a carrier protein from Neisseria meningitides. Several clinical trials have been carried out, showing increase in anti-EGF antibody titers, decrease in plasma EGF concentration and survival advantage in vaccinated patients bearing advanced non-small cell lung cancer (NSCLC) [29]. This EGF-based cancer vaccine (CIMAvax) has been approved by the Cuban regulatory Agency, CECMED, in 2008 for treating advanced patients with NSCLC.

In a recent article, authors reported that 58 patients were vaccinated monthly for more than one or two years, and this long term vaccination was feasible and safe, and there was no evidence of wound healing or any cumulative toxicity [30].

In a non-clinical study, authors from the same group investigated the possible role of the EGF-vaccine in the croton-oil-induced ear edema and in the wound healing experimental animal models.

Mice were immunized with an EGF-vaccine by intramuscular injections and serum titers against EGF were measured through ELISA techniques. Control animals received saline.

Immunized mice produced antibodies against EGF while no antibody titers could be measured in control animals. Croton oil applied to the inner ear surface of EGF-vaccine treated mice caused a 61.3% lower ear punch weight and a 60.2% lower myeloperoxidase activity than control mice. In the EGF-vaccine treated animals, planimetry measurements and histological analysis did not lead to significant impairment in tissue repair.

The authors concluded that EGF-vaccination in mice decreased the normal croton-oil-induced inflammation response, without apparent impairment in tissue healing [31].

HER-1 vaccine

HER-1 vaccine is a cancer vaccine which is in its very beginning clinical steps in Cuba. In non-clinical pharmacodynamics studies Ramírez and colleagues [32] immunized mice with the extracellular domain of murine EGFR (mEGFR-ECD) in adjuvants with the objective of circumvent tolerance to self EGFR by inducing an immune response with consequent antitumor effect. The study demonstrated that despite mEGFR expression in thymus, strong DTH response was induced by inoculation of mice with the mEGFR-ECD. This self-immunization, using both Freund's Complete Adjuvant (FCA) and very small sized

proteoliposomes from Neisseria meningitidis (VSSP), promoted highly specific IgG titers, predominantly IgG2a and IgG2b. Sera from mice immunized with mEGFR-ECD/VSSP not only recognized EGFR1 tumor cell lines by FACS, but also inhibited the *in vitro* growth, even in the absence of complement. Noteworthy, vaccination of mice with mEGFR-ECD/VSSP stimulated a potent anti-metastatic effect in the EGFR Lewis lung carcinoma model; while reproduction associated side effects were absent. Curiously, mice immunized with the human EGFR-ECD (Her1-ECD) in VSSP though induced highly specific IgG antibodies with strong *in vitro* cytotoxic effect over EGFR human cell lines, showed low cross-reactivity with the mEGFR-ECD.

Fuentes et al. [33], explore the possible role of mEGFR-ECD vaccine in the croton-oil-induced ear edema and the wound healing in experimental animal models. Mice were immunized four times biweekly with 50 μg of mEGFR-ECD/VSSP/Montanide or with PBS/VSSP/Montanide by intramuscular way. Mice treated with mEGFR-ECD/VSSP/Montanide had a similar inflammatory response compared to control ones in the croton oil inflammation model. No differences in healing speed were found for the skin wounds in the mice vaccinated with mEGFR-ECD, with respect to the control animals. Planimetry measurements, histological and morphometrical analysis did not lead to significant impairment in tissue repair. These data suggest that application of Her1-ECD/VSSP vaccine as a therapeutic approach in cancer patients would not elicit a poor healing process after surgery or other invasive procedures.

An immune-active dose-escalation Phase I Clinical Trial in hormone refractory prostatic cancer patients treated with the ECD of the human EGFR is on-going in Cuba, and especial emphasis is performing in detecting any skin or wound healing adverse event.
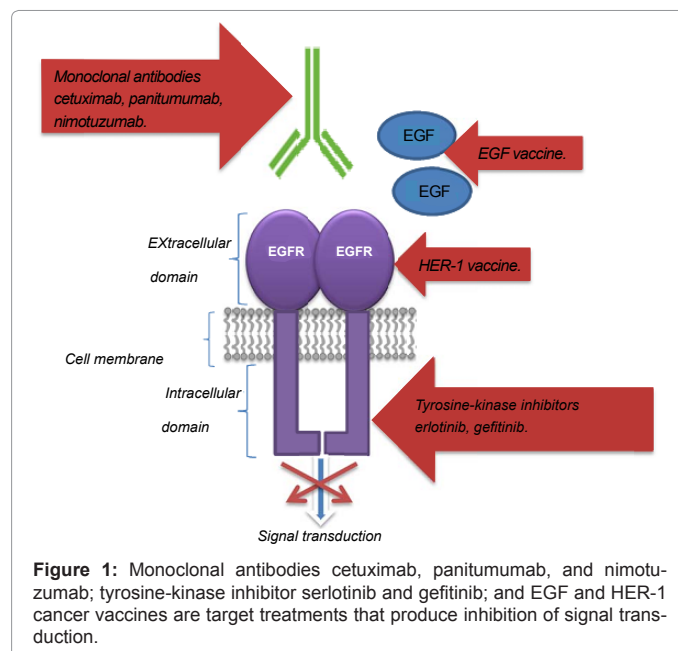
A schematic representation of the anti-EGF/EGFR therapeutic anti-cancer drugs and their mechanisms of actions are shown in figure 1.

Reflections and Conclusions

Several reports have shown that post-operative leakage may have a negative impact on long-term survival in patients with malignancies [34-39].

Evidences suggest that recurrence of resected head and neck squamous cell carcinomas (HNSCCs) is due to the outgrowth of unrecognized residual tumor cells as well as to the premalignant and/or precursor-field epithelial cells. Licitra et al. [40] studied the impact of processes triggered by HNSCC surgery in stimulating both residual tumor cells, demonstrated to overexpress epidermal growth factor receptor (EGFR), and premalignant cells surrounding the resected lesion.

They showed that primary tumors have intermediate/high EGFR expression (91%), EGFR phosphorylation and EGFRpositive FISH (35%). Normal, metaplastic and dysplastic epithelium surrounding the resected tumor displayed EGFR overexpression. EGFR activation and gene amplification were observed in normal and dysplastic epithelium, respectively. Each tested wound healing drainage induced cells to proliferate and the proliferation was significantly higher in relapsed compared with non-relapsed HNSCC patients. Anti-EGFR treatments inhibited the drainage-induced proliferation, with the highest inhibitory efficiency by cetuximab on A431 cells, while CAL27 cell growth was more efficiently inhibited by tyrosine kinase inhibitors.

Figure 1: Monoclonal antibodies cetuximab, panitumumab, and nimotuzumab; tyrosine-kinase inhibitor serlotinib and gefitinib; and EGF and HER-1 cancer vaccines are target treatments that produce inhibition of signal transduction.

They concluded that surgery could favor the proliferation of cells showing EGFR overexpression/activation/amplification such as residual tumor cells and/or precursor-field epithelial cells already present after surgery and that treatment with anti- EGFR reagents inhibits wound-induced stimulation, according to the EGFR family status. Similar results have been obtained in breast cancer with the administration of preoperative chemotherapy and trastuzumab [41].

Brusevold et al. [42] showed, using a scratch wound assay *in vitro*, that EGF and hepatocyte growth factor (HGF) induce cell migration in human oral squamous carcinoma cell lines and that addition of EGFR-specific inhibitors cetuximab or gefitinib abolishes cell migration elicited by EGF.

In a more recently study, Sano et al. [43] used a nice combination of preclinical animal models. In the first study an athymic nude BALB/c mouse model of human microscopic residual oral squamous cell carcinoma (OSCC) were used, mice were treated with cetuximab with the objective of measuring tumor growth, survival, and cervical lymph node metastases.

In the second study, a BALB/c normal mouse model of wound healing was established to assess the effect of an EGFR antibody on the wound healing process, in this experiment authors used the rat-antimouse EGFR antibody ME-1.

They hypothesized that EGF and transforming growth factor (TGF)-a are present in the wounds of patients who have undergone resection of head and neck cancers and could be stimulating the early repopulation of residual tumor cells and that inhibition of EGFR signaling in this setting could inhibit this tumor regrowth and thereby improve treatment outcomes.

They demonstrated that cetuximab inhibited tumor progression in the *in vivo* model of microscopic residual disease and that the use of a rat–anti mouse EGFR antibody does not significantly delay the process of wound healing in a mouse wound healing model. They demonstrated in a preclinical model that intervention in the EGFR pathway with

cetuximab treatment soon after surgery could inhibit the repopulation of OSCC cells without impairing wound healing [43].

On the other hand, it has been recently shown, using a wound healing assay, that EGFR is also involved in radiation induced migration of HNSCC cells and therefore EGFR might be a target for the treatment of tumors to improve the efficacy of radiation [44].

Considering the above discussed elements, seems that treatment with anti-EGF/EGFR drugs, apparently, does not elicit a deleterious effect in the wound healing process, inhibits wound-induced tumor cell proliferation, and radiation induced cell migration; we suggest that these kinds of drugs would still be useful in the wound healing post-operative process. The treatment might be maintained and their effects tested, with very special surveillance, at least in HNSCC patients during the post-surgical period. Obviously, further researches are needed for better understanding of these pathophysiological processing HNSCCs and in other EGFR over-expressing cancers from epithelial origin.

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