Epidermal growth factor in clinical practice – a review of its biological actions, clinical indications and safety implications

Jorge Berlanga-Acosta, Jorge Gavilondo-Cowley, Pedro López-Saura, Tania González-López, María D Castro-Santana, Ernesto López-Mola, Gerardo Guillén-Nieto, Luis Herrera-Martinez

Berlanga-Acosta J, Gavilondo-Cowley J, López-Saura P, González-López T, Castro-Santana MD, López-Mola E, Guillén-Nieto G, Herrera-Martinez L. Epidermal growth factor in clinical practice–a review of its biological actions, clinical indications and safety implications. Int Wound J 2009; 6:331–346

ABSTRACT

Chemotaxis, mitogenesis, motogenesis and cytoprotection are common cellular events involved in both tumourigenesis and tissue repair, which appear amplified upon growth factors exposure. Epidermal growth factor (EGF) promotes these events in epithelial and mesenchymal cells through the binding to a specific tyrosine kinase receptor. In experimental oncology settings, EGF does not initiate malignant transformation but exhibits 'tumour promotion'. These observations have raised doubts on the clinical use of EGF despite solid demonstrations of efficacy in experimental conditions and clinical trials. The results of a Pubmed and Bioline investigation on EGF clinical uses and preclinical safety data are presented here. EGF topical administration has been used since 1989 to enhance the healing process of a variety of peripheral tissues wounds (16 clinical reports), as well as its intravenous, oral and rectal administration for gastrointestinal damages (11 clinical reports). EGF therapeutic efficacy and excellent tolerability seem demonstrated. Lack of long-term adverse effects is highlighted in those studies with 6, 12 and 24 months of patients follow-up. Although post-treatment follow-up may fall short for malignant growth, there are no reports on evidences linking EGF clinical use with cancer. A multicentre, nationwide survey in Cuba, 15 years after randomly using silver sulphadiazine with EGF or not in burn victims yielded that cancer incidence was comparable between EGF-treated and control subjects and that such incidence rate does not differ from the age-matched national incidence for those 15-year period. All the animal species subjected to long-term EGF systemic administration exhibit dose-dependent and reversible epithelial organs hyperplasia with no changes in cells phenotypic differentiation. Histotypic pre-malignant markers were not identified. The results emerged from co-carcinogenesis studies and from transgenic mice over-expressing EGF are conflicting and indicate that EGF overexposure, either innate or postnatal, may not be sufficient to transform cells. The ability of EGF to heal injured tissues in life-threatening scenarios or to assist in preventing physical and social disability advocates for its clinical use under a rational medical risk/benefit balance.

Key words: EGF • cancer • malignant • ulcers • wounds

Authors: J Berlanga-Acosta, PhD, Tissue Repair and Cyto-protection Research Project, Pharmaceutical Division, Biomedical Research, Center for Genetic Engineering and Biotechnology, Havana, Cuba; J Gavilondo-Cowley, PhD, Cancer Research Project, Pharmaceutical Division, Biomedical Research, Center for Genetic Engineering and Biotechnology, Havana, Cuba; P López-Saura, PhD, Clinical Trials and Regulatory Affairs Direction, Center for Biological Research, Havana, Cuba; T González-López, MD, Clinical Trials and Regulatory Affairs Direction, Center for Biological Research, Havana, Cuba; MD Castro-Santana, MSc, Center for Genetic Engineering and Biotechnology, Camagüey, Cuba; E López-Mola, MSc, Tissue Repair and Cyto-protection Research Project, Pharmaceutical Division, Biomedical Research, Center for Genetic Engineering and Biotechnology, Havana, Cuba; Gerardo Guillén-Nieto, PhD, Tissue Repair and Cyto-protection Research Project, Pharmaceutical Division, Biomedical Research, Center for Genetic Engineering and Biotechnology, Havana, Cuba; L Herrera-Martinez, PhD, Center for Genetic Engineering and Biotechnology, Havana, Cuba
Address for correspondence: J Berlanga-Acosta, PhD, Tissue Repair and Cyto-protection Research Project, Pharmaceutical Division, Biomedical Research, Center for Genetic Engineering and Biotechnology, Ave 31 e/ 158 & 190, Cubanacan, Playa, PO Box 6162, Havana 10 600, Cuba
E-mail: jorge.berlanga@cigb.edu.cu

international wound journal

Key Points

- this review is intended to be a contribution towards the unresolved concern about the potential cancer-enhancing properties of exogenously administered EGF when aimed at therapeutic purposes
- impaired wound healing is a significant clinical and economic problem particularly within the diabetes mellitus patients' population
- diabetics are plagued by a high incidence of systemic endothelial disease, a failure in anti-bacterial defences, and frail tissue repair machinery
- converging evidences suggest a deficit in the production of different growth factors such as platelet-derived growth factor (PDGF), keratinocyte growth factor (KGF) and vascular endothelial growth factor (VEGF)
- the above described growth factors deficit in diabetes leads to: (i) fibroblasts functionality impairment with limited extracellular matrix formation, maturation and remodelling, (ii) poor myofibroblasts population with negligible wound contraction and (iii) limited or failed angiogenic response
- these findings substantiate the rationale of introducing the exogenous administration of growth factors as an instrumental therapy to enhance and sustain the healing process in this particular patients' population
- the most recent clinical evidences converge to convincingly document the favourable impact of the growth factors therapy in the healing process of diabetic foot ulcers
- we review here the biological actions and the clinical contribution of EGF therapy in the field of tissue repair in parallel with preclinical and clinical safety data

INTRODUCTION

Epidermal growth factor (EGF) is a 53-aminoacids polypeptide originally isolated from mouse salivary glands. EGF discovery was presided by its ability to stimulate epithelial growth and differentiation upon its injection to newborn mice (1,2). The interpretation of this finding was that EGF exogenous administration reprogrammed biological events chronologically established within specific temporary windows. EGF biological activities depend upon its binding to a specific cell membrane receptor, through which it exerts a potent mitogenic effect on the majority of epithelial tissues, fibroblasts and endothelial cells (3). EGF–receptor interaction triggers complex biochemical processes that eventually lead to cell-cycle progression (4) and that somewhat mirror biochemical features of transformed cells (5). Consequently, this brought concern and disappointment over the growing expectation about the clinical use of polypeptide growth factors as 'magic tissue sealers'. Eventually, growth factors accumulated medical merit in the realm of recalcitrant and problem wounds (6,7). This review is intended to be a contribution towards the unresolved concern about the potential cancer-enhancing properties of exogenously administered EGF when aimed at therapeutic purposes.

Tissue repair is a megaprocess controlled in each of its events by a variety of growth factors and cytokines in which the former constitute the broadest and most representative population of soluble messengers (8,9). Impaired wound healing is a significant clinical and economic problem particularly within the diabetes mellitus patients' population. Diabetics are plagued by a high incidence of systemic endothelial disease, a failure in anti-bacterial defences, and frail tissue repair machinery. The combination of these factors results in a high incidence of lower-extremity amputation within this population (10). The mechanisms by which diabetes impedes tissue repair remain unclear. Converging evidences suggest a deficit in the production of different growth factors such as platelet-derived growth factor (PDGF), keratinocyte growth factor (KGF) and vascular endothelial growth factor (VEGF) (11–13). Furthermore, a particularly severe deficit of EGF has been described in diabetes. EGF receptor has been identified as a target for advanced glycation-end products precursors in a time and dose-dependent manner, leading to the abrogation of the receptor autophosphorylation and activation cascade (14).

The above described growth factors deficit in diabetes leads to: (i) fibroblasts functionality impairment with limited extracellular matrix formation, maturation and remodelling, (ii) poor myofibroblasts population with negligible wound contraction and (iii) limited or failed angiogenic response. All these factors converge to render a wound cronification phenotype with significant arrest of the repair process (15,16).

These findings substantiate the rationale of introducing the exogenous administration of growth factors as an instrumental therapy to enhance and sustain the healing process in this particular patients' population. Growth factors 'replacement therapy' has included the topical administration of recombinant human PDGF (17), EGF (18,19), VEGF (20), a growth factors' cocktail generated from an autologous platelet-rich plasma gel (21) and *in vitro* engineered skin substitutes serving as local growth factor bioreactors (22). The most recent clinical evidences converge to convincingly document the favourable impact of the growth factors therapy in the healing process of diabetic foot ulcers. In June 2008 the United States Food and Drug Administration (http://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm094969.htm) announced that upon reviewing information, concluded that there was a five times higher increase in the risk of death from cancer in patients who used three or more Regranex tubes than in those patients who did not use Regranex.

We review here the biological actions and the clinical contribution of EGF therapy in the field of tissue repair in parallel with preclinical and clinical safety data. The literature investigation was based in two main fields: (i) the clinical use of EGF for internal and external tissues repair and (ii) the *in vivo* long-term preclinical toxicology data derived from studies in which EGF was repeatedly administered for a period of time. We also focused on related articles including EGF-mediated tumour transplants, transgenic animals over-expressing EGF and relevant *in vitro* models. The clinical search strategy was conducted as follows: (i) Direct and unrestricted Pubmed search introducing 'Epidermal Growth Factor

or EGF and Clinical Trial' as key words; (ii) similar search through Bioline International (www.bioline.org.br) data source and (iii) Pubmed restricted search for: humans, randomised-controlled trial, case reports, clinical trial, phases I–IV and comparative study. Articles where the drug tested was an anti-EGF agent were discarded. The 13 titles retrieved from the Pubmed in the field of EGF clinical interventions in Ophthalmology (corneal healing) and Otolaryngology (healing of tympanic membrane perforations) were declined because of the scarceness of critical data in the abstracts and the limitations confronted to review old papers. The retrieved information was analysed and presented in tables including: [clinical indication] [EGF dose + duration and route] [number of patients] [study outcome] and [safety]. The EGF clinical trials or case reports for peripheral tissue and gastrointestinal tract healing were reviewed and are summarised in Tables 1 and 2, respectively. For the preclinical toxicology and related studies, a similar strategy was followed through Pubmed direct and via Reference Manager using the following key words: 'EGF + carcinogenesis + rodent', 'EGF + malignant + rodent' and 'EGF + longterm + toxicity'. The data presented here were selected from a total of 12 461 downloaded references. Those articles describing the phenotype of EGF transgenic mice, so as classic research and review articles from the 1980s and 1990s, were also used.

EGF MODE OF ACTION

The first evidence suggesting a role for EGF in tissue repair derived from Stanley Cohen in the early 1960s (Prof. Stanley Cohen–unpublished observations, personal communication) in rabbits with controlled corneal burns that received eye drops based on natural EGF, purified from mouse submandibular glands. In addition to EGF itself, an EGF family of ligands is described today thus comprising transforming growth factor-α (TGF-α), heparin-binding EGF-like growth factor (HB-EGF), amphiregulin, epiregulin, betacellulin, neuregulins, etc. (48). All of them exhibit mitogenic activity upon binding to four different high-affinity receptors: EGFR/ErbB1, HER2/ErbB2, HER3/ErbB3 and HER4/ErbB4. Upon ligand binding, the formation of a functionally active EGFR–EGFR dimer (homodimer) or of an EGFR–HER2, EGFR–HER3 or EGFR–HER4 dimer (heterodimer) causes the adenosine triphosphate-dependent phosphorylation of specific tyrosine residues in the EGFR intracellular domain. This phosphorylation triggers a complex programme of intracellular signals to the cytoplasm and then to the nucleus.

There are two major intracellular pathways activated by the EGFR that invoke the two most important biological actions of EGF in tissue repair: *cell proliferation* and *cytoprotection.* It means that EGFR agonistic stimulation may shift toward mitogenic and pro-survival programmes that concertedly translate into an increase in cell population number. For the mitogenic response, the RAS–RAF–MEK–MAPK pathway that controls cell-cycle progression from the G1 phase to the S phase is important, whereas the PI3K–Akt pathway activates a cascade of anti-apoptotic and cytoprotective mediators thus rescuing injured cells (49). Illustrative examples of the PI3K–Akt pathway anti-apoptotic effect are that of keratinocytes exposure to ultraviolet radiation (50) and oxidative stress (51).

Another biological action unleashed by the EGF–EGFR binomium is the stimulation of locomotion in epithelial and fibroblastic cells (52) through the phosphorylation and consequent activation of the phospholipase Cγ1 and the ERK/MAP kinase cascades (53). This pro-motogenic impulse induced by the EGF–EGFR complex on keratinocytes is of paramount importance for re-epithelialisation. Keratinocytes migration is in part mediated by: (i) the shedding of EGFR ligands from the damaged cells (54), (ii) collagenase-1 activation and sustained production by EGFR autocrine activation (55) and (iii) disruption of the $\alpha 6\beta 4$ integrins at hemidesmosomes and their disassembly by an EGFR phosphorylation-mediated mechanism (56). It has been also observed that EGF can control fibroblasts extension, attachment or detachment directly or indirectly via modifications of the injured tissue extracellular matrix composition (57).

EGF receptor is expressed on most human cell types including those which play critical roles for wound repair such as fibroblasts, endothelial cells and keratinocytes (undifferentiated, marginal, leading edge, hair follicles, sweat ducts and sebaceous glands) (58). The EGF-induced mitogenic, motogenic and cytoprotective actions are instrumental for healing

Table 1 EGF in peripheral tissue healing

Author/Ref.	Indication	Route/dose and duration	Number of patients	Study	Outcome	Safety
Brown (23)	Acceleration of epidermal regeneration of donor sites	Topic; EGF 10 mcg/ml until re-epithelialisation	12 patients; controlled within patient, side/side (EGF/SS)	DBRCT	Acceleration of the rate of healing	Not described
Alert (24)	Prevention of skin burns by radiotherapy	Topic; EGF 10 mcg/g of SSC/twice a day, for the whole radiotherapy programme	23 patients for EGF cream	POL	Potent radioprotection	Not described
Brown (25)	Healing stimulation of different types of chronic wounds	Topic; EGF 10 mcg/g of SSC until healing	9 patients crossed over to EGF	POL-crossover	Wounds closure in eight patients	†
Falanga (26)	Healing of venous ulcers	Topic; EGF 10 mcg/ml/10 weeks or until healing	17 EGF/18 PL	DBRCT	Greater reduction in ulcer size and larger number of ulcers healed	Follow-up not mentioned. Well tolerated
Borges (27)	Burn wounds healing enhancement	Topic; EGF 10 mcg/g of cream/48 hours until healing	10 pediatric patients for EGF group	Phase II. DBRCT	Wound healing and re-epithelialisation enhancement	Well tolerated
Cohen (28)	Healing of controlled wounds	Topic; EGF 10 mcg/g of SSC/twice daily, no longer than day 21.	17 healthy volunteers/wounds for EGF	DBRCT	No difference in wound healing	Well tolerated
Gonzalez (29)	Healing stimulation of venous ulcers	Topic; EGF 10 mcg/g of cream; thrice a week / 6 weeks	40 patients for EGF creams	DBRCT	Wound healing enhancement by EGF	Well tolerated
Rodriguez (30)	Acne progress control and scars amelioration	Topic; EGF 10 mcg/g of cream/24 hours for 6 weeks	30 patients for EGF	DBRCT	Acne control and scars attenuation	Well tolerated
Tsang (18)	Healing of diabetic foot ulcers	Topic; EGF 0·02–0·04% for 12 weeks	21 per EGF concentration group	DBRCT	Enhancement of healing and healing time reduction	Follow-up–6 months. Well tolerated

Table 1 (*Continued*)

Author/Ref.	Indication	Route/dose and duration	Number of patients	Study	Outcome	Safety
Hong (19)	Healing of diabetic foot ulcers	Topic; EGF 0·05% + dressing until healing	68 patients crossed over to EGF	POL-Crossover	Enhanced healing of neuropathic foot ulcer	Follow-up–6 months. Well tolerated
Viswanathan (31)	Efficacy and safety of EGF gel in patients with Grade I or II DFU	Topic; EGF 150 mcg/g up to 16 weeks*	30 EGF / 30 PL	Phase III-DBRCT	Enhancement of healing and healing time reduction	Well tolerated. Follow-up for 2 years
Berlanga (32)	Efficacy of EGF local infiltrations in terminal DFU	Local EGF (intralesionally) injected at 25–125 mcg/ulcer, thrice a week up to 8 weeks	29 patients for EGF	POL	Stimulation of ulcer granulation	Well tolerated. 1-year follow-up
Fdez-Montequin (33)	Efficacy of EGF local infiltrations in terminal DFU	IDEM. injected at 25 or 75 mcg/ulcer, thrice a week up to 8 weeks	41 patients for either EGF dose	Phase II. DBR-dose controlled	Stimulation of ulcer granulation	Well tolerated. 1-year follow up
Mohan (34)	Healing rate, reduction of healing time	Topic; EGF 150 mcg/g for 15 weeks or total healing	135 only for EGF	Phase IV (PMS)	Enhancement and speeding for the healing process of DFU	Well tolerated
Tabrizi (35)	Reduction of healing time for PV lesions	Topic; EGF 10 mcg/in SSC until healing	20 patients. Controlled within patient, left/right	DBRCT	Significant reduction in healing time	Well tolerated
Betancourt (36)	Efficacy of EGF local infiltrations for ulcer healing	Injected into the ulcer 75 mcg, thrice a week up to re-epithelialisation	20 patients	POL	Stimulation of ulcer granulation and re-epithelialisation	Well tolerated

*Treatment was prolonged even if wounds healed earlier to check for adverse effects.

†Although safety data are not explicitly described, it can be inferred that patients were followed for years when the authors described ulcers recurrence in a period between 1 and 4 years.

EGF, epidermal growth factor; DBRCT, double-blind randomised-controlled trial; POL, prospective open label; PL, placebo; Mcg, microgram; PV, pemphigus vulgaris; PMS, post-marketing surveillance study; DFU, diabetic foot ulcers; SS, silver sulphadiazine cream.

Table 2 EGF for the gastrointestinal system. Clinical studies and case reports

Author/Ref.	Indication	Dose, duration, route	No. of patients	Study	Outcome	Safety
Elder (37)	Research study in HV and ZES	EGF 0·25 μg/kg/h for 1 hour IV	4 ZES patients and 4 normal subjects	POL	Reduction in gastric hypersecretion. Ulcer pain relieved	Well tolerated
Koffman (38)	Research study	Daily infusions of 1 hour. EGF 0·25 μg/kg/h for 5 days IV	5 duodenal ulcer patients	POL	EGF modifies gastric acid secretion	Not described
Walker-Smith (39)	Microvillous atrophy	EGF 100 ng/kg/h for two 6-day periods IV	1 pediatric patient with microvillous atrophy	CR	EGF-induced crypt cells proliferation	Well tolerated
Drumm (40)	Microvillous atrophy	EGF 100 ng/kg/h for 21 days. IV and enteral	2 pediatric patients	CR	EGF-stimulated intestinal cells mitosis	Well tolerated
Sullivan (41)	NEC	EGF 100 ng/kg/h for 6 days IV	1 pediatric patient	CR	EGF-stimulated intestinal cells mitosis	Well tolerated
Itoh (42)	Gastric ulcer healing	IV 6 mcg/patient, twice a week for 8 weeks	86 patients for EGF	DBRCT	Enhanced ulcer healing	Well tolerated
Haedo (43)	Duodenal ulcer healing	Oral. EGF at 450 or 600 mg/day for 6 weeks	47 patients for EGF	DBRCT	EGF treatment shortened healing time	Well tolerated
Palomino (44)	Stimulation of duodenal ulcer healing	Oral. 450 or 2250 mcg/day/6 weeks or until complete healing	68 patients for EGF	DBRCT	EGF-stimulated ulcer healing in a dose–response manner	Well tolerated
Sinha (45)	UC healing by EGF	Daily rectal enemas. EGF 5 mcg/in 100 ml of carrier for 14 days	14 patients	DBRCT	EGF enemas are an effective treatment for active UC	Well tolerated
Sigalet (46)	Intestinal physiology improvement	Oral. 100 mcg/kg/day for 6 weeks	5 pediatric patients	POL	EGF improved different parameters	Well tolerated
Sullivan (47)	Trophic effect on the GITM	IV EGF continuous infusion at 100 ng/kg/h for 6 days	8 neonates	PRDB	Enhancement of mucosal remodeling and trophism	Well tolerated

EGF, epidermal growth factor; DBRCT, double-blind randomised-controlled trial; POL, prospective open label; CR, case report; PRDB, prospective randomised, double blind; Mcg, microgram; ZES, Zollinger–Ellison syndrome; HV, healthy volunteers; UC, ulcerative colitis; mg, milligram; IV, intravenous, GITM, gastrointestinal tract mucosa; NEC, necrotising enterocolitis.

events that at the gross expression may be summarised as: (i) stimulation of productive cells migration toward and homing within the injured area, (ii) stimulation of granulation tissue outgrowth–including extracellular matrix accumulation, maturation and *de novo* angiogenesis, (iii) stimulation of wound contraction by stimulating myofibroblast activation and proliferation and (iv) stimulation of the damaged area resurfacing by epithelial cells migration and proliferation (for excellent reviews, see references (59,60))

CLINICAL INDICATIONS AND OUTCOMES

The mechanisms whereby EGF and other growth factors stimulate tissue repair are the same ones involved in tumours development. Cell proliferation, survival, *de novo* angiogenesis and cell migration are common critical processes (61). An early concern grew associated with the fact that some growth factors were identified in the conditioned culture medium of different cancer cell lines or experimentally transformed cells (62). It was also concerning the homology between some growth factors receptor-coding genes and viral oncogenes such as EGF receptor and v-erb (4). In spite of all these laboratory findings, rhEGF was subjected to a blinded-controlled clinical evaluation in the USA as early as 1989 in the area of peripheral tissue repair (23). Afterwards, EGF interventions have been addressed to treat a variety of peripheral wounds from chronic venous ulcers to *Pemphigus vulgaris* lesions, the later published 2 years ago (see Table 1, including references (23–36)). Two issues must be highlighted: (i) EGF treatments have been well tolerated (including the example of the intra-ulcer local infiltration) and (ii) some of these studies have introduced follow-up periods of 6–12 months up to 4 years; the later representing an appropriate biological window for an EGF-mediated long-term adverse reactions.

Table 2 (including references (37–47)) focuses on case reports and clinical studies in which EGF has been aimed to enhance the restoration of the gastrointestinal tissue following diverse luminal and transmural damages. These lesions exhibit different pathophysiological mechanisms and thus different clinical evolution and prognosis. EGF has been locally or systemically administered at different doses, administration schedules and through varied pharmaceutical compositions. There has been no evidence that any of these therapeutic interventions has led to long-term adverse events. However, the number of clinical interventions, patients included, and follow-up periods described fall short to achieve definitive safety conclusions. It is notorious that the first systemic administration of EGF dates back to 1975 in patients with Zollinger–Ellison syndrome. Obviously, this work and others appear worthless in terms of long-term safety as the EGF exposure time appears negligible (37). The most recent clinical trial involving EGF systemic administration was conducted in the UK by Sullivan and co-workers in eight critical neonates affected by necrotising enterocolitis (see Table 2, reference (47)). Systemically administered EGF exhibits a broad pharmacological spectrum potentially useful for threatening clinical processes of the gastrointestinal tract (acute ischemia, intestinal barrier failure, necrotising enterocolitis, short bowel syndrome, etc.), which still remain as orphan or with limited therapeutic options (63,64). Recent clinical experiences confirm wisdom anticipations in that EGF could be the remedy for a variety of gastrointestinal mucosal damages demanding replenishment, cytoprotection, phenotypic maturation and re-adaptation, cell migration and proliferation (47). In addition to have been extensively used about two decades ago in the forms of eye and ear drops, other epithelial context of inflammation and ulceration as ulcerative colitis or chemotherapy-induced oral mucositis has been treated with EGF (65). Incontrollable haemorrhagic cystitis because of leukaemia chemotherapy was successfully treated since the fourth day of EGF continuous intrabladder irrigation through a suprapubic catheter. The patient was followed for 5 months with no recurrence neither adverse side effects (66).

Convincing experimental data indicate that EGF plays an important role in cancer development but not as an 'initiating' agent. Classic experiments established that EGF promotes chemical- and viral-induced cancer growth (for review, see reference (67)). These findings hardly reach clinical translation because of the existence of multiple endogenous mechanisms that protect normal cells against a non programmed mitogenic event. In many, if not all human epithelial tumours, EGF receptor is amplified or overexpressed and its signalling

Key Points

- the mechanisms whereby EGF and other growth factors stimulate tissue repair are the same ones involved in tumours development
- recent clinical experiences confirm wisdom anticipations in that EGF could be the remedy for a variety of gastrointestinal mucosal damages demanding eplenishment, cytoprotection, phenotypic maturation and readaptation,cell migration and proliferation

Key Points

- before EGF or another peptide growth factor administration, a careful patient selection should be performed based on the personal and family background and on the basis of the risk/benefit balance
- 'the capacity of cancer cells to produce their own growth factors and to respond to them (autocrine secretion) has become a central concept, which links the oncogene to the growth factor

system deregulated with a 'gain-of-function' profile (68). It becomes obvious that in this context, any therapy with EGF or any other growth factor is absolutely contraindicated. Therefore, before EGF or another peptide growth factor administration, a careful patient selection should be performed based on the personal and family background and on the basis of the risk/benefit balance (41,69). In fact, this rule is ordinarily applied in clinical practice for many approved drugs, including hormones, mutagens and mitogens.

PRECLINICAL TOXICOLOGY AND SAFETY DATA. LONG-TERM EXPOSURE TO EGF IS NOT SUFFICIENT TO INITIATE MALIGNANT TRANSFORMATION

There is an extensive battery of preclinical studies documenting that EGF does not induce genotoxicity, mutagenicity or cytotoxicity (70). Several experiments have shown that EGF acts as a potent cytoprotective agent in the treatment of mucositis associated with cancer chemotherapy (71) and protects against rectal cancer induction in azoxymethane-exposed rats (72). These results are not surprising in the light of classic experiments that showed the EGF-mediated cytoprotective effect (73). In animals with intestinal mucosa atrophy and exposed to repeated co-administrations of EGF and KGF, the intestinal tissue was rapidly repaired by a synergic mitogenic co-stimulation of the two growth factors, without evidence of abnormal growth or malignant or pre-malignant transformation (74). All these evidences, however, do not exclude that the proliferative and anti-apoptotic effects of EGF could contribute to enrich the population of genetically damaged cells.

EGF inhibited the growth of cancer cell lines of epithelial origin (75,76). In line with the above findings, it was recently shown that EGF failed to enhance *in vitro* proliferation of three human gastric adenocarinoma lines, as had no effect on tumour growth when these cells were implanted in nude mice. Surprisingly, for both the *in vitro* and *in vivo* approaches, the experimental protocols and the EGF doses have been largely used and known to facilitate proliferation and tumourigenesis (77). Long-term exposure of *Min* mice to EGF, as a more idiopathic and non manipulated precancer model, did not stimulate the appearance of more polyps nor their transformation to malignancy (78).

As shown in Table 3, numerous preclinical studies based on long-term systemic administration of EGF at supra-physiological concentrations concluded that EGF induces epithelial hyperplasia that appeared to depend on the dose and length of the exposure time (references (79–88)). The excessive epithelial growth was reversible upon treatment withdrawal. Interestingly, no histological evidences of dysplasia and/or anaplasia were detected in these tissue populations committed to proliferate. Table 3 also includes the findings of carcinogenesis studies in mice, rats and hamsters in which EGF was administered with well-known chemical mutagenic carcinogens. The promoter effect of EGF was not reproducibly shown in every case, which may depend upon the particular characteristics of the experimental system (89–92).

A number of *in vivo* mechanisms take control of the cells response to a mitogenic impulse. These include EGF bioavailability and peptidases processing in the pericellular milieu, EGF receptor down-regulation, and the protein kinase C(PKC)-mediated counter regulation of the tyrosine kinase activity through phosphorylation of serine and threonine residues (93). In another scenario, classic pharmacokinetic studies have shown that following its parenteral administration, EGF is rapidly cleared from the central bloodstream, rapidly biodistributed and eliminated through the kidneys (94). A recent study with the cognate HB-EGF showed the similarity with EGF in terms of no-toxicity, no-accumulation and rapid clearance (95).

It seems that a constant constitutive ligand secretion that allowed for an autocrine loop with the receptor is more important than exogenous growth factor administration to transform normal cells, as shown 20 years ago in fibroblasts induced to secrete EGF (96). The essence of all these findings was wisely anticipated by Anita Roberts and Michael Sporn: 'The capacity of cancer cells to produce their own growth factors and to respond to them (autocrine secretion) has become a central concept, which links the oncogene to the growth factor. Oncogenes confer cells autonomy of growth factors, not only by direct codification of autocrine peptidic growth factors or their receptors, but also by

Table 3 EGF preclinical safety studies

Study goal	Reference	Animal sp.	Dose / route	Findings
Broad toxicological evaluation of EGF	70	Rats, mice, monkeys	Dose scale up to 3 mg/kg for 4 weeks. IV/SC	Hyperplasia and hypertrophy of most epithelial and mesenchymal structures of internal organs and the skin. Cellular growth and proliferation were not associated with altered differentiation
Effect of chronic administration of EGF especially at the urinary tract	79	Minipigs	EGF 30 μg/kg/day for 4 weeks SC	An EGF-mediated growth effect was observed in ureters, kidneys and heart. A milder enlargement was also found in pancreas, esophagus, salivary glands and lungs. Pre-malignant or malignant lesions were not detected
Impact of chronic treatment with EGF on the esophageal epithelia in pigs and rats	80	Minipigs rats	EGF 30 and 150 μg/kg/day / 4 weeks SC	Significant thickening of the esophageal mucosa in both species, without the occurrence of changes in the normal pattern of cellular differentiation
Impact of chronic systemic treatment with EGF in the mucosal surface of the small intestine	81	Rats	EGF 150 μg/kg for 4 weeks SC	EGF treatment for 1–4 weeks caused a time-dependent increase in intestinal weight. The growth was characterised by increased wall thickness, increased cross-sectional area and reduced wall stiffness
Impact of chronic treatment with EGF in the growth of the urinary tract	82	Rats	EGF 150 μg/kg for 4 weeks SC	Ureters enlargement and urothelial hypercellularity not associated to changes in the epithelial differentiation pattern according to lectin histochemistry
Effect of prolonged systemic treatment with EGF in the luminal surface and colonic mass	83	Rats	EGF 150 μg/kg for 4 weeks SC	Systemic treatment with EGF for 1 week increased the luminal surface area relatively more than the mass of the colon. Treatment for more than 1 week caused a colonic mass increase in a time-dependent manner. No malignant changes are reported
Effects of long-term EGF treatment on the normal rat colon	84	Rats	EGF 150 μg/kg for 4 weeks SC	EGF has a stimulating role on the mucosa and luminal surface area of the entire functioning colon and a trophic effect on the submucosa of the distal colon
Effects of chronic administration of EGF on the levels of endogenous EGF	85	Rats	EGF at 150 μg/kg for 4 weeks SC	Chronic systemic treatment with EGF causes growth of the SMG with concomitantly reduced contents of EGF, and growth of the kidneys with unchanged content and excretion of EGF
Effects of chronic treatment with EGF in the urinary tract	86	Minipigs	EGF at 30 μg/kg/day × 4 weeks SC	Treatment with EGF induces the growth of all wall layers in the urinary tract with remarkable hyperplastic and hypertrophic changes of the smooth muscle cells in the muscular coat

Table 3 (*Continued*)

Study goal	Reference	Animal sp.	Dose / route	Findings
Impact of chronic systemic treatment with EGF induces gastric physiological changes	87	Minipigs	EGF at 30 mg/kg/day for 4 weeks SC	EGF-induced increase in serum gastrin, increased the number of antral G-cells, and decreased the density of antral D-cells. The acidity of gastric fluid was reduced, and the protein concentration increased
Effect of EGF systemic treatment in pancreatic ductal proliferation	88	Minipigs	EGF at 30 mg/kg/day for 5 weeks. Later 3 weeks of recovery SC	Interlobular ducts appeared hyperplastic, with increased number of PCNA positive cells. The epithelia of these ducts were increased in height, with accumulations of glycoconjugates in the columnar cells and in an increased number of goblet cells
Effect of EGF in polyps formation and its progression to cancer in MIN mice.	78	MIN mice	EGF by osmotic mini-pumps (6·7 μg/day) for 28 days	EGF did not stimulate the appearance of polyps nor its transformation to malignant tumours
The effects of intra-colonic EGF on mucosal growth and carcinogenesis	72	Mice	EGF (12 nM) in saline solution, rectal, 24 weeks	Showed that the intra-colonic treatment with EGF did not strengthen the carcinogenic effect of the chemical azoxymethane. EGF does not promote colonic carcinogenesis in this model
Effect of EGF on experimental ulcerative mucositis by cancer chemotherapy.	71	Hamster	EGF 0·5 μl/h × 2 weeks	EGF leads to sensitise tumour cells under the effects of anti-neoplastic agents
Effect of EGF on rat stomach chemical carcinogenesis	89	Rats	EGF 10μg/ml/day x 30 weeks with carcinogen MNNG	The findings suggest a possible enhancing effect of EGF on stomach carcinogenesis in rats
Effect of EGF on the development of salivary gland carcinomas	90	Mice	EGF 2 μg/ml/day × 8 weeks with carcinogen DMBA	EGF does not promote tumour induction in mouse salivary gland carcinogenesis assay
Effect of EGF administration on chemically induced tumours	91	Hamster	EGF + DMBA × 6weeks	Results suggest that EGF applied from the luminal side of the mucosa stimulates tumour formation in the hamster cheek pouch and forestomach
Effect of EGF in pancreatic and bronchial cancer when administered with a chemical carcinogen	92	Hamsters	EGF (5 μg) three injections/week × 3weeks + 19 weeks of N-nitroso-bis(2-oxopropyl)amine	EGF increased the incidence of animals with bronchial cancer doubled. Suggested as a cocarcinogen as a result of its mitogenic activity

EGF, epidermal growth factor; mg, milligram; mcg, microgram; IV, intravenous; SC, subcutaneous; SMG, submandibular gland; MNNG, N-methyl-N′-nitro-N-nitro-soguanidine; DMBA, dimethyl-1,2-benzanthracene.

amplification of mitogenic signals generated by a growth factor or its receptor' (97).

In genetically engineered animal models, malignant transformation is not *a sine qua non* after EGF undue exposure. Tönjes and co-workers (98) showed that hepatocarcinogenesis appears in transgenic mice that express a genetic construction (Alb-DS4) which codifies for EGF in the liver. This was the first *in vivo* demonstration of the transforming potential of EGF in terms of initiation (if the term is applicable for transgenics). In 1999, another transgenic mouse for EGF was reported over-expressing the growth factor in the small intestine of rats. The effects of the transgene translated into a local salutary effect: trophic and pro-adaptogenic for the insulted mucosa, supporting the existence of a direct autocrine/paracrine effect of EGF on the enterocytes (99). A recent transgenic mouse rendered contradictory results. The animals did not develop malignant or pre-malignant lesions but exhibited a remarked delay in their somatic growth as main phenotypic change. None of the well-known neoplastic changes reported in the mouse over-expressing TGF-α were detected in spite of the local over-expression of EGF in sensitive organs (100). Another study performed with the construction of IgEGF effectively reproduced the consequences of an altered EGF signalling by its receptor (101), as previously described by Tönje et al. (98). The mice again developed hepatocarcinomas. The general interpretation of the EGF murine transgenic model as for other EGF receptor ligands is that EGF or TGF-α over-expression seems necessary but not sufficient to induce carcinogenesis in mice. Nevertheless their role in promoting multistage carcinogenesis in conjunction with accumulated mutations has been shown (102). The interaction between cooperative forces to transform cells has been detected via molecular and epidemiological studies on the incidence of cancer in relation to age. From four to six genetic events are necessary for tumour development, whereas the genetic instability is necessary for an individual cell to develop these alterations (103,104).

The model of multistage murine skin carcinogenesis continues to be fundamental for the *in vivo* study of cancer (105) so that successively generated double transgenics for oncogenes and growth factors have confirmed the importance of 'hits accumulation' cooperatively acting to transform cells (106,107). Colon is another niche where cancer is frequent, EGF receptor is overexpressed and its ligands are known to fuel up the tumour (108). The expected tumours as a consequence of the TGF-α transgene over-expression were not observed in the organ. On the contrary, mice colonic mucosa appeared to be protected against the effect of ulcerogenic and necrogenic chemicals (109). Amidst this plethora of conflicting evidences, the only possible conclusion is that undue exposure to this growth factor and likely others do not irrevocably transform cells.

MARJOLIN ULCERS INCIDENCE DOES NOT APPEAR INCREASED FOLLOWING GROWTH FACTORS INTERVENTIONS

Healing wounds and progressing tumours are dynamic niches of biological interaction among different cell lineages that share numerous biological features (110). For both, cell proliferation, survival, *de novo* angiogenesis and migration are operational forces controlled by locally secreted growth factors (61). The relationship between malignant transformation and wounds was initially established by the French surgeon Jean-Nicholas Marjolin (111). In general terms, the MU is a well-known, not rare and particularly aggressive cutaneous tumour that appears in a previously injured and protractedly inflamed skin, especially after burn injuries and chronic wounds (112). The role of local wounding for the development and progression of tumours was showed and the concept that prolonged exposure to 'wound signals' at the tumour site causes cancer progression was no longer a speculation. Growth factors such as transforming growth factor-beta and fibroblast growth factors had the ability to replace wounding to enhance tumour development (113).

In spite of the ancestral and well-characterised association between chronic wounds and squamous carcinomas, and the well-documented participation of most growth factors in tumour promotion, the historical evolution of the clinical evidences points out that the topical administration of growth factors to stimulate the repair process of either acute or chronic wounds has been salutary, safe and has not increased the onset of MUs. This fact confirms that these wound-derived

Key Points

- for both, cell proliferation, survival, *de novo* angiogenesis and migration are operational forces controlled by locally secreted growth factors
- growth factors such as transforming growth factor-beta and fibroblast growth factors had the ability to replace wounding to enhance tumour development
- in spite of the ancestral and well characterised association between chronic wounds and squamous carcinomas, and the well-documented participation of most growth factors in tumour promotion, the historical evolution of the clinical evidences points out that the topical administration of growth factors to stimulate the repair process of either acute or chronic wounds has been salutary,safe and has not increased the onset of MUs

Key Points

- growth factors therapeutic interventions do not appear to contribute to the malignant transformation of wound bed cells
- classic experiments have shown that EGF promotes but does not initiate tumourigenesis that is clinically meaningful
- experimental and clinical evidences qualify parenterally administered EGF as a first line therapeutic intervention because of its broad spectrum of biologically salutary actions, which concertedly may lead toward full restitution of severely injured tissues and organs
- EGF has therefore the privilege to be reserved for life-threatening conditions (necrotising enterocolitis) or disabling processes (i.e. high-grade diabetic foot ulcers) where no other therapeutic choices are available
- these well-delimited indications for 'nearly orphan conditions' are not in contradiction with ethical considerations and will gauge to benefit when balanced versus individual patient's risk

tumours progress via multiple carcinogenic stimuli in which the local selection, genetic instability and cumulative genetic changes involving suppressor genes and oncogenes are indispensable for a definitive tumourigenesis (114). In conclusion, growth factors therapeutic interventions do not appear to contribute to the malignant transformation of wound bed cells.

We recently conducted a nationwide survey among burned subjects that had received topical EGF cream in a double-blind, randomised, controlled study during 1993–1994. Five hundred patients (<60 years old, <40% body surface burned, superficial and/or deep dermal burns) were randomised to receive 2% silver sulphadiazine cream with (243 patients) or without (257 patients) 10 μg/g EGF up to wound closure or 25 days maximum. After 15 years, 337 individuals (67%) were localised and screened for malignancies and, if deceased, causes of death. Among paediatric patients (152), no tumours had developed at all. Among adults, three patients in the EGF group and one in the control group presented neoplasia (larynx, osteosarcoma, colon and uterus, respectively). These cancer incidence rates 3·3% (95% confidence interval: 0–7%) and 1·1% (0–3·2%) match with the actual ones for Cuban 15–60 years old population during the 15-year period (1·8%) (Anuario Estadistico de Cuba; http://www.one.cu/). Remarkably, none of the burned patients developed Marjolin carcinoma on the injured area.

CONCLUDING REMARKS

Classic experiments have shown that EGF promotes but does not initiate tumourigenesis that is clinically meaningful. Experimental and clinical evidences qualify parenterally administered EGF as a first line therapeutic intervention because of its broad spectrum of biologically salutary actions, which concertedly may lead toward full restitution of severely injured tissues and organs. EGF has therefore the privilege to be reserved for life-threatening conditions (necrotising enterocolitis) or disabling processes (i.e. high-grade diabetic foot ulcers) where no other therapeutic choices are available. These well-delimited indications for 'nearly orphan conditions' are not in contradiction with ethical considerations and will gauge to benefit when balanced versus individual patient's risk.

REFERENCES

1 Cohen S. Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the newborn animal. J Biol Chem 1962;237:1555–62.
2 Scott J, Urdea MS, Quiroga M, Sánchez-Pescador R, Fong N, Selby M, Rutter WJ, Bell GI. Structure of a mouse submaxillary messenger RNA encoding epidermal growth factor and seven related proteins. Science 1983;221:236–40.
3 Bennet N, Schultz G. Growth factors and wound healing: biochemical properties of growth factors and their receptors. Am J Surg 1993;165:728–37.
4 Pusztai L, Lewis CE, Lorenzen J, McGee JOD. Review article-Growth factors: regulation of normal and neoplastic growth. J Pathol 1993;(169): 191–201.
5 Tannock IF, Rotin D. Acidic pH in tumors and its potential for therapeutic exploitation. Cancer Res 1989;49:4373–84.
6 Davis MD, Weed B, Felty CL, Rooke T. Treatment of recalcitrant lower extremity ulcers with topical becaplermin. J Am Acad Dermatol 2004;50:981–2.
7 Embil JM, Papp K, Sibbald G, Tousignant J, Smiell JM, Wong B, Lau CY. Recombinant human platelet-derived growth factor-BB (becaplermin) for healing chronic lower extremity diabetic ulcers: an open-label clinical evaluation of efficacy. Wound Repair Regen 2000;8:162–8.
8 Takehara K. Growth regulation of skin fibroblasts. J Dermatol Sci 2000;24(Suppl 1):S70–7.
9 Mast BA, Schultz GS. Interactions of cytokines, growth factors and proteases in acute and chronic wounds. Wound Repair Regen 1996;4:411–20.
10 Lerman OZ, Galiano RD, Armour M, Levine JP, Gurtner GC. Cellular dysfunction in the diabetic fibroblast. Impairment in migration, vascular endothelial growth factor production, and response to hypoxia. Am J Pathol 2003;162:303–12.
11 Doxey DL, Ng MC, Dill RE, Iacopino AM. Platelet-derived growth factor levels in wounds of diabetic rats. Life Sci 1995;57:1111–23.
12 Werner S, Breeden M, Hubner G, Greenhalgh DG, Longaker MT. Induction of keratinocyte growth factor expression is reduced and delayed during wound healing in the genetically diabetic mouse. J Invest Dermatol 1994;103:469–73.
13 Mansbridge JN, Liu K, Pinney RE, Patch R, Ratcliffe A, Naughton GK. Growth factors secreted by fibroblasts: role in healing diabetic foot ulcers. Diabetes Obes Metab 1999;1:265–79.
14 Portero-Otín M, Pamplona R, Bellmunt MJ, Ruiz MC, Prat J, Salvayre R, Nègre-Salvayre A. Advanced glycation end product precursors impair epidermal growth factor receptor signaling. Diabetes 2002;51:1535–42.
15 Berlanga J, Cibrian D, Guillen I, Freyre F, Alba JS, Lopez-Saura P, Merino N, Aldama A, Quintela AM, Ajamieh H, Urquiza D, Ahmed N, Thornalley P. Methylglyoxal administration induces diabetes-like microvascular changes and perturbs the healing process of cutaneous wounds. Clin Sci (Lond) 2005;109:83–95.

16 Hansen SL, Young DM, Boudreau NJ. HoxD3 expression and collagen synthesis in diabetic fibroblasts. Wound Repair Regen 2003;11:474–80.
17 Steed DL. Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity ulcers. Plast Reconstr Surg 2006;117(suppl 7):143S–9S.
18 Tsang MW, Wong WK, Hung CS, Lai KM, Tang W, Cheung EY, Kam G, Leung L, Chan CW, Chu CM, Lam EK. Human epidermal growth factor enhances healing of diabetic foot ulcers. Diabetes Care 2003;26(6):1856–61.
19 Hong JP, Jung HD, Kim YW. Recombinant human epidermal growth factor (EGF) to enhance healing for diabetic foot ulcers. Ann Plast Surg 2006;56:394–8.
20 Hanft JR, Pollak RA, Barbul A, van Gils C, Kwon PS, Gray SM, Lynch CJ, Semba CP, Breen TJ. Phase I trial on the safety of topical rhVEGF on chronic neuropathic diabetic foot ulcers. J Wound Care 2008;17:30–2, 34–7.
21 Driver VR, Hanft J, Fylling CP, Beriou JM, Autologel Diabetic Foot Ulcer Study Group. A prospective, randomized, controlled trial of autologous platelet-rich plasma gel for the treatment of diabetic foot ulcers. Ostomy Wound Manage 2006;52:68–70.
22 Zaulyanov L, Kirsner RS. A review of a bi-layered living cell treatment (Apligraf) in the treatment of venous leg ulcers and diabetic foot ulcers. Clin Interv Aging 2007;2:93–8.
23 Brown LG, Nanney BL, Griffen J, Cramer AB, Yancey JM, Curtsinger LJ, Holtzin L, Schultz GS, Jurkiewicz MJ, Lynch JB. Enhancement of wound healing by topical treatment with epidermal growth factor. N Engl J Med 1989;321:76–9.
24 Alert J, Rodríguez J, Lombardero J, Pírez R. Acción radioprotectora local del factor de crecimiento epidérmico humano recombinante: reporte preliminar. Interfer Biotecnol 1989;6:62–6.
25 Brown GL, Curtsinger L, Jurkiewicz MJ, Nahai F, Schultz G. Stimulation of healing of chronic wounds by epidermal growth factor. Plast Reconstr Surg 1991;88:189–94.
26 Falanga V, Eaglstein WH, Bucalo B, Katz MH, Harris B, Carson P. Topical use of human recombinant epidermal growth factor (h-EGF) in venous ulcers. J Dermatol Surg Oncol 1992;18:604–6.
27 Borges H, Martínez A, López LD, Ung Lu E, Gonzalez T, Lopez-Saura P. El Factor de Crecimiento Epidérmico humano recombinante acelera la cicatrización de quemaduras en niños. Estudio a doble ciegos. Biotecnol Apl 1994;11:205–9.
28 Cohen IK, Crossland MC, Garrett A, Diegelmann RF. Topical application of epidermal growth factor onto partial-thickness wounds in human volunteers does not enhance reepithelialization. Plast Reconstr Surg 1995;96:251–4.
29 Gonzalez T, Quiñones M, Labarta V, Fernandez M, Charles-Edouard D y Lopez-Saura P. Aplicación tópica de factor de crecimiento epidérmico humano recombinante en ulceras post-flebíticas Biotecnol Apl 1995;12:185–6.
30 Rodríguez J, Fernandez G, Gonzalez T, Castro MD, Hernández F, Díaz A y Lopez-Saura P. Uso del factor de crecimiento epidérmico humano recombinante en el tratamiento del acne Biotecnol Apl 1995;12:186–7.
31 Viswanathan V, Pendsey S, Sekar N, Murthy GSR. A phase III study to evaluate the safety and efficacy of recombinant human epidermal growth factor (REGEN-D^{TM} 150) in healing diabetic foot ulcers. Wounds 2006;18:186–96.
32 Acosta JB, Savigne W, Valdez C, Franco N, Alba JS, del Rio A, López-Saura P, Guillén G, Lopez E, Herrera L, Férnandez-Montequín J. Epidermal growth factor intralesional infiltrations can prevent amputation in patients with advanced diabetic foot wounds. Int Wound J 2006;3:232–9.
33 Fernández-Montequín JI, Infante-Cristiá E, Valenzuela- Silva C, Franco-Pérez N, Savigne-Gutierrez W, Artaza-Sanz H, Morejón-Vega L, González-Benavides C, Eliseo-Musenden O, García-Iglesias E, Berlanga-Acosta J, Silva-Rodríguez R, Betancourt BY, López-Saura PA; Cuban Citoprot-P Study Group. Intralesional injections of Citoprot-P (recombinant human epidermal growth factor) in advanced diabetic foot ulcers with risk of amputation. Int Wound J 2007;4:333–43.
34 Mohan VK. Recombinant human epidermal growth factor (REGEN-D 150): effect on healing of diabetic foot ulcers. Diabetes Res Clin Pract 2007;78:405–11.
35 Tabrizi MN, Chams-Davatchi C, Esmaeeli N, Noormohammadpoor P, Safar F, Etemadzadeh H, Ettehadi HA, Gorouhi F. Accelerating effects of epidermal growth factor on skin lesions of pemphigus vulgaris: a double-blind, randomized, controlled trial. J Eur Acad Dermatol Venereol 2007;21:79–84.
36 Fernández-Montequín JI, Betancourt BY, Leyva-Gonzalez G, Mola EL, Galán-Naranjo K, Ramírez-Navas M, Bermúdez-Rojas S, Rosales F, García-Iglesias E, Berlanga-Acosta J, Silva-Rodriguez R, Garcia-Siverio M, Martinez LH. Intralesional administration of epidermal growth factor-based formulation (Heberprot-P) in chronic diabetic foot ulcer: treatment up to complete wound closure. Int Wound J 2009;6:67–72.
37 Elder JB, Gillespie IE, Ganguli P, Delamore WI, Gregory H. Effect of urogastrone in the Zollinger-Ellison syndrome. Lancet 1975;306:424–5.
38 Koffman GG, Elder JB, Ganguli PC. Effect of urogastrone on gastric secretion and serum gastrin concentrations in patients with duodenal ulcerations. Gut 1982;23:951–6.
39 Walker-Smith JA, Phillips AD, Walford N, Gregory H, Fitzgerald JD, Maccullagh K, Wright NA. Intravenous epidermal growth factor/urogastrone increases small intestinal cells proliferation in the congenital microvillous atrophy. Lancet 1985;326:1239–40.
40 Drumm B, Cutz E, Tomkins KB, Cook D, Hamilton R, Sherman P. Urogastrone/epidermal growth factor in treatment of congenital microvillous atrophy. Lancet 1988;331:111–2.
41 Sullivan PB, Brueton MJ, Tabara ZB, Goodlad RA, Lee CY, Wright NA. Epidermal growth factor in necrotizing enteritis. Lancet 1991;338:53–4.

42 Itoh M, Matsuo Y. Gastric ulcer treatment with intravenous human epidermal growth factor: a double-blind controlled clinical study. J Gastroenterol Hepatol 1994;9(Suppl 1):S78–S83.

43 Haedo W, Gonzalez T, Mas JA, Franco S, Gra B, Soto G, Alonso A, López-Saura P. Oral human recombinant epidermal growth factor in the treatment of patients with duodenal ulcers. Rev Esp Enferm Apar Dig 1996;88:409–13.

44 Palomino A, Hernández-Bernal F, Haedo W, Franco S, Más JA, Fernández JA, Soto G, Alonso A, González T, López-Saura P. A multicenter, randomized, double-blind clinical trial examining the effect of oral human recombinant epidermal growth factor on the healing of duodenal ulcers. Scand J Gastroenterol 2000;35:1016–22.

45 Sinha A, Nightingale J, West KP, Berlanga-Acosta J, Playford RJ. Epidermal growth factor enemas with oral mesalamine for mild-to-moderate left-sided ulcerative colitis or proctitis. N Engl J Med 2003;349:350–7.

46 Sigalet DL, Martin GR, Butzner JD, Buret A, Meddings JB. A pilot study of the use of epidermal growth factor in pediatric short bowel syndrome. J Pediatr Surg 2005;40:763–8.

47 Sullivan PB, Lewindon PJ, Cheng C, Lenehan PF, Kuo BS, Haskins JR, Goodlad RA, Wright NA, de la Iglesia FA. Intestinal mucosa remodeling by recombinant human epidermal growth factor (1-48) in neonates with severe necrotizing enterocolitis. J Pediatr Surg 2007;42:462–9.

48 Yarden Y. The EGFR family and its ligands in human cancer: signaling mechanisms and therapeutic opportunities. Eur J Cancer 2001;37(Suppl 4):3–8.

49 Citri A, Yarden Y. EGF-ERBB signalling: towards the systems level. Nat Rev Mol Cell Biol 2006;7:505–16.

50 Canguilhem B, Pradines A, Baudouin C, Boby C, Lajoie-Mazenc I, Charveron M, Favre G. RhoB protects human keratinocytes from UVB-induced apoptosis through epidermal growth factor receptor signaling. J Biol Chem 2005;280:43257–63.

51 Wang X, McCullough KD, Franke TF, Holbrook NJ. Epidermal growth factor receptor-dependent Akt activation by oxidative stress enhances cell survival. J Biol Chem 2000;275:14624–31.

52 Barrandon Y, Green H. Cell migration is essential for sustained growth of keratinocyte colonies: the roles of transforming growth factor-alpha and epidermal growth factor. Cell 1987;50:1131–7.

53 Wells A. EGF receptor. Int J Biochem Cell Biol 1999;31:637–43.

54 Tokumarua S, Higashiyam S, Endo T, Nakagawa T, Yamamori K, Hanakawa Y, Ohmoto H, Yoshino K, Shirakata Y, Matsuzawa Y, Hashimoto K, Taniguchi N. Ectodomain shedding of epidermal growth factor receptor ligands is required for keratinocyte migration in cutaneous wound healing. J Cell Biol 2000;151:209–20.

55 Pilcher BK, Dumin JA, Sudbeck BD, Krane SM, Welgus HG, Parks WC. The activity of collagenase-1 is required for keratinocyte migration on a type I collagen matrix. J Cell Biol 1997;137:1445–57.

56 Mainiero F, Pepe A, Yeon M, Ren Y, Giancotti FG. The intracellular functions of $\alpha 6\beta 4$ integrin are regulated by EGF. J Cell Biol 1996;134:241–53.

57 Maheshwari G, Wells A, Griffith LG, Lauffenburger DA. Biophysical integration of effects of epidermal growth factor and fibronectin on fibroblast migration. Biophys J 1999;76:2817–23.

58 Wenczack BA, Lynch JB, Nanney LB. Epidermal growth factor receptor distribution in burn wounds. Implications for growth factor-mediated repair. J Clin Invest 1992;90:2392–401.

59 Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiol Rev 2003;83:835–70.

60 Pastore S, Mascia F, Mariani V, Girolomoni G. The epidermal growth factor receptor system in skin repair and inflammation. J Invest Dermatol 2008;128:1365–74.

61 Bissell MJ, Radisky D. Putting tumours in context. Nat Rev Cancer 2001;1:46–54.

62 Anzano MA, Roberts AB, Smith JM, Sporn MB, De Larco JE. Sarcoma growth factor from conditioned medium of virally transformed cells is composed of both type alpha and type beta transforming growth factors. Proc Natl Acad Sci USA 1983;80:6264–8.

63 Berlanga J, Prats P, Remirez D, Gonzalez R, Lopez-Saura P, Aguiar J, Ojeda M, Boyle JJ, Fitzgerald AJ, Playford RJ. Prophylactic use of epidermal growth factor reduces ischemia/reperfusion intestinal damage. Am J Pathology 2002;161:373–9.

64 Warner BW, Erwin CR. Critical roles for EGF receptor signaling during resection-induced intestinal adaptation. J Pediatr Gastroenterol Nutr 2006;43(Suppl 1):S68–S73.

65 Girdler NM. The effect of epidermal growth factor mouthwash on cytotoxic-induced oral ulceration. A phase I clinical trial. Am J Clin Oncol 1995;18:403–6.

66 Dorticós E, Pavon V, Jaime JC, Reboredo M, Lopez-Saura P, Berlanga-Acosta J, Hernandez P. Successful application of epidermal growth factor for the treatment of hemorrhagic cystitis after bone marrow transplantation. Bone Marrow Transplant 2003;31:615–6.

67 Stoscheck CM, King LE. Role of epidermal growth factor in carcinogenesis. Cancer Res 1986;46:1030–37.

68 Arteaga CL. Epidermal growth factor receptor dependence in human tumors: more than just expression? Oncologist 2002;7:31–9.

69 Nair RR, Warner BB, Warner BW. Role of epidermal growth factor and other growth factors in the prevention of necrotizing enterocolitis. Semin Perinatol 2008;32:107–13.

70 Maraschin fnmR, Bussi R, Conz A, Luciana O, Pirovano R, Nyska A. Toxicological evaluation of u-hEGF. Toxicol Pathol 1995;23(3):356–66.

71 Sonis ST, Costa JN, Evitts SM, Lindquist LE, Nicolson M. Effect of epidermal growth factor on ulcerative mucositis in hamsters that receive cancer chemotherapy. Oral Surg Oral Med Oral Pathol 1992;74:749–55.

72 Reeves JR, Richards RC, Cooke T. The effects of intracolonic EGF on mucosal growth and experimental carcinogenesis. Br J Cancer 1991;63:223–6.
73 Brzozowski T, Kont PC, Kont SJ, Konturek P. Mucosal irritant, adaptive cytoprotection, and adaptation to topical ammonia in the rat stomach. Scand J Gastroenterol 1996;31:837–46.
74 Sasaki M, Fitzgerald AJ, Mandir M, Berlanga-Acosta J, Goodlad RA. Keratinocyte growth factor and epidermal growth factor can reverse the intestinal atrophy associated with elemental diets in mice. Exp Physiol 2003;88:261–7.
75 Knowles AF, Salas Prato M, Villela J. Epidermal growth factor inhibits growth while increasing the expression of an ecto-calcium-ATPase of a human hepatoma cell line. Biochem Biophys Res Commun 1985;126:8–14.
76 Barnes DW. Epidermal growth factor inhibits growth of A431 human epidermoid carcinoma in serum-free culture. J Cell Biol 1982;93:1–4.
77 Xia L, Yuan YZ, Xu ChD, Zhang YP, Qiao MM, Xu JX. Effects of epidermal growth factor on the growth of human gastric cancer cell and the implanted tumor of nude mice. World J Gastroenterol 2002;8:455–8.
78 Bashir O, Fitzgerald AJ, Mandir M, Berlanga-Acosta J, Playford RJ, Goodlad RA. Effect of epidermal growth factor administration on intestinal cell proliferation, crypt fission and polyp formation in multiple intestinal neoplasia (Min) mice. Clin Sci 2003;105:323–30.
79 Vinter-Jensen L, Juhl CO, Poulsen SS, Djurhuus JC, Dajani EZ, Nexo E. Chronic administration of epidermal growth factor to pigs induce growth especially of the urinary tract with accumulation of epithelial glycoconjugates. Lab Invest 1995;73:788–93.
80 Juhl CO, Vinter-Jensen L, Poulsen SS, Orntoft RF, Dajani EZ. Chronic treatment with epidermal growth factor causes esophageal epithelial hyperplasia in pigs and rats. Dig Dis Sci 1995;40:2717–23.
81 Vinter-Jensen L, Smerup M, Kissmeyer-Nielsen P, Poulsen SS. Chronic systemic treatment with epidermal growth factor in the rat increases the mucosal surface of the small intestine. Regul Pept 1995;60:117–24.
82 Vinter-Jensen L, Smerup M, Jorgensen PE, Juhl CO, Orntoft T, Poulsen SS, Nexo E. Chronic treatment with epidermal growth factor stimulates growth of the urinary tract in the rat. Urol Res 1996;24:1521–5.
83 Kissmeyer-Nielsen P, Vinter-Jensen L. Time-dependent changes in the luminal surface and mass of the rat colon during prolonged systemic treatment with epidermal growth factor. Scand J Gastroenterol 2000;35:300–5.
84 Kissmeyer-Nielsen P, Vinter-Jensen L, Smerup M. Effects of longterm epidermal growth factor treatment on the normal rat colon. Gut 1996;38:582–6.
85 Vinter-Jensen L, Jorgensen PE, Poulsen SS, Nexo E. The effects of chronic administration of epidermal growth factor (EGF) to rats on the levels of endogenous EGF in the submandibular glands and kidneys. Regul Pept 1996;67:179–85.
86 Vinter-Jensen L, Juhl CO, Dajani EZ, Nielsen K, Djurhuus JC. Chronic systemic treatment with epidermal growth factor induces smooth muscle cell hyperplasia and hypertrophy in the urinary tract of mature Goettingen minipigs. Br J Urol 1997;79:532–8.
87 Vinter-Jensen L, Juhl CO, Eika B, Gregersen H, Dajani EZ. Chronic systemic treatment with epidermal growth factor induces hypergastrinaemia in Goettingen minipigs. Scand J Gastroenterol 1995;30:422–7.
88 Vinter-Jensen L, Juhl CO, Teglbjaerg PS, Poulsen SS, Dajani EZ, Nexo E. Systemic treatment with epidermal growth factor in pigs induces ductal proliferations in the pancreas. Gastroenterology 1997;113:1367–74.
89 Yasui W, Takekura N, Kameda T, Oda N, Ito M, Ito H, Tahara E. Effect of epidermal growth factor on rat stomach carcinogenesis induced by N-methyl-N′-nitro-N-nitrosoguanidine. Acta Pathol Jpn 1990;40:165–71.
90 Tsujimoto H, Yura Y, Yoshioka Y, Kusaka J, Yoshida H, Sato M. Effect of epidermal growth factor administration on the development of mouse salivary gland carcinomas. J Oral Pathol Med 1999;28:30–6.
91 Harada K, Shiota G, Kawasaki H. Effect of local administration of epidermal growth factor on 9,10-dimethyl-1,2-benzanthracene-induced tumour formation in hamster cheek pouch. Eur J Cancer B Oral Oncol 1995;31:27–31.
92 Malt RA, Chester JF, Gaissert HA, Ross JS. Augmentation of chemically induced pancreatic and bronchial cancers by epidermal growth factor. Gut 1987;28(Suppl):):249–51.
93 Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. Cell 1990;61:203–12.
94 Kuo BS, Kusmik W, Pole J, Elsea S, Chang J, Hwang KK. Pharmacokinetic evaluation of two human epidermal growth factors (hEGF51 and hEGF53) in rats. Drug Metab Dispos 1992;20:23–30.
95 Coowanitwong I, Keay SK, Natarajan K, Garimella TS, Mason CW, Grkovic D, Bauer KS. Toxicokinetic study of recombinant human heparin-binding epidermal growth factor-like growth factor (rhHB-EGF) in female Sprague Dawley rats. Pharm Res 2008;25:542–50.
96 Stern DF, Hare DL, Cecchini MA, Weinberg RA. Construction of a novel oncogene based on synthetic sequences encoding epidermal growth factor. Science 1987;235:321–5.
97 Sporn MB, Roberts AB. Autocrine growth factors and cancer. Nature 1985;313:745–7.
98 Tönjes RR, Löhler J, O'Sullivan JF, Kay GF, Schmidt GH, Dalemans W, Pavirani A, Paul D. Autocrine mitogen IgEGF cooperates with c-myc or with the Hcs locus during hepatocarcinogenesis in transgenic mice. Oncogene 1995;10:765–8.
99 Erwin CR, Helmrath MA, Shin CE, Falcone RA Jr, Stern LE, Warner BW. Intestinal overexpression of EGF in transgenic mice enhances adaptation after small bowel resection Am J Physiol 1999;277(3 pt 1):G533–40.

100 Chan SY, Wing-Chuen RW. Expression of epidermal growth factor in transgenic mice causes growth retardation. J Biol Chem 2000;275:38693–8.
101 Borlak J, Meier T, Halter R, Spanel R, Spanel-Borowski K. Epidermal growth factor-induced hepatocellular carcinomas: gene expression profiles in precursor lesions, early stage and solitary tumors. Oncogene 2005;24:1809–19.
102 Giraud AS. Lessons from genetically engineered animal models X. Trefoil peptide and EGF receptor/ligand transgenic mice. Am J Physiol Gastrointest Liver Physiol 2000;278:G501–6.
103 Loeb LA. Mutator phenotype may be required for multistage carcinogenesis. Cancer Res 1991;51:3075–9.
104 Owens DM, Caroline Wei SJ, Smart RC. Carcinogenesis: A multihit, multistage model of chemical carcinogenesis. Carcinogenesis 1999;9:1837–44.
105 Chan KS, Carbajal S, Kiguchi K, Clifford J, Sano S, DiGiovanni J. epidermal growth factor receptor-mediated activation of Stat3 during multistage skin carcinogenesis. Cancer Res 2004;64: 2382–9.
106 Wang XJ, Liefer KM, Greenhalgh DA, Roop DR. 12-O-tetradecanoylphorbol-13-acetate promotion of transgenic mouse epidermis coexpressing transforming growth factor-alpha and v-fos: acceleration of autonomous papilloma formation and malignant conversion via c-Ha-ras activation. Mol Carcinog 1999;26:305–11.
107 Greenhalgh DA, Wang XJ, Roop DR. Multistage epidermal carcinogenesis in transgenic mice: cooperativity and paradox. J Investig Dermatol Symp Proc 1996;1:162–76.
108 Kuwai T, Nakamura T, Sasaki T, Kitadai Y, Kim JS, Langley RR, Fan D, Wang X, Do KA, Kim SJ, Fidler IJ. Targeting the EGFR, VEGFR, and PDGFR on colon cancer cells and stromal cells is required for therapy. Clin Exp Metastasis 2008;25:477–89.
109 Egger B, Carey HV, Procaccino F, Chai NN, Sandgren EP, Lakshmanan J, Buslon VS, French SW, Buchler MW, Eysselein VE. Reduced susceptibility of mice overexpressing transforming growth factor to dextran sodium sulfate induced colitis. Gut 1998;43:64–70.
110 Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 1986;315:1650–9.
111 Kowal-Vern A and Criswell BK. Burn scar neoplasms: A literature review and statistical analysis. Burns 2005;31:403–13.
112 Sabin SR, Goldstein G, Rosenthal HG, Haynes KK. Aggressive squamous cell carcinoma originating as a Marjolin's ulcer. Dermatol Surg 2004;30(2 Pt 1):229–30.
113 Martins-Green M, Boudreau N, Bissell MJ. Inflammation is responsible for the development of wound-induced tumors in chickens infected with Rous sarcoma virus. Cancer Res 1994;54: 4334–41.
114 Bartle EJ, Sun JH, Wang XW, Schneider BK. Cancers arising from burn scars. A literature review and report of twenty-one cases. J Burn Care Rehabil 1990;11:46–9.