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Considerations on the transforming potential of the epidermal growth factor

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ABSTRACT

The epidermal growth factor (EGF) is a potent mitogen for a variety of epithelial and mesenchyma derived cells. Cells treated with EGF exhibit transient metabolic changes and a morphologic phenotype which mirror those found in malignant counterparts. EGF binds to a specific membrane receptor with intrinsic tyrosine kinase activity coded by a cellular proto-oncogene, whose mutated form has been largely implicated in malignancy. Experimental evidence indicates that EGF potentiates chemical carcinogenesis *in vivo* as well as viral transformation *in vitro*. Thus EGF has been historically considered as a tumor promoter. Nevertheless, recent experimental findings, mostly derived from preclinical models, strongly suggest that EGF treatment is insufficient to initiate benign or malignant transformation. On the contrary, in certain scenarios EGF may act as a potent cytoprotective agent for normal cells, and sensitizes cancer cells to anti-tumor therapy. All these experimental data provide fundamental basis for the clinical use of EGF in terms of safety. The clinical experience accumulated so far suggests that EGF does not seem to initiate carcinogenesis.

Key words: EGF, carcinogenesis, malignancy, toxicity

RESUMEN

El factor de crecimiento epidérmico (FCE) es un potente mitógeno para células epiteliales y de origen mesenquimatoso. Las células tratadas con FCE muestran cambios metabólicos y morfológicos de carácter transitorio que son comparables con aquellos encontrados en células malignas. La respuesta celular al FCE está condicionada a la interacción con un receptor específico de membrana, modelo de actividad tirosina-cinasa, codificado por un proto-oncogen celular y cuya forma mutada ha sido vista asociada a la transformación maligna. Algunas evidencias experimentales han demostrado que el FCE potencializa el efecto transformante de carcinógenos químicos y virus oncogénicos, por lo que ha sido considerado como un agente promoter de la carcinogénesis. No obstante, hallazgos recientes, mayormente obtenidos en modelos animales,

sugieren que el tratamiento a largo plazo con FCE es insuficiente para iniciar eventos premalignos o malignos. Contrariamente, el FCE ha mostrado ser un potente factor citoprotector para células normales, que además sensibiliza las células malignas al tratamiento con agentes antitumorales. Los datos aquí revisados son argumento para el uso clínico del FCE en términos de seguridad toxicológica. El seguimiento a largo plazo de pacientes que han recibido FCE, evidencia que este factor de crecimiento no parece ser un agente iniciador de carcinogénesis.

Palabras claves: FCE, carcinogénesis, malignización, toxicidad

Introduction

The epidermal growth factor (EGF), is a 53-amino acid polypeptide that was originally isolated from the mouse submandibular glands as a concomitant of the nerve growth factor and recognized by its ability to stimulate precocious incisor eruption and eyelid opening in newborn mice (1). EGF is synthesized in a variety of tissues in mammals as a large precursor (prepro-EGF) of 1217 amino acids, which includes at least seven EGF-like sequences (2).

Mature EGF binds to a specific cellular receptor exerting a potent mitogenic activity on most epithelial tissues, fibroblasts, and endothelial cells (3). Besides, profound biochemical events are induced upon EGF-receptor interaction including protein phosphorylation, diacyl glycerol, inositol phosphate, prostaglandin and cyclic nucleotide generation, as alterations in intracellular ion concentrations. These events bring about cytoskeletal and morphological changes, alterations in the metabolic pathways of glucose and protein synthesis, which eventually culminate in cell-cycle progression (4). These EGF-induced metabolic effects mirror certain biochemical features found in tumor cells (5).

Several lines of evidence appeared more than ten years ago suggesting that the EGF-stimulated cell regulatory system may play a role in carcinogenesis. In brief, this evidence indicated that EGF elicits transformation-associated phenotypes in certain target cells, that EGF potentiates chemical transformation *in vivo* and viral transformation *in vitro*, and that polypeptide growth factors included in the EGF-like peptide family are secreted by transformed cells, thus enhancing tumor development (for review see 6).

This has, for years, brought concern on the clinical use of EGF. Recent data, mainly derived from animal models, suggest that EGF does not initiate cell transformation. Furthermore, it is likely that its role in carcinogenesis is only as a promoting agent by acting in an epigenetic manner when there is a prone genetic cell type toward malignancy.

Experimental evidence

Here we briefly mention classical findings related to the effect of EGF on cell metabolism, which have been considered as responses associated to neoplastic transformation. Accordingly, other data are in conflict with the first line of evidence:

1. EGF induces a partial loss of the dependence of growth on density inhibition and the dependence on serum growth (7-9) and increases the level of phosphotyrosine in proteins (10, 11). These examples describe only transient effects which are common to other growth factors and cytokines, that do not necessarily imply neoplastic transformation. Furthermore, these effects are reversible, so normal cells regain their functional pattern upon EGF removal. The fact that EGF increases the level of phosphorylation in tyrosine is, on the other

hand, an ordinary and standard mechanism in cell signal transduction, which takes place in both normal or transformed cells (12).

2. EGF induces the expression of some cellular proto-oncogenes (13). Proto-oncogenes play a critical role in normal cell physiology (mitosis, migration, anchoring, differentiation), and they are depicted as membrane receptors, signal transducers, and transcription factors (14). Furthermore, the indispensability of certain proto-oncogenes for normal mammal organogenesis and development has been clearly illustrated by knock out animal models (15).
3. Growth of cells in soft agar and thereby colony formation is potentiated by EGF (16, 17). This is a phenotype-associated event, and not a gene-commanded behavior. It has been proved to be a transient and reversible event upon EGF removal (18).
4. EGF enhances viral transformation in cultured cells (19, 20). The effect of EGF on these cells mirrored those observed by 12-O-tetradecanoylphorbol13-acetate, a well-known tumor promoter. In other experiments, the presence of EGF was interpreted as a mandatory condition to perpetuate the transformed phenotype. However, EGF alone was unable to induce phenotypic changes in noninfected cells. Classical tumor promoters do not have or show very weak tumorigenicity by themselves, but can enhance the carcinogenic potential of chemical substances (21). Thus, EGF may be considered a tumor promoter, but as judged by these experiments it was insufficient to initiate malignant transformation.
5. EGF enhances the carcinogenic potential of methylcholanthrene in rodent skin (22, 23). However, it should be highlighted that repeated EGF intravenous administrations in rats, provoked a marked hypercellularity in several epithelial organs, including the skin, in a dose-dependent fashion. These changes were not associated to cell indifferentiation, benign or malignant tumorigenesis, nor they were detrimental to animal health (24).

Other evidence suggesting that EGF does not initiate tumorigenesis

1. According to preclinical models, EGF administration does not induce mutagenicity, clastogenicity nor cytotoxicity (24). Rather it was seen that EGF antagonizes the effects of some well-known mutagenic drugs as cyclophosphamide, cisplatin and thiotepa (Bello JL, National Institute of Oncology and Radiobiology, Havana, Cuba. Unpublished data).
2. EGF inhibits the growth of several lines of mammary, liver, pituitary, and cutaneous cancer cells (25-30).
3. Certain concentrations of EGF transiently inhibit tumor growth *in vivo* (31).
4. EGF is a cytoprotective agent for some cell systems (*i.e.* digestive tract). In this context, the role of EGF is linked to mucosal damage prevention against irritating and necrotizing agents (32).
5. EGF protects some peripheral tissues against radiogenic treatment secuelae (33).
6. Some recent experimental evidence showed that EGF sensitizes *in vitro* and *in vivo* cancer cells to anti-tumor therapy (34).
7. EGF was shown to prevent abnormal healing in animal models by preventing excess fibrous proliferation and scarring in the esophagus (35).
8. The fact that EGF stimulates the proliferation of basal epithelial cells has been recently interpreted as of therapeutic value in the treatment of cancer-chemotherapy associated mucositis (36).
9. Recent data from *in vivo* models suggested that EGF might prevent lipid peroxidation (37). Indeed, free radicals are considered as carcinogenic agents (38).
10. A constitutive over-expression of the EGF receptor and of a potent receptor ligand, transforming growth factor alpha (TGF-a) is seen in psoriasis (39). However psoriasis is not a malignant disease. The excess production of TGF-a does not seem to be a determinant

requisite to initiate carcinogenesis. The TGF-a transgenic mouse further supports this assumption. In these animals, the growth factor over-expression was targeted to the epidermal suprabasal layer by a specific keratin gene promoter. The consequences of the excess production of the TGF-a paracrine in relation to malignant transformation, were mostly limited to the appearance of papillomas while most of these tumors appeared in skin areas subjected to traumas (40). The presence of benign tumors primarily at wound sites suggests that factors produced in wounds must act together with TGF-a overexpression to cause papilloma formation.

11. The consequences associated to long term administrations of EGF in laboratory animals have recently been described. These data are briefly summarized here:

- Male Wistar rats received EGF at 150 μg/kg/day for 4 weeks. At the end of the treatment period, the animals exhibited a significant increase in mucosal weight and surface area of the functioning small intestine (41).
- A similar experiment was done in mini pigs receiving EGF at 30 μg/kg/day for 4 or 5 weeks. The experiment showed a significant size increase of the ureters and the urothelium. These findings were associated to the enhancement of the mitotic activity in the basal cells of the urothelium (42).
- In mini pigs and rats, the subcutaneous administration of EGF at 30 and 150 μg/kg/day, respectively during 4 weeks provoked an increase in the thickness of the esophageal epithelium. However, this hypercellularity was accompanied by a normal pattern of differentiation as judged by the characterization of the lectin binding affinity (43).
- A consistent ovarian growth was observed in Wistar rats receiving EGF at 150 μg/kg/day for 4 weeks. The ovarian growth was due to an increased number and size of follicular cysts and an increase in the quantity of luteinizing cells (44).
- Finally, we (Bello JL, Berlanga J, and López-Saura P, unpublished data) treated Beagle dogs (both sexes) with human recombinant EGF (hr-EGF) at 900, 180, and 54 μg/kg/day during 47 days. After seven days of treatment the animals treated with the largest EGF dose, exhibited systemic alopecia, sebaceous dermatitis, constant sialorrea and corneal illness. Days later, these changes appeared in the other dose-treatment groups. Ascitis and a marked increase in cutaneous thickness were onset at about day 20 of administration. At autopsy we found: (i) increase of the dermal thickness, (ii) a dramatic fibrotic proliferation of the connective tissue surrounding thoracic and abdominal organs, (iii) up to 100 mL of peritoneal liquid, and (iv) several visceral adherences to cavity walls. The microscopic study revealed a consistent thickness in kidneys and liver capsules and the corresponding organ stroma, hair follicle apoptosis, hyperplasia of several epithelial organs (salivary glands, thyroid epithelium, duodenum, etc.). The intensity of these changes was expressed in a dose and sex dependent fashion, where the most dramatic effects were observed in males.

All these experiments have confirmed the potent mitogenic capacity of EGF on a wide variety of epithelial responding tissues, as well as for some mesenchymally derived cells. They have also demonstrated that, at least under the experimental conditions established so far, the EGF-mediated hypercellularity is not associated to malignancy in animals with a conceivable normal cellular substrate. Indeed, it should be taken into account that these experiments were done in different animal species, sexes, and using a varied range of doses of the growth factor.

Some other indirect evidence:

a. As judged by pharmacokinetic studies, EGF is rapidly cleared from the central bloodstream, and rapidly eliminated via urine after its parenteral administration (45). Furthermore, there

is no data to indicate that EGF may be stored in any body compartment after a bolus infusion.

b. An illuminating experiment (46) showed that at least the events involved in skin healing wounds (cell migration, mitosis, angiogenesis, etc.) demand a sustained release of the peptide to achieve a significant clinical effect. In a similar context, we also observed that for EGF in semisolid vehicles to enhance wound healing, a frequent treatment schedule is required (47).
c. Wounding is considered a tumor promoter event at least in the skin (for review see 48). However, tumorigenesis has never been reported as a consequence of EGF treatments in wounded skin areas in humans (49, 50).
d. There are some mechanisms for the control of EGF availability in the pericellular milieu, in regard to cellular uptake and signaling pathways, so that cells may become insensitive to further EGF activation. These include cellular and extracellular peptidases (51, 52) and molecular events involving signal transduction as a "down-regulation" receptor, PKC-mediated tyrosine kinase regulation, etc. (for review see 53, 54).

Conclusions

EGF is identified as a tumor promoter according to *in vitro* and *in vivo* models. However, the experimental evidence obtained so far, indicate that EGF does not initiate carcinogenesis. The appearance of recent data indicating that EGF may be beneficial by preventing mutagenicity, lipid peroxidation and by sensitizing tumors to anti-cancer therapy, support the concept that EGF is, however, a potent cytoprotective polypeptide for certain cell systems.

The fact that: (i) EGF has contributed in saving lives under threatening condition, (ii) for such entities the treatment period is short, (iii) EGF is rapidly cleared from body compartments, and (iv) the clinical use of certain drugs is presided by a risk-benefit ratio analysis, in a case-by-case manner, provides a fundamental basis for a wider clinical use of EGF in terms of safety.

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