ISSN:2157-2518

Journal of Carcinogenesis & Mutagenesis

The International Open Access Journal of Carcinogenesis & Mutagenesis

Available online at: OMICS Publishing Group (www.omicsonline.org)

Digital Object Identifier: http://dx.doi.org/10.4172/2157-2518.1000115

Carcinogenesis & Mutagenesis

Berlanga-Acosta et al. J Carcinogene Mutagene 2011, 2:1
http://dx.doi.org/4172/2157-2518.1000115

Review Article Open Access

Epidermal Growth Factor (EGF) and Platelet-Derived Growth Factor (PDGF) as Tissue Healing Agents: Clarifying Concerns about their Possible Role in Malignant Transformation and Tumor Progression

Jorge Berlanga-Acosta[1]*, Jorge Gavilondo-Cowley[2], Diana García del Barco-Herrera[1], Jorge Martín-Machado[3] and Gerardo Guillen-Nieto[3]

[1]Tissue Repair and Cyto-Protection Research Group, Biomedical Research Direction. Center for Genetic Engineering and Biotechnology, PO Box 6162, Cuabanacan, Playa 10600. Havana City, Cuba
[2]Cancer Research Group, Biomedical Research Direction. Center for Genetic Engineering and Biotechnology, PO Box 6162, Cuabanacan, Playa 10600, Havana City, Cuba
[3]Vaccine Division, Biomedical Research Direction. Center for Genetic Engineering and Biotechnology, PO Box 6162, Cuabanacan, Playa 10600, Havana City, Cuba

Abstract

EGF and PDGF, reminiscent of the early hopes of solution for problem wounds have attained a niche by healing diabetic ulcers. Although they belong to unrelated families, multiple biological features are shared. Mounting evidences reviewed here; document however, divergent and opposing roles for EGF and PDGF in both tissue repair and tumorigenesis. Wounds: As EGF receptor is not expressed by inflammatory cells, its ligand does not quantitatively or qualitatively modifies the course of inflammation. In contrast, PDGFB recruits and perpetuates inflammation. These infiltrated inflammatory cells turns and additional local source of growth factors. EGF enhances matrix synthesis via gene expression, while PDGF increases wound fibroblasts and myofibroblasts population density and exhibits far more chemotactic and angiogenic effect. Epithelialization is distinctly stimulated by EGF. Oncogenesis: EGF is not an oncogene-derived product and does not render perpetual or irreversible transformation in vitro or in vivo. Its promoting effect is not uniformly reproduced and seems to depend upon the animals genetic background, targeted tissue biology, and/or the chemical carcinogen-induced mutations. A variety EGF receptor mutated forms may confer cell's self-sufficiency for no need of exogenous growth factors supply. PDGFB is an oncogen product, establishes growth-perpetuating autocrine loops and confers self-sufficiency to glial tumorigenesis. Its role as a co-carcinogen as in tumor stroma and neoangiogenesis appears far more defined. Understanding the cellular and molecular imprinting of EGF and PDGF would allow for the judicious medical balance in terms of risk-benefit for the patient.

Keywords: Wounds; Cancer; Growth factors; EGF; PDGF

Introduction

Growth factors and their receptors are members of the six broad groups of oncogene products that under finely tuned conditions play crucial biological roles in cell differentiation, organogenesis, development, tissues repair and apoptosis [1]. These molecules have been conserved along the evolution and appear to be irreplaceable for the multiplicity and survival of metazoan organisms [2].

It is likely that Stanley Cohen's (Stanley Cohen-personal communication) was the first to exogenously administer a growth factor in pharmacological concentrations to an animal model, thus evoking an expected clinical response (corneal burns healing in rabbits). Since then, several growth factors have been scrutinized as candidates within the tissue repair scenario. With peaks and troughs EGF and PDGF opened up the first pharmacological attempts to improve the healing process and have evolved to stand as major players within the clinical armamentarium of hard-to-heal lower limb wounds [3-10].

Although these growth factors belong to different families and drive particular biological roles over specific cell populations, some targets overlapping do exist. In contrast to their salutary effect in wound healing; EGF, PDGF as well as as their receptors are implicated in experimental and human malignant diseases. Wounds and developing tumors are biologically similar niches of dynamic interaction between a variety of cell types sharing many histological features [11]. For tissue repair and tumorigenesis, cell proliferation, migration, survival, and angiogenesis are instrumental events whereas all these processed appeared governed by a plethora of growth factors [12].

Given that ground-breaking evidences have accumulated and document biologically divergent roles for EGF and PDGF in tumorigenesis, and inspired by the potential usefulness of the dissection of each of these ligands participation, in cancer and tissue repair; we have embarked to review and clarify these issues for basic and clinical researchers. This review could be potentially useful for practitioners who care for chronic wounds and prescribe growth factors-based interventions.

The information analyzed for this work was retrieved from Pubmed introducing a series of relevant key words: ("EGF / PDGFB signaling pathways", "EGF and PDGFB and wound healing or wound repair", "EGF and PDGFB and cell and transformation, "EGF and PDGFB and transgenic mice and malignant or tumorigenesis" and "EGF and PDGFB and malignant or tumors or cancer"). All the articles reviewed were restricted to English language and grouped according to publication dates from 1982 to 2010.

***Corresponding author:** Jorge Berlanga-Acosta PhD, Tissue Repair and Cyto-Protection Research Group, Biomedical Research Direction Center for Genetic Engineering and Biotechnology, PO Box 6162, Cuabanacan, Playa 10600, Havana City, Cuba, Tel: (53-7) 2716221; Fax: (53-7) 2714764; E-mail: Jorge.berlanga@cigb.edu.cu

Received Janurary 03, 2011; **Accepted** February 22, 2011; **Published** February 25, 2011

Citation: Berlanga-Acosta J, Gavilondo-Cowley J, del Barco-Herrera DG, Martín-Machado J, Guillen-Nieto G (2011) Epidermal Growth Factor (EGF) and Platelet-Derived Growth Factor (PDGF) as Tissue Healing Agents: Clarifying Concerns about their Possible Role in Malignant Transformation and Tumor Progression. J Carcinogene Mutagene 1:115. doi:10.4172/2157-2518.1000115

Epidermal Growth Farctor (EGF)

The EGF system- The receptor and signaling pathways

EGF is not an oncogene-derived product thus; it is likely that for most malignant contexts it may not be the major culprit. However, EGF receptor (EGFR) and a sequence of a virus producing erythroblastosis and fibrosarcomas (v-erbB) in birds were shown to be similar [13]. Subsequent studies unraveled that the activated EGFR and the Rous sarcoma virus transforming factor SRC, were related proteins that performed a newly discovered enzymatic function: tyrosine phosphorylation, underscored so far. As described by Normanno [14] EGFR expression has been found increased in a broad variety of epithelial tumors (Table 1) and this typically correlated with a worse prognosis. Approximately one-third of all human epithelial cancers exploit deregulated signaling by the *ERBB* family for growth, survival and other functions toward tumor perpetuation (for excellent review see [1]); henceforth this family of receptors represented the paradigm of aberrant signaling by a growth factor receptor in human neoplasia.

Four receptor genes encoding the EGF receptor family are described: epidermal growth factor receptor (*EGFR*), v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 (*ERBB2*), v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (*ERBB3*), and v-erb-a erythroblastic leukemia viral oncogene homolog 4 (*ERBB4*), this includes four variations of the full-length receptor. As mentioned, these receptors are endowed with protein tyrosine kinase (PTK) activity and are privative of metazoans [15].

The cytoplasmic region of the EGFR comprises three distinct domains: 1. the juxtamembrane domain (required for feedback by protein kinase C) 2. the noncatalytic carboxy-terminal tail (possessing six tyrosine transphosphorylation sites mandatory for recruitment of adaptor/effectors proteins) 3. the central tyrosine kinase domain (SRC homology domain) responsible for mediating transphosphorylation of the six carboxyterminal tyrosine residues [16].

This signal transduction system shows a complex bow-tie pathway architecture while showing several feedback loops that ensure cell response control [17]. Receptors activation is promoted by eleven ligands identified so far in mammals. These include EGF, transforming growth factor-α (TGFA), heparin-binding EGF-like growth factor (HBEGF), betacellulin, amphiregulin, epiregulin, epigen and the 4 isoforms of neuregulins [18]. In the absence of a ligand, monomeric receptors reside within the cell membrane in an inactive state. When the ligand is present, the receptor sites become occupied by monomeric EGF and a two stable 1:1 EGF:EGFR complex persists. Sequential receptor dimerisation and oligomerisation ensues. Such dimerisation is accomplished through the interaction of one EGFR S1 domain with that of a second ERBB2 in a 2:2 complex. While the ligands may overlap with respect to binding to receptors, they have their own specificities and affinities for each of the respective receptors. This redundancy and overlapping nature of ligand-receptor binding enhances robustness in sensing the molecules in the environment, as dysfunction in one of the receptors may be compensated for by other receptors that have an affinity for the overlapping ligand molecule [17]. Subsequent to transphosphorylation of the receptor dimer, second messenger proteins possessing domains that recognize site-specific phosphorylation as SH2 structure and PTB domain interact with the receptors. The enzyme phospholipase C gamma (PLCG) is a well-characterized second messenger recruited. Upon phosphorylation of the EGFR, PLCG interacts with the receptor and among other events, causes relocation to the membrane where it makes contact with the substrate ultimately generating second messengers as Ins(1,4,5)P3 and diacylglycerol [15]. The functional significance of PLCG activation relates to epithelial and fibroblast cells migratory capability. Inhibition of the enzyme's signaling abrogates cell locomotion without disturbing proliferation.

Tissue / Organ	Expression %
Lung	40-80
Breast	14-91
Stomach	33-74
Colon	25-77
Pancreas	30-50
Prostate	40-80
Kidney	50-90
Ovary	35-70
Head and neck	36-100

Table 1: Frequency of expression of the EGFR in human carcinomas. EGFR belongs to the ERB family of tyrosine kinase receptors. As shown in table 1, a variety of human malignant tumors over-express the EGFR which has been correlated with poor prognosis.

Other physiologically significant signaling pathways related to EGFR activation are mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K)/ v-akt murine thymoma viral oncogene homolog (AKT) (PI3K/AKT). These pathways promote two major cell responses: proliferative and pro-survival or cytoprotection (by inhibiting apoptotic signals). The mitogenic response mediated by the RAS–RAF–MEK–MAPK pathway is associated to cyclin D1 increased levels which sequester the cyclin kinase inhibitor p27 and release Cdk2 thus enhancing G1/S cell-cycle progression [19]. AKT promotes cell survival by phosphorylating the B-cell CLL/lymphoma 2 (BCL2) family member BAD and other downstream cell death pathway substrates as caspase-9 [20]. Other alternative signaling substrates have been described elsewhere [21,22]. In addition to the traditional cytoplasmic EGFR signaling pathways, evidence from several groups indicates that EGFR family receptors can be shuttled from the cell surface to the nucleus, where they transduce signals [23]. In general terms the three main functional signaling pathways evoked by EGFR agonistic stimulation in wound repair events (Figure 1), appear to be relevant but amplified in tumorigenesis. Alternatively, EGFR can be transactivated by a series of G protein–coupled receptor agonists, phorbol esters, cytokines, chemokines, estrogen, and cell stress signals [24]. EGFR transactivation has also been shown through other growth factors receptors tyrosine kinases as the PDGFRB [25].

EGF in Tissue Repair

Today EGF has achieved a central niche as a pharmacological resource to trigger and sustain the healing process of diabetic lower limbs wounds. As a topical composition it has proved efficacy in low-grade (neuropathic) Wagner's grades I and II diabetic foot ulcers [26,27]. Poor-prognosis, Wagner's III-IV ulcers have been successfully healed by the wound's base and contours deep infiltration of an EGF-based pharmaceutical composition [28,29]. As briefly mentioned above, the first evidence for a role of EGF in tissue healing derived from Dr. Stanley Cohen by using EGF eye-drops on rabbit's corneal healing. Later on, an EGF-like factor was detected in wound fluid collected from rats which contained a chemotactic factor for endothelial cells, whereas this effect appeared neutralized with anti-EGF antisera [30]. In addition, substantial levels of EGF and TGFA were found in wound fluid from skin graft donor site wounds in patients with burn injuries [31]. Different studies have identified marginal surface keratinocytes, wound fibroblasts and hair follicle epithelial cells as the main cellular sources of the EGF like growth factors involved in wound healing [32,33].

Nevertheless it seems that EGF is neither the most abundant nor the most potent healer as compared to TGFA [34,35] or other

cognate ligands as the HBEGF. As a matter of fact, EGF appears to be functionally distinct from TGFA and other EGFR ligands [36]. This supports the notion that signaling effectors and thus cells responses are specified by the receptor and the type of ligand itself. It certainly may bring differences in terms of pharmacological efficacy and clinical safety.

EGFR becomes rapidly over-expressed following tissue injury which is followed by a progressive decline paralleling the re-epithelialization process [33]. The receptor is detected in fibroblasts, endothelial cells and keratinocytes which suggest the establishment of autocrine and paracrine loops by the wound's cells. It has been demonstrated that the healing deficit observed in aged animals and humans may be related to a sensitive loss of the EGFR expression in fibroblasts, which could afterwards deter wound contraction [37]. EGF-mediated healing events are summarized in Table 2 [33,38].

EGF in Tumorigenesis

In vitro and in vivo experiments- Conflicitng results

Three major factors justify the involvement of the EGF/EGFR axis in malignant cells biology: (a) the proliferative and transforming competence of the ligands, (b) the receptor's performance (amplification, over-expression and activating mutations), and (c) the active cross-talk of the receptor with other oncogene-derived products.

Transfection experiments indicated that constitutive expression of preproEGF is sufficient to establish autocrine growth and focal transformation of NIH3T3 expressing low levels of EGFR [39]. Afterwards, when the same mammalian host was transfected with multicopies of the native EGFR gene, traits of *in vitro* transformed phenotype were detected only upon adding EGF. EGFR overexpression appeared to amplify normal EGF signal transduction [40]. By 1990 Schlessinger and Ullrich announced for the first time a mechanism for disrupting the cellular control and proto-oncogene activation, by identifying ligand-independent mitogenic and transforming activities derived from a mutated EGFR [41]. This new finding paved the way for the subsequent demonstration that highly tumorigenic epithelial cell lines are distinguished by their EGF independence for proliferation [42]. The EGF-ligand independence concept became clinically meaningful by the recently discovered mutations variants of the EGFR in human cancers, which exhibit oncogenic potential as to transform vertebrate cells in the absence of exogenous growth factor supply [43]. The EGFR vIII variant is for instance a frequently detected mutation in glioblastomas. This contains a deletion in the EGFR extracellular domain that causes constitutive EGFR dimerization, phosphorylation and coupling with downstream effectors [44]. In addition to the fact that mutations in the tyrosine kinase domain of the EGFR results in ligand-independent gene activation, an increased ligand affinity by up to 30 fold has also been described [45]. David Riese's laboratory recently demonstrated that artificially mutated forms of the EGFR can display increased affinity for a ligand that normally displays minimal affinity, thus inducing an unusual downstream signaling that deregulates cellular control. Therefore mutations in the EGFR extracellular domain may expand the repertoire of EGFR agonists and permit ligand-induced signaling in tissues that normally do not exhibit such signaling [46]. Consequently, EGFR (ERBB1) and its cognate ERBB2 have turned a

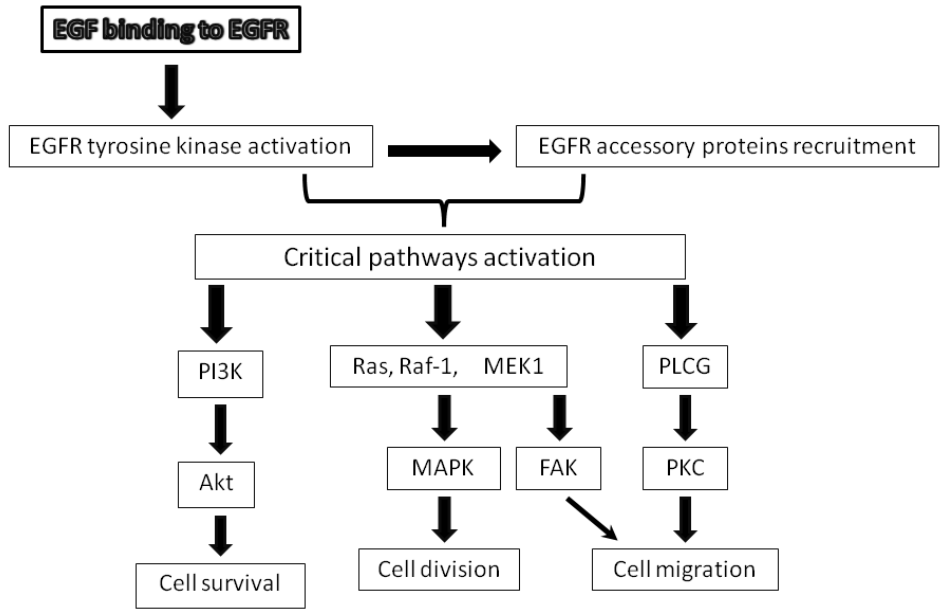

Figure 1: Main EGFR signaling pathways in wound healing and cancer. EGFR occupation by EGF or other cognate agonistic ligand triggers a conformational change within the receptor's topography leading to carboxy-terminal tyrosines phosphorylation and accessory proteins recruitment. Three major signaling pathways have been described upon EGFR occupation. PI3K, phosphatidil inositol 3-kinase, involved in cyto-protection and cell tolerance to hypoxia. PI3K phosphorylates downstream substrates as A*kt* or PKB on serine 473. Consequently A*kt* inhibits apoptosis via BAD and BAX inactivation. This pathway assists in cell survival and appears to be involved in wound bed and tumor cells survival when angiogenesis is not accomplished, thus contributing to tumor metastasis. Cell proliferation involves the RAS-RAF-MAPK pathway, where phosphorylated EGFR recruits accessory proteins which activate the oncogene derived proteins RAS, subsequently RAF, and the Mitogen-Activated Protein Kinase (MAPK) pathway leading to cell cycle inhibitors blockade, cyclins synthesis and cell proliferation. This pathway may participate in wound bed re-population as in tumor invasion and metastasis. Phospholipase C-gamma (PLCG) activation via phosphorylation, renders the hydrolysis of phosphatidylinositol 4,5 biphosphate (PIP2) into inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG), resulting in activation of protein kinase C (PKC). This pathway is involved in cell migration cooperating with focal adhesion kinase complex (FAK). Besides PKC activation is also responsible for controlling receptor down-regulation and protein kinase activity by phosphorylating the yuxtamembrane residue of threonine 654. This results in a temporary inhibition of the tyrosine kinase activity.

Biological event	Effectors cells	Brief description
Chemotaxis	Fibroblasts	Local fibroblasts remain dormant in intact skin. Upon injury and growth factors receptors activation, the cells are committed to migrate and anchor with the provisional fibrinoid matrix.
Cell proliferation	Fibroblasts, endothelial cells, keratinocytes	Except inflammatory cells, fibroblasts, myofibroblasts and keratinocytes which express the EGFR are committed to divide. This contributes to wound fibroplasias, neovascularization and re-epithelialization.
Matrix elaboration	Fibroblasts and myo fibroblasts	EGF stimulates collagen and fibronectin gene expression in activated fibroblasts. Myofibroblasts are also committed in matrix protein secretion and accumulation. This contributed to granulation tissue growth.
Angiogenesis	Endothelial cells	EGF stimulates angiogenesis via EGFR stimulation and through trans activation of other growth factors.
Contraction	Myofibroblasts	EGF enhances the transdifferentiation of fibroblasts to myofibroblasts. Smooth muscle actin type alpha fibers are extracellularly deposited which precipitates wound contraction.
Epithelial resurfacing	Keratinocytes	EGF stimulates wound re-epithelialization by stimulating migration of the leading edge cells. Backward located cells proliferate and push forward the advancing cells

Table 2: Events included in cutaneous tissue repair cascade stimulated by EGF.

preferential target for most epithelial cancers therapeutic approaches [47], which have also justified large-scale screening for EGFR mutations in patients candidates for tyrosine kinase inhibitors [45].

EGF exhibits tumor-promotion activity as has been shown in both *in vitro* and *in vivo* experimental models of co-carcinogenesis. However, EGF mediated-promotion has not shown reproducibility through most diverse experimental settings as discussed below. EGF promotes chemical and viral-initiated tumorigenesis [3]. Nevertheless, in non-transformed cells, endogenous mechanisms prevent non-programmed mitogenic waves. Among them the dominant negative regulatory effect of protein kinase C (PKC) via Thr-654 phosphorylation and the well-known receptors' down-regulation as an attenuation mechanism were early described [48].

EGF has paradoxically shown to inhibit the growth of several cancer cell lines of epithelial origin according to classic studies [3]. 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 tumorigenesis [49]. In a series of *in vivo* co-carcinogenesis experiments, EGF has often failed to promote colonic and salivary gland carcinogenesis in mice while in others (gastric, pancreatic and bronchial carcinogenesis), its promoter action appeared somewhat clear [3]. As a corollary for this plethora of controversial data, it was recently shown that progression from metaplasia to invasive gastroesophageal carcinoma in patients, formerly associated with autonomous activation of EGFR and/or AKT, is in fact associated to suppression of the EGFR-AKT axis [50].

Another controversial result added to the list involves the *Adenomatosis poliposi coli* / Multiple intestinal neoplasia (ApcMin) murine model. These animals spontaneously develop benign adenomas in the small intestine and colon tumors; thus representing an idiopathic and non-manipulated colorectal cancer model. Although Apc mutation is among the earliest alterations leading to colorectal cancer, and that EGFR activity is up-regulated in Min mice enterocytes [51]; systemically administered EGF did not stimulate the onset of more polyps nor their transformation toward a malignant phenotype [52]. It does not disqualify however the biological significance of the EGFR system in the biology of the transition from intestinal dysplasia and neoplasia.

Studies in which different animal species have been subjected to EGF long-term systemic administration at phramacological concentrations concluded that EGF induces epithelial hyperplasia which appeared to depend on the dose and length of exposure. The pattern of epithelial growth appeared controlled, differentiated and remarkably reversible upon treatment withdrawal. No histological evidences of pre-neoplastic changes were detected. Although with intrinsic limitations, these models have confirmed that EGF is not sufficient to transform cells *in vivo* and that constitutive autocrine/paracrine loops may be more meaningful than exogenous growth factor surges. Aside from this, recent studies indicate that most human cancers express a variety of members of the EGF-receptor family ligands others than EGF which may play particular roles in tumorigenesis and tumor progression. This sustains the need for broadening the studies' scope across the whole family of EGF receptors [53].

EGF transgenic animals: Among the different transgenic animals generated that display innate EGF overexposure, cancer debut has been noted as exceptional [3]. Tönjes and co-workers demonstrated that hepatocarcinogenesis appears in transgenic mice that express a genetic construction which codifies for EGF in the liver. This was the first and unique in vivo demonstration of the transforming potential of EGF so far. In 1999, another transgenic mouse for EGF was reported overexpressing 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. In that year another line of mice was generated harbouring human EGF and keratinocyte growth factor (KGF) constructs under the control of the human insulin promoter. Expression of either EGF or KGF resulted in significant morphological changes including cellular proliferation and disorganized islet growth. Surprisingly, the intercrossing included even extensive intra-islet fibrosis but not premalignant or malignant changes [54]. Another EGF transgenic mice line rendered surprising results. In addition to be free of developing malignant lesions, the mice exhibited a remarkable delay in their somatic growth as a major phenotypic change (proportionate dwarfism). None of the well known neoplastic changes reported in mice overexpressing TGFA were detected in spite of the local over-expression of EGF in sensitive organs [3]. Although a large volume of data have shown the effects of EGF on the proliferation of Sertoli and Leydig cells; male transgenic mice overexpressing human EGF under the control of β-actin promoter exhibited spermatogenesis failure instead of testicular tumours [55].

The fact that most human cancers express ligands other than EGF,

unleashed the generation of transgenic mice for TGFA, amphiregulin and other family ligands. The lessons derived from these engineered subjects reinforced that privative features and biological differences exist among members of the EGFR ligands [53]. TGFA organs targeted overexpression have shown to cause pancreas ductal cancer which appears dramatically accelerated following crossbreeding with p53 knockout mice [56,57]. Similarly, mammary [58] and hepatocellular [59-61] carcinomas have been ascribed to TGFA with enhanced aggressiveness upon synergizing with other oncogene transgenic lines. Transgenic mice harboring amphiregulin epidermal overexpression exhibit among other changes epidermal hyperplasia and aberrant differentiation [62].

Although the transforming role of EGFR in human tumour biology has been described, genetically manipulated models have rendered more conservative data. Overexpression of the receptor in mouse urothelium limitedly leads to local hyperplasia indicating that the receptor overexpression alone appeared insufficient for malignant transformation [63]. Similarly, a mutated version of the EGFR introduced into the glioma-prone RasB8 (V12Ha-Ras overexpression) mouse strain showed that glial expression of EGFRvIII by itself does not initiate gliomagenesis [64].

Based on data from transgenic animals, several conclusions can be drawn: (I) EGF overexpression may be necessary but not sufficient to induce carcinogenesis (II) As the exploited EGF constructs have been ubiquitous and organ-targeted coinciding with the EGFR expression; it is reasonable to suggest that the loop's signal is tightly controlled and thus not sufficient to transform cells. (III) Wild type EGFR overexpression appears not to be sufficient for malignant transformation. (IV) Given the reproducibility of the malignancy findings on TGFA mice lines, it is tenable to suggest that TGFA is endowed with larger transforming potential than EGF (V). The interaction among cooperative forces to transform cells and endorse tumours with further malignant behaviour is a mandatory attribute for transformation.

Ligands other than EGF Transform Cells

Cultured cell models have illustrated that different EGF family ligands that bind EGFR promote divergent biological outcomes due to differences in ligand intrinsic activity. EGFR is able to sense who the binding ligand is, which contributes to select its cognate receptor phosphorylation partner. These observations highlight the importance of understanding the biological peculiarities of other EGFR ligands included in the EGF family [16,65].

Recent findings indicate that the expression of some EGF family members, most notably TGFA, AR, and HB-EGF, are associated with poorer patient prognosis or resistance to chemotherapeutics. For the latter (HB-EGF), its overexpression is associated to apoptotic resistance to chemotherapy. Contrary to an ideal pharmacology, a variety of chemotherapy treatments provoke HB-EGF expression, processing and extradomain shedding which leads to epidermal growth factor receptor phosphorylation and a downstream signaling that clearly amplifies tumor cells cytoprotective reserves [66].

EGF expression in breast tumor samples is associated with a more favorable prognosis, whereas TGFA expression is associated with more aggressive tumors [67]. Likewise, microarray analyses reveal that early hyperplastic precursors of breast cancer display increased AR transcription and decreased EGF transcription relative to normal breast tissue [68]. In non-small-cell lung carcinoma (NSCLC) patients, TGFA and AR serum concentrations correlate with tumor aggressiveness, but the serum concentration of EGF does not. In fact, the serum concentration of EGF is significantly higher in healthy individuals than in NSCLC patients [69]. Moreover, NSCLC tumors that are refractory to the EGFR tyrosine kinase inhibitor gefitinib display increased TGFA and AR transcription than do tumors that are sensitive to gefitinib [70]. In animals TGFA overexpression and the consequent autocrine loop presides papillomas malignant transformation [71]. These data extend to human pathology so that excessive TGFA loop appears to prevail in transformed squamous epithelium and premalignant dysplasias. Furthermore, increased levels of TGFA are detected in normal mucosa from cancer patients compared with levels in normal mucosa from healthy individuals suggesting that activation of TGFA/EGFR autocrine growth is an early event in head and neck tumorigenesis [72].

As mentioned, distinct physical and biochemical properties of the ERBB receptor in interaction with each ligand may underlie particular cells' behavior. Ligands such as EGF, whose receptor interaction is relatively pH resistant, target the receptor to lysosomes, whereas TGFA and neuregulin-1 readily dissociate from their respective receptors at endosomal pH, resulting in receptor recycling. Thus, receptor rerouting plays a major role in signal potentiation. These ligand-specific characteristics provide an extra level of control [18].

Taken together these data suggest that TGFA and AR stimulate EGFR coupling, leading to the onset of tumor aggressive phenotype while EGF fails to do so; and may in fact antagonize the stimulation of pathogenic signaling by TGFA and AR [53]. These clinical findings appear to be anticipated by the observations derived from the transgenic models in which TGFA for instance, exhibited a potent transforming action in a variety of epithelial organs which resembled their human counterparts.

Platelet-derived growth factor (PDGF)

PDGF family members and expression pattern: Platelet-derived growth factor (PDGF) was identified in 1974 by Dr. Russell Ross, an experimental pathologist who first proposed and demonstrated the 'response-to-injury' hypothesis. His major and everlasting scientific contribution, PDGF discovery, is indeed closely tied to tissue repair in essence. PDGF is a family of growth factors of paramount biological relevance, since it is expressed at early developmental stages ranging from sea urchins to humans. PDGF acts as a temporo-spatial organizer that through autocrine and paracrine loops contributes to tissue differentiation.

PDGF was originally identified as a disulfide-linked dimer of two different polypeptide chains, A and B which can arrange to organize three proteins: PDGFAA, PDGFAB, and PDGFBB, encoded by the genes PDGFA and PDGFB [73]. More recently, genomic and biochemical efforts identified two additional PDGF genes and proteins: PDGFC and PDGFD [74]. The B-chain (PDGFB) was characterized by amino acid sequencing, revealing a close homology between PDGFB and the product of the retroviral oncogene v-sis of simian sarcoma virus (SSV) By contrast, early in the 80's Doolittle and others showed that the transforming gene of the simian sarcoma virus (v-*sis* retrovirus) is PDGF [75]. It stud as an example of an oncogene-derived growth factor. Subsequent studies confirmed that the human cellular counterpart (c-sis) was identical to PDGFB and that autocrine PDGF activity was sufficient for transformation [74]. In higher vertebrates there are two PDGF receptors (PDGFR), PDGFR-alpha (PDGFRA) and PDGFR-beta (PDGFRB) that form both homo and heterodimers. All PDGF ligands except PDGFD induce PDGFRA dimerization, whereas PDGFB and PDGFD activate PDGFRB dimers. In addition, all ligands except PDGFA activate both receptor types in cells coexpressing the α and β receptors [76].

The expression pattern of the individual PDGF ligands and receptors is complex and seems to exhibit some sort of tissue specificity which may render a physiological control tool. PDGFA and PDGFC are expressed in epithelial cells, muscle, and neuronal progenitors. PDGFB is mainly expressed by vascular endothelial cells, megakaryocytes, and neurons. PDGFD expression has been observed in fibroblasts and smooth muscle cells. PDGFA is expressed in mesenchymal progenitor cells in lung, skin, and intestine and in oligodendrocyte progenitors. PDGFRB is expressed in mesenchyme, particularly in vascular smooth muscle cells and pericytes

Mice deficient for PDGFB die perinatally and display renal, cardiac, vascular, and hematological abnormalities [77]. The data collected from site-directed mutagenesis, as those from gain-of-function and especially from loss-of-function transgenic models substantially justify its evolutionary conservation.

PDGFB and its receptor are members of the repair mechanisms that become activated upon tissue injury. As a matter of fact, PDGFB is expressed by a constellation of cells including: epithelial, placental, testicular, vascular, neuronal, skeletal, inflammatory and retinal. It is released in large amounts from degranulating platelets remaining in the wound fluid early after injury [33]. This growth factor assists in the healing of soft and hard tissues as human bone fractures [78].

PDGFB protects, prevents and rescues cells from death while it exhibits a particularly strong neurotrophic and neuroprotective effect [78]. The fact that PDGFB signals are transduced by the serine/threonine kinase AKT pathway (among others) supports its cytoprotective effect [79].

PDGFB is an angiogenic growth factor and has shown to recruit the pericytes required to support the structural integrity of the vessels. Its effect is significant and peculiar, since it appears to act on the micro-vasculature in an organ-specific manner. Its administration has shown to induce functional anastomoses *in vivo*. PDGF also controls the vascular tone and reduces platelet aggregation. Another role of PDGF is to maintain the interstitial fluid pressure probably through its ability to stimulate interactions between connective tissue cells and molecules from the extracellular matrix [78].

PDGFB dark side is given by its unpaired implication in three major groups of human pathological processes as oncogenesis, vascular diseases, and fibrotic disorders. PDGFB system's commitment in cancer is broad including both the action of the ligand and the receptor so that tumor growth, migration, neovascularization and stromagenesis are enhanced. In addition, PDGF is expressed by both tumor and tumor stromal cells participating in the process of cellular transdifferentiation. Signaling through an autocrine PDGF/PDGFR loop is an early oncogenic event in gliomagenesis and other tumors and the increased expression of its receptor, correlates with the degree of malignancy [80].

In general, two types of cells appear to respond in a pathological fashion to PDGF(s): smooth muscle cells and fibroblasts; promoting vessel wall pathologies and fibrotic tissue scarring, respectively. Another general remark is that PDGFRB appears to be the dominant PDGFR involved in vascular pathology, whereas growing evidence instead suggests a pivotal role for the alpha isoform in various types of mesenchymal cell/fibroblast-driven pathologies [81].

PDGF signaling pathways

PDGF(s) biosynthesis and processing are controlled at multiple levels and differ for the different PDGF(s) forms. The major part of expressed PDGFB becomes trapped on the cell surface or in the extracellular matrix, where it can be subsequently released. All these events represent points-of-control for PDGFB local availability and therefore biological activity [82].

Because PDGF isoforms are dimeric molecules, they bind two receptors simultaneously and thus dimerize receptors upon binding. PDGFB binds all three dimeric combinations of α- and β-receptors while these receptor combinations transduce overlapping, but not identical cellular signals [83]. Importantly, the level of PDGFR expression on cells appears not to be constant. For instance, the expression of PDGFRB on connective tissue cells *in vivo* is ordinarily low but increases during inflammation suggesting that PDGF may play a role in the stimulation of mesenchymal cell proliferation that often accompanies chronic inflammatory processes [84].

Activation of a tyrosine kinase receptor is induced upon ligand binding and thus receptor dimerization, which allows autophosphorylation of tyrosine residues between the two receptors in the dimmer [85]. PDGFR are not uniformly distributed. Ligand binding induces internalization of the ligand-receptor complex into endosomes. The complex then dissociates, and the receptor recycles or, alternatively, the ligand-receptor complex is degraded in the lysosomes which contribute to regulate many aspects within the cell signaling process [86]. PDGFR undergoes lysosomal and cytoplasmic degradation after ubiquitination. Experimental observations illustrates that the rate of deactivation of receptors is an important parameter in the regulation of the mitogenic response [87].

PDGFRB engages several well-characterized signaling pathways as the Ras-MAPK, PI3K, and PLCG which are known to be targets of the activated EGFR and that are deeply involved in diverse cellular responses. As described for the EGFR, here again a number of adaptor proteins play important roles connecting the PDGFR to signaling pathways proteins. However a peculiar feature for PDGF signaling is that the strength of signals is modulated by the simultaneous activation of both stimulatory and inhibitory signals. Some mechanisms for modulation of signaling via PDGFR have been established so that the tyrosine phosphorylation is balanced by activation of tyrosine phosphatases. This fact, obviously contribute to the finely tuned regulation of PDGF activity [88].

One of the most described effects of PI3K signaling pathway is the downstream phosphorylation of the AKT/PKB serine/threonine kinase which triggers a potent antiapoptotic response [89,90]. PDGFR-mediated PI3K pathway activation also promotes actin reorganization, cell movements and growth stimulation [85].

PLCG is another phosphorylation target of PDGFR [76] which leads to intracellular calcium mobilization and PKC activation. Full activation of PLCG is dependent on PI3-kinase. Similar to EGFR activation PDGF-mediated PLCG activation induces cell motility. Other additional signaling molecules are engaged by PDGFR including enzymes, adaptors, and transcription factors which are involved in mitogenic responses, cell proliferation, differentiation, migration and survival [91].

PDGFB in wound repair

PDGFB is of particular relevance due to its chemotactic, mitogenic, angiogenic, and stimulatory effects leading to matrix formation and wound bed granulation [92]. Globally speaking three lines of studies support the role of PDGF in wound healing: (a) Expression of the ligand and the receptor during the healing process; (b) evidences from *in vitro* effects of PDGF on cells involved in wound healing; and (c) studies on the effect of topical application of PDGF to healing wounds.

PDGFB as other growth factors is secreted primarily from the alpha granules of platelets, but also from activated macrophages and fibroblasts. PDGF was the first growth factor shown to be chemotactic for cells migrating into the healing wound, such as neutrophils and monocytes/macrophages, in addition to fibroblasts [33]. The fact that PDGF recruits inflammatory cells into the wound bed establishes a significant distinction versus EGF as the later does not recruit neutrophils, monocytes or macrophages nor is its receptor expressed by these populations.

Experiments by Pierce and co-workers [93] demonstrated the effect of PDGFB administration in terms of intense inflammatory cell infiltration early after wounding, as the marked increase in granulation tissue formation and increased fibrosis. These indicated that PDGFB effect turns the wound matrix hyperpopulated. In subsequent studies these data were confirmed, and PDGF proved to be a more potent chemoattractant for wound macrophages and fibroblasts. Thus PDGFB treated wounds regularly exhibit an increased numbers of fibroblasts and granulation tissue [94].

One of the most relevant aspects of PDGF within the wound bed is its ability to accelerate extracellular matrix deposition. As in correspondence for its role in tumor biology, PDGF appeared to transduce its signal through wound macrophages triggering the induction of positive autocrine feedback loops and synthesis of endogenous wound PDGF and other growth factors, thereby enhancing the sequence of tissue repair events [95] (Figure2). This biological particularity of PDGF does not seem to be described for EGF [74].

Dermal fibroblasts are one of the major target cells of PDGF in the initiation and propagation of skin tissues repair. They secrete PDGFB and express PDGFRB receptor. PDGFRB targeted deletion studies on dermal fibroblasts have demonstrated its role in transducing wound healing signals [96] accounting for an 85% reduction of granulation tissue mass. Converging studies have demonstrated that PDGFB via the PDGFRB exerts mitogenic and motogenic input on dermal fibroblasts with a minor pro-contractile effect on fibroblasts-populated gels [97].

The functional significance of PDGFB signaling system during the early phases of wound healing became substantiated when after 7 days of using the PDGFRB inhibitor imatinib mesylate, the healing process was delayed with a significant reduction in the closure rate. Inhibiting PDGFRB restricted the distribution of collagen-synthesizing cells to wound margins and dramatically reduced cell proliferation and migration. PDGFRB inhibition was accompanied by abnormal microvascular morphogenesis reminiscent of that observed in PDGFRB–/– mice as it appeared to be also impaired by pericyte proliferation and migration inhibition [98]. Analyses of knock-out mice for PDGFB and PDGFRB have shown that PDGF signaling via PDGFRB is critically involved in recruitment of pericytes and vascular smooth muscle cells (vSMCs) to blood vessels [76].

Within the wound bed PDGF(s) and PDGFR expression patterns suggest a paracrine mechanism of action, since the ligands are predominantly expressed in the epidermis, whereas the receptors are mostly found in the dermis and the granulation tissue. As a matter of fact PDGFA and PDGFB chains are constitutively expressed in normal epidermis as are expressed by cultured keratinocytes [99]. Based on these data of expression pattern in the healing wound and its known *in vitro* activities; PDGF has been suggested to have two major but distinct roles in wound repair: an early function to stimulate fibroblast proliferation and a later function to induce the myofibroblast phenotype which presumes contraction stimulation. This hypothesis was supported by the finding that addition of neutralizing PDGF antibodies to human wound fluid caused a 45% reduction in the mitogenic effect of the wound fluid for cultured fibroblasts [33]. Nevertheless, this compartmentalization system accounting for a paracrine-mediated activity may represent one among the different control points of PDGF biological activity. Classic studies demonstrated that PDGF expression levels correlate with the

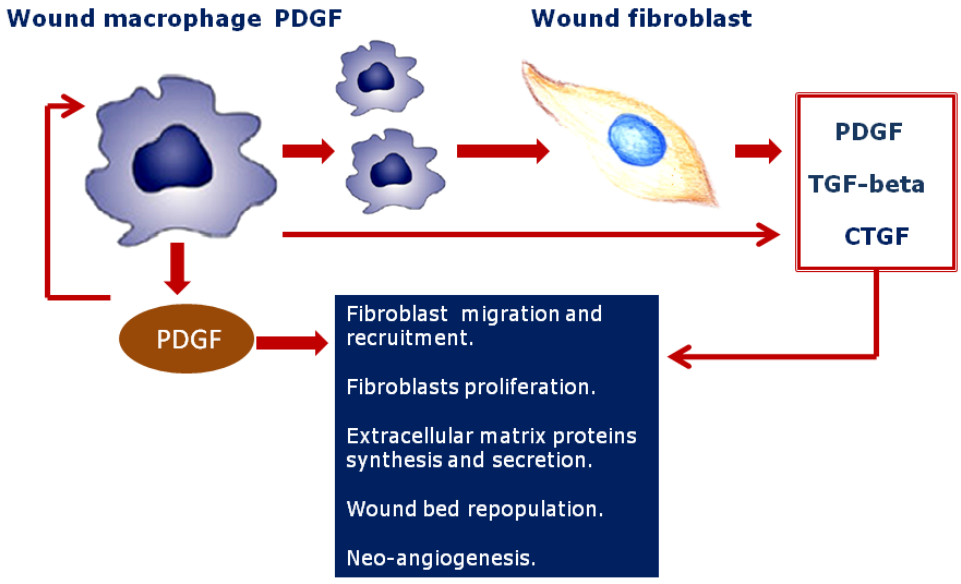

Figure 2: PDGF signal amplification through infiltrated macrophages. As described elsewhere for tumors, infiltrated macrophages plays an important role in wound healing. PDGF is one of the few growth factors distinguished by the ability to recruit monocytes/macrophages to the wound bed. PDGF protein expression is further amplified by macrophages while more cells are attracted and homed. Via this propagating cross-talk, PDGF stimulates a number of physiological activities on fibroblasts, myofibroblasts, and endothelial cells, thus favoring granulation tissue growth and wound cellularization. PDGF enhances its own secretion by wound fibroblasts as to synthesize other pro-fibrogenic growth factors which in the form of cocktail enhances wound bed granulation, contraction and vascularization.

stage of wound repair process, being highest during the initial repair stages and declining at the time of complete re-epithelialization and tissue remodeling; reflecting the need for a fine balanced regulation in PDGF activity [100]. PDGF mediates wound healing events within a close and specific temporary. During the early stages at 4 days post-wounding, PDGF initiates the migration of keratinocytes and fibroblasts to the epidermal edges promoting the formation of granulation tissue including angiogenesis. However, these contributions appeared counteracted in later stages of wound healing. The prolonged inflammatory response and extensive cell proliferation prevented the advancement of the migratory edges towards a physiological wound re-epithelialization. The authors concluded that PDGF can accelerate some stages of healing in certain types of wounds when administered during specific periods; while its prolonged administration can undermine the granulation tissue due to excessive chronic inflammation [101]. All these evidences have raised the hypothesis that cells choose to proliferate or migrate depending on the sensed gradients of PDGF in the environment. Low PDGF concentration response consists of directional migration along the gradient. Upon sensing a precise PDGF threshold cells switch from a migrating phenotype to a proliferating one, leading, in the case of wound healing to efficient tissue repair. In addition to accomplish a principal role in granulation tissue formation and contraction, one of the most outspoken biological actions of PDGFB is its involvement in angiogenesis. In 1994 Battegay and co-workers discerned basic principles of PDGFB-mediated angiogenesis using *in vitro* models. Cultured monolayer endothelial cells secrete PDGFB but do not express the receptor. In contrast, once the cultured cells form cords and tubes, they express the receptor and respond to the ligand that they no longer produce. Thus ligand and receptor appeared in an inversely correlated time-event window. The authors suggested that PDGFB might amplify angiogenesis in a paracrine manner via direct action on endothelial cells expressing the receptor [102]. The PDGF-promoted angiogenic activity within the wound bed may also be regulated by a retention motif identified as heparan sulphate (HS) proteoglycans. Physiologically, the motif seems to localize secreted PDGFB to proteins or proteoglycans on the endothelial cell surface or in the periendothelial matrix, thereby promoting its recognition by neighboring receptor-carrying cells. Retention is required for proper location of pericytes nearby microvessel wall [103]. In line with this, other angiogenesis-committed cell populations are recruited and stimulated by PDGF and other growth factors which cooperatively act [104]. Defective mutant mice for either PDGFB or PDGFRB die in utero from widespread hemorrhaging attributable to impaired recruitment of mural pericytes and smooth muscle cells to nascent vessels [105].

Another salutary effect of the PDGFB and its receptor extends to cytoprotection. Depletion of the PDGFRB protein significantly attenuated among other functions, the protection from H_2O_2-induced apoptosis in genetically manipulated dermal fibroblasts. The *in vivo* translation of this finding may impact into wounds with unsatisfactory oxygenated blood supply and in which pharmacological doses of PDGFB are introduced [106].

Another line of evidences documenting the role of PDGF in wound biology derives from studies with chronic wound fibroblasts, aged mice and pathological conditions. Genetically diabetic C57BL/KsJ-db/db mice wounds treated with recombinant PDGFB had many more fibroblasts and capillaries in the wound bed than did wounds of the vehicle alone [107]. Further experiments demonstrated that in genetically diabetic db/db mice exhibiting wound healing impairment; a significant reduction in PDGFA and its receptor expression is found in either intact or wounded skin; whereas PDGFB receptor expression is reduced during the repair process. Similarly, exposure to systemic glucocorticoid treatment for the onset of a healing impairment phenotype was accompanied by reduced expression of PDGFA and B and of the β-type receptor in the early phase of wound healing. Thus, PDGF system expression appears essential for normal repair [108]. This notion was further reinforced by the finding of Ashcroft and colleagues who showed a delay in the expression of PDGFA and B isoforms and their receptors in wounded aged mice. This observation was paralleled by a similar finding for EGF and its receptor [109]. Reduced angiogenic response in transplanted hearts in aged recipient mice appeared to be related to reduced PDGF(s) expression [110].

It has been shown that the mitogenic response of chronic wounds-derived fibroblasts to human recombinant PDGFB become reduced with ulcer age irrespective to the amount of receptors expressed by the fibroblasts. This observation could explain the non-healing state and therapy resistance to topical PDGFB interventions in leg ulcers [111]. In line with the above observation, Pierce and co-workers showed that the levels of PDGF(s) in non-healing human dermal ulcers were strongly reduced [112]. Taken together all these findings testify that PDGF system is critical for the onset and the progression of at least the first stages of the healing machinery and that its deficit may be an ethiopathogenic player for the wound chronification process. A chronic injury may induce irreversible gene expression leading to pathologic, unregulated cell growth as well as decreased growth factors and receptors expression [108].

PDGF in tumorigenesis

Although PDGFB in its pharmaceutical formulation (Regranex) was the first growth factor approved by the United States Food and Drug Administration for the treatment of human ulcers; concern about its indication has increased following the "Warning" (http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm048471.htm) addressing the increased risk of cancer death in patients who use three or more tubes of the product. This warning is unfortunately not enriched by further scientific data at least for the public audience.

Although PDGFB does not disrupt the normal sequence of healing events as TGFB does, its advantageous biological effect in wounds may turn cloudy due to the amount of experimental evidences documenting its transforming competence. Aberrant synthesis of PDGF and concomitant autocrine growth stimulation may be an important step in the neoplastic conversion of PDGFR-positive cells. Other studies document the carcinogenic potential of PDGFB as a "self-sufficiency" factor for malignant transformation establishing PDGF as a unique autocrine transforming factor, particularly for central nervous system cell populations.

More than 2 decades ago it was shown that both v-sis and its cellular counterpart (c-*sis),* cause transformation by an autocrine mechanism when expressed by PDGF-responsive cells [78]. Since then, PDGF hyperactivity has been observed in multiple types of human solid tumors. Chromosomal translocation involving PDGF genes has been observed to cause deregulation of PDGF expression in certain solid tumors and myeloproliferative diseases. The role of constitutive PDGF in human malignant tumorigenesis is out of discussion although still remains how to fix up the pieces of the multistep transformation process under an excessive PDGF signaling [113].

During the early 80's it was announced that the transforming protein of the primate sarcoma virus and PDGF are derived from the same or a closely related gene [75] given the extensive sequence similarity. It was also indicated that the mechanism whereby v-sis transforms cells could involve the constitutive expression of a protein with functions similar or identical to those of a factor involved in normal cell growth.

Soon after, a region of 104 contiguous amino acids was identified as showing identity with the predicted sequence of the p28sis, the putative transforming protein of the SSV [114]; thus suggesting a SSV-mediated transformation mechanism in which expression of growth factors appeared to be involved [115]. During those years the first transformation by a human c-*sis* cDNA using NIH-3T3 cells was shown, whereas it was subsequently shown that SSV-transformed cells release a material that is functionally similar to PDGF and that is able to induce PDGFR down-regulation by an autocrine and reversible mechanism [116].

This line of evidences was enriched when it was found that the SSV-transforming gene product is capable of specifically binding PDGFR, stimulating tyrosine phosphorylation, and inducing DNA synthesis in quiescent fibroblasts. Moreover, viral infection of a variety of cell types revealed a strict correlation between possessing PDGFR and susceptibility to transformation by the simian sarcoma retrovirus [117]. Further characterization of cells transformed by SSV revealed PDGF-related proteins in subcellular organelles and in conditioned media, consistent with an autocrine stimulation of cell growth [118]. It is important to highlight, however, that autocrine PDGF stimulation in vitro has the same effects as prolonged exogenous administration to the same cells. Thus, it means that such cells are not provided with full nor permanent repertoire of malignant behavior capabilities. In supporting this assertion, stands the evidence that anti-PDGF antibodies inhibited proliferation, and the SSV-induced morphological changes in human diploid fibroblasts [119].

More convincing evidences on the sis gene transforming sufficiency have, however, stemmed from *in vivo* models of carcinogenesis. For this aim, different methodological approaches from retroviral transduction systems to transgenic mice have been utilized. Despite differences in technical refinement, all the studies have pursued to achieve local or systemic PDGFB constructs over-expression. Since early observations suggested the existence of a PDGF autocrine mechanism for the development and maintenance of astrocytomas and other nervous system-derived tumors; emphasis was addressed to this subject.

In 1989 mice harboring fibrosarcomas were generated by perinatally injecting a retrovirus that expresses PDGFB and mimics the alternative splicing pattern of the v-sis oncogene. The emerging tumors were invasive, tumorigenic in athymic mice and morphologically similar to spontaneous fibrosarcomas of the monkeys from which the SSV was originally isolated. This was a robust demonstration that PDGFB is capable of generating a sufficiently large cells population to allow for selection of a fully neoplastic clone [120]. Similar findings were obtained years later by injecting mice with a retrovirus coding for the PDGF B-chain. Several mice developed brain tumors resembling to glioblastoma multiforme or of a primitive neuroectodermal tumor possibly derived from an immature glial progenitor. The study suggested that an autocrine mechanism of transformation may be an initial event in neuro-oncogenesis as described for human brain tumors [121]. These evidences were afterward enriched when Dai and co-workers demonstrated that PDGF autocrine stimulation reverts astrocytes differentiation program. PDGFB overexpression caused a significant increase in proliferation rate of cultured astrocytes and neural progenitors, moreover, it converted cultured astrocytes into cells with morphologic and gene expression characteristics of glial precursors. In vivo, PDGF gene transfer to neural progenitors and astrocytes originated the formation of oligodendrogliomas and of either oligodendrogliomas or oligoastrocytomas, respectively. Loss of Ink4a–Arf was also detected in those tumors with shortened latency and enhanced malignancy. Importantly, this mutation is frequently found in high-grade human gliomas. These data not only sustained the PDGF autocrine view, yet it showed it is potentially sufficient to induce gliomagenesis [122].

Subsequently, this group demonstrated that PDGFB may play a dose-dependent role in glial tumorigenesis. At lower doses, PDGF establishes an autocrine loop to promote gliomagenesis whereas an increased cellularity results from high-expressing PDGFB tumors. These tumors also exhibited higher grade histological features correlating with more extensive angiogenesis due to an activated AKT pathway in the recruited vascular smooth muscle cells (vSMC). The data indicated that by elevating PDGFB expression, a tumor can support large vessel formation. Based on the observation that tumors reverted from higher to lower grade by inhibiting the PDGFB receptor, the authors envision the therapeutic impact of this finding by reducing tumor malignancy and angiogenesis [123].

A definitive experimental argument supporting that PDGFB could induce transformation of human cells was provided by Govindarajan and co-workers [124] who demonstrated that, human cells (SV7tert) infected with retroviruses encoding PDGFB and thus constitutively overexpressing the growth factor were transformed and were tumorigenic upon implantation to 6-week-old nude male mice. The study describes other phenotypic changes associated to the deregulated PDGF axis as up-regulation of VEGF, reactive oxygen species production, resistance to apoptosis and other epigenetic changes that occur *in vivo* as silencing of p16 and increased glycolysis.

Infecting adult rats' white matter progenitor cells with PDGFB high expression retroviruses, induced the rapid formation of tumors that closely resembled human glioblastomas. Since tumors arose from the massive expansion of both infected and uninfected (recruited) glial progenitors, it was stated that PDGF was driving tumor formation via both autocrine and paracrine stimulation of glial progenitor cells. The reporter used in this retroviral-mediated transfection, allowed for the identification of cells from clonal expansion of the "tumor-initiating cells". PDGF constitutive over-expression proved to be self-sufficient to "initiate" and give rise to malignant gliomas, although other genetic or epigenetic alterations are at play [125].

Transgenic mice that expressed human PDGFB in GFAP-expressing glia responsive to doxycycline administration were generated by Hitoshi and co-workers while pursuing for the role of PDGF expression in the pathogenesis of gliomas. These mice expressed high levels of PDGFB in the spinal tissue and developed spinal cord neoplasms resembling human mixed oligoastrocytoma. In this context, PDGFB was self-sufficient as a single growth factor to mediate the development of spinal tumors that are histologically indistinguishable from human intramedullary spinal tumors. The study of the individual genetically engineered mice lines provided evidence of the importance of PDGFB for the "initiation" of glial tumors arising in the spine [113]. In a subsequent study, mouse gliomas were generated following the overexpression of PDGFB in embryonic neural progenitors. The induced tumors appeared to be a very uniform class of gliomas in which only those PDGFB-overexpressing cells were tumorigenic [126].

The evidences above enlisted point toward the full competence of PDGFB over-expression to initiate malignant transformation on particular target cells. As judged from these experiments, PDGFB: (a) Overrides glial cell cycle control inducing hyperproliferation *in vitro* and *in vivo*. (b) Initiates gliomagenesis *in vivo* and reverts astrocytes differentiation *in vitro*. (c) Gliomas' malignant phenotype appears in a PDGF dose-dependent manner. (d) Its gliomagenic ability appears in virtue of autocrine and paracrine signaling mechanisms. (e) Ensures

tumor angiogenesis by stimulating vascular smooth muscle cells recruitment [74].

Aside from its described "initiating" role in neurotumors, PDGFB has also shown to promote carcinogenesis in cooperation with ordinary chemical carcinogens. Although the genetic basis of hepatocellular carcinoma is well-established and major signaling pathways have been depicted [127], the involvement of PDGF in the genesis of liver cirrhosis and the common onset of hepatic carcinomas within this aberrant fibrotic environment, explain why the family of PDGF has shifted to the center of interest. The "promoting" role of PDGFB in hepatocarcinogenesis was investigated in transgenic mice that exhibit liver PDGF overexpression and that at some stage develop organ's fibrosis. PDGFB transgenic mice displayed pre-malignant lesions as hepatocellular carcinomas. The finding extends PDGF transforming potential beyond gliomagenesis [128]. Accordingly, PDGFB tumor-promoting activity has been demonstrated in an experimental model of human squamous cell carcinoma. Transfection of non-tumorigenic PDGFR-deficient HaCaT keratinocytes with PDGFB resulted in tumorigenic transformation giving rise to benign cystic tumors on subcutaneous injection. This demonstrated a tumorigenic conversion of the preneoplastic keratinocytes by paracrine effects [129].

The role of uncontrolled PDGFB expression resulting in autocrine loop in the development of several forms of human cancers including glioblastomas, sarcomas and epithelial tumors has turned into solid science [130]. Furthermore, among the vast human tumors histotypes, malignant gliomas represent a paradigm of aberrant PDGF system [131,132]. Proteomic analysis of surgical glioma samples emphatically described the participation of the PDGF pathway activation in which the primary trigger is ligand-driven. The study also revealed the pathogenic signature of the EGFR activation due to amplification and mutation [132]. Others have underlined that this EGFR mutant form (vIII) lacking extracellular binding domain and with intrinsic tyrosine kinase activity confers advantages to growth, survival, invasion, and angiogenesis [133]. Hence, devotedly following the "oncogene addiction" concept, EGFR-receptor and PDGF-ligand induced downstream tyrosine kinase inhibition, are accepted as key targets in contemporary therapeutic strategies [21].

Compelling evidence for an involvement of PDGFR in tumorigenesis is provided by findings of structural aberrations of the corresponding genes that lead to overexpression or expression of an abnormal protein. In cases of dermatofibrosarcoma protuberans and giant-cell sarcoma, the PDGFRB gene rearrangement leads to the synthesis of a chimeric protein with transforming activity. In chronic myelomonocytic leukemia, the PDGFRB gene is fused to an ets-like gene, (TEL), originating a chimeric protein that behaves as a constitutively active PDGFRB [74]. Transgenic mice in which the TEL/PDGFRB expression was directed to the lymphoid compartment by the immunoglobulin heavy-chain enhancer/promoter developed lymphoblastic lymphomas. The study confirmed the role of the aberrant PDGFR tyrosine kinase activity as a PDGFR-specific tyrosine kinase inhibitor, prolonged disease latency both in transgenic mice and in transplanted tumor cells [134]. The transforming role of the tyrosine kinases TEL–PDGFRB in chronic chronic myelomonocytic leukemogenesis has received extensive validation [135].

In addition to being an autocrine growth factor, PDGF is also involved in paracrine stimulation of tumor stroma growth. In several tumor types, there is a complementary expression of the PDGF ligand by the tumor cell while the stroma cells express the cognate receptor. This crosstalk is critical for tumor progression, invasion and metastasis. Furthermore, the development of myelofibrosis in chronic myelogenous leukemia has been ascribed to PDGF [74,136].

PDGFB plays an instrumental role in tumor stroma development a demonstrated 20 years ago. Xenotransplanted human melanoma cells expressing PDGFB formed stroma-rich and highly vascularized tumors that were devoid of necroses. In the absence of PDGF, these cells formed poorly vascularized and necrotizing tumors with no detectable stroma [137]. PDGF is among the tumoral chemoattractants that ensures macrophages and fibroblasts recruitment originating the so called "tumor-associated macrophages" (TAM) and "cancer-associated fibroblasts" (CAF) respectively [138] (Figure 3). Through cross-talks with cancer and other stromal cells, macrophages become instructed to promote tumor growth, angiogenesis and metastasis. Thus, Wyckoff and co-workers described a paracrine loop between cancer cells and macrophages in which cancer cells secrete colony stimulating factor (CSF-1), a chemoattractant agent for macrophages; while the stimulated macrophages secrete EGF which promotes directed cancer cells chemotaxis. At the end, this EGF/ CSF-1 self-propagating paracrine favors carcinoma cells migration and invasiveness and represents a model of cell lineages interaction and growth factors cooperation [139]. Another self-perpetuating loop including PDGF and recruited macrophages is that under hypoxic conditions, these cells up-regulate

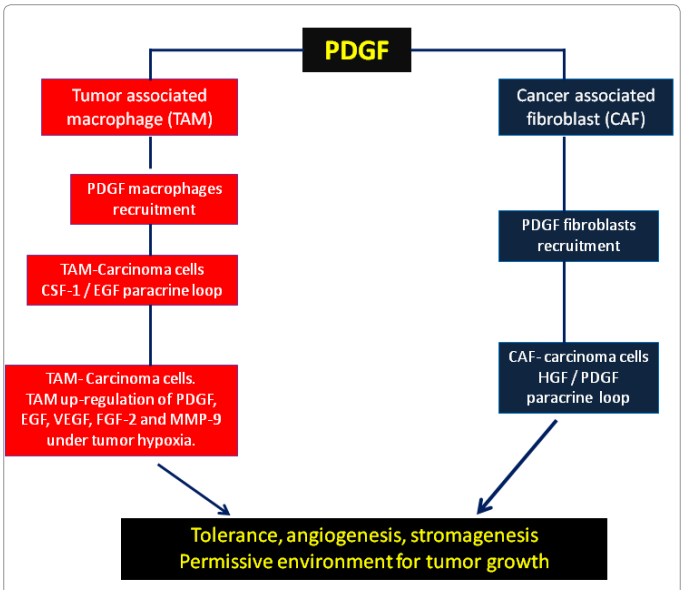

Figure 3: PDGF interactions and cross-talk in tumorigenesis. As described for wounds, PDGF may create a favorable environment for macrophages and fibroblasts recruitment into the tumor parenchyma. Macrophages-secreted PDGF as by other cells within the tumor recruits more macrophages and activates fibroblasts creating and interactive networking that instructs both cells to act as key players in tumor promotion/progression. As described by Wickoff and Condeelis, a paracrine self-propagating loop is created between tumor macrophages and tumor epithelial cells so that the former secrete EGF, which is a positive stimulus for the malignant epithelial cells that express EGFR. In turn, carcinoma cells secrete CSF-1 which attracts macrophages and induces them to secrete more EGF, thus completing the loop. Another example of tumor-perpetuating loop is given by TAM's response to hypoxia, so that growth factors with special tropism for epithelial (EGF), stromal (PDGF, fibroblast growth factor: FGF), and vascular cells (PDGF and vascular endothelial growth factor: VEGF) are released; thus enhancing carcinoma cells survival, stromagenesis by fibroblasts activation and angiogenesis. In this context metaloproteases (MMP) are also released by macrophages which remodel tumor storma. PDGF is involved in another loop which connects CAF and carcinoma cells. CAF secrete hepatocyte growth factor (HGF) which enhances carcinoma cells proliferation and migration. These cells in turn secrete PDGF which is used as activating and mitogenic factor for CAF.

an array of growth factors genes including EGF and PDGF itself that promote tumor proliferation, invasion, and angiogenesis [140] (Figure 3).

As depicted in Figure 3, fibroblasts (cancer-associated fibroblasts, CAF) have been recognized inside carcinomas and are increasingly implicated as functional participants. Inhibition of stromal PDGFR reduces proliferation of tumor cells and angiogenesis in cervical lesions, through a mechanism involving suppression of CAF-derived growth factors. Treatment with neutralizing antibodies to the PDGFR recapitulated these effects [141]. In line with this, PDGF is involved in another carcinoma cells and CAF cross talk. The carcinoma cells-derived PDGF induces the expression of hepatocyte growth factor (HGF) by CAF, and in turn, fibroblast-derived HGF leads to invasive growth of the carcinoma cells [142]. Finally, mounting evidences also implicate PDGF system and PDGFRB phosphorylation in lymphatic vessels growth and metastasis enhancement [136,143].

Concluding Remarks

Growth factors brought hopes to scientists and clinicians involved in the field of wound repair. For a while these agents promised to heal the so panicky chronic wounds so that for an orphan and challenging medical need, a valuable solution was about to be met. Soon after, laboratory evidences started to unveil the underlying connection of growth factors and cancer. Caution, disappointment and apprehensiveness clouded such times. After many years only EGF and PDGF have entered as prescribed medications for chronic ulcers. These polypeptides belong to different families and bind different receptors. Nevertheless, they share tyrosine kinase activity, similar accessory proteins and transduce signals through common canonical pathways – which trigger proliferation, migration, survival, etc.

However, the mechanisms whereby EGF and PDGFB impact on both wound repair and tumor growth are different. EGF does not modify inflammation and is far more prone to act on epithelial lineages. In contrast, PDGF recruits monocytes/macrophages to both scenarios which engender a first self-perpetuating loop with macrophages and fibroblasts. Thus, the exogenous administration of PDGF to chronic wounds may perturb the healing trajectory by protracting macrophages infiltration. PDGF is biologically endowed to repair tissues as it seems to influence of the fate and the behavior of most mesenchymal-derived cells. However, further studies are demanded to define the appropriate ulcer niche to be targeted, and the therapeutic window of administration as to ensure that PDGF therapy could be thoroughly useful.

Dissimilarities are also found in the role of these two growth factors in malignant transformation. An overwhelming amount of data converges to indicate that EGF over-exposure is not able to endow cells with full or permanent transforming ability. Over-expression of a wild type form of the EGFR appears to amplify normal EGF signal transduction, but does not impose irreversible transformation traits. Although it may look paradoxical or controversial, the expression of some other EGF family members rampantly harnesses tumor cells behavior more than EGF does.

PDGFB is experimentally entitled as a "self-sufficiency" factor for tumorigenesis in human diploid cells, nude mice and transgenic animals that bear PDGFB over-expressing vectors. Its role as an "initiation" agent as to cooperate in other co-carcinogenesis settings has been fully determined. The hyperproliferative and dedifferentiation states (in some cells) imposed by the PDGFB autocrine loop seems to be proximal to the accumulation of other transforming mutations. These evidences give rise to an additional line of caution before its clinical use in diabetic patients.

Nevertheless, despite the unequivocal evidences of PDGF sufficiency to transform cells in vivo; and despite the large casuistic of subjects treated with this growth factor; there is no scientific evidence that its exogenous administration to chronic ulcers has increased the incidence of Marjolin's ulcers. Therefore, further studies are demanded to set up clear-cut clinical indications and regulatory constrains for the medical use of each of these growth factors.

References

1. Mendelsohn J (2000) Blockade of receptors for growth factors: an anticancer therapy--the fourth annual Joseph H Burchenal American Association of Cancer Research Clinical Research Award Lecture. Clin Cancer Res 6: 747-753.
2. Croce CM (2008) Oncogenes and cancer. N Engl J Med 358: 502-511.
3. Berlanga-Acosta J, Gavilondo-Cowley J, Lopez-Saura P, Gonzalez-Lopez T, Castro-Santana MD, et al. (2009) Epidermal growth factor in clinical practice - a review of its biological actions, clinical indications and safety implications. Int Wound J 6: 331-346.
4. Robson MC, Phillips LG, Thomason A, Altrock BW, Pence PC, et al. (1992) Recombinant human platelet-derived growth factor-BB for the treatment of chronic pressure ulcers. Ann Plast Surg 29: 193-201.
5. Pierce GF, Tarpley JE, Allman RM, Goode PS, Serdar CM, et al. (1994) Tissue repair processes in healing chronic pressure ulcers treated with recombinant platelet-derived growth factor BB. Am J Pathol 145: 1399-1410.
6. Mustoe TA, Cutler NR, Allman RM, Goode PS, Deuel TF, et al. (1994) A phase II study to evaluate recombinant platelet-derived growth factor-BB in the treatment of stage 3 and 4 pressure ulcers. Arch Surg 129: 213-219.
7. Wieman TJ, Smiell JM, Su Y (1998) Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers. A phase III randomized placebo-controlled double-blind study. Diabetes Care 21: 822-827.
8. Smiell JM (1998) Clinical safety of becaplermin (rhPDGF-BB) gel. Becaplermin Studies Group. Am J Surg 176: 68S-73S.
9. LeGrand EK (1998) Preclinical promise of becaplermin (rhPDGF-BB) in wound healing. Am J Surg 176: 48S-54S.
10. Wieman TJ (1998) Clinical efficacy of becaplermin (rhPDGF-BB) gel. Becaplermin Gel Studies Group. Am J Surg 176: 74S-79S.
11. Dvorak HF (1986) Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 315: 1650-1659.
12. Bissell MJ, Radisky D (2001) Putting tumours in context. Nat Rev Cancer 1: 46-54.
13. Downward J, Yarden Y, Mayes E, Scrace G, Totty N, et al. (1984) Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. Nature 307: 521-527.
14. Normanno N, Maiello MR, De Luca A (2003) Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs): simple drugs with a complex mechanism of action? J Cell Physiol 194: 13-19.
15. Bazley LA, Gullick WJ (2005) The epidermal growth factor receptor family. Endocr Relat Cancer 1: S17-S27.
16. Higashiyama S, Iwabuki H, Morimoto C, Hieda M, Inoue H, et al. (2008) Membrane-anchored growth factors, the epidermal growth factor family: beyond receptor ligands. Cancer Sci 99: 214-220.
17. Oda K, Matsuoka Y, Funahashi A, Kitano H (2005) A comprehensive pathway map of epidermal growth factor receptor signaling. Mol Syst Biol 1: 2005.
18. Olayioye MA, Neve RM, Lane HA, Hynes NE (2000) The ErbB signaling network: receptor heterodimerization in development and cancer. EMBO J 19: 3159-3167.
19. Citri A, Skaria KB, Yarden Y (2003) The deaf and the dumb: the biology of ErbB-2 and ErbB-3. Exp Cell Res 284: 54-65.
20. Vincenzi B, Zoccoli A, Pantano F, Venditti O, Galluzzo S (2010) Cetuximab: from bench to bedside. Curr Cancer Drug Targets 10: 80-95.
21. Takeuchi K, Ito F (2010) EGF receptor in relation to tumor development: molecular basis of responsiveness of cancer cells to EGFR-targeting tyrosine kinase inhibitors. FEBS J 277: 316-326.

22. Pines G, Kostler WJ, Yarden Y (2010) Oncogenic mutant forms of EGFR: lessons in signal transduction and targets for cancer therapy. FEBS Lett 584: 2699-2706.

23. Wang SC, Hung MC (2009) Nuclear translocation of the epidermal growth factor receptor family membrane tyrosine kinase receptors. Clin Cancer Res 15: 6484-6489.

24. Gschwind A, Zwick E, Prenzel N, Leserer M, Ullrich A (2001) Cell communication networks: epidermal growth factor receptor transactivation as the paradigm for interreceptor signal transmission. Oncogene 20: 1594-1600.

25. Li J, Kim YN, Bertics PJ (2000) Platelet-derived growth factor-stimulated migration of murine fibroblasts is associated with epidermal growth factor receptor expression and tyrosine phosphorylation. J Biol Chem 275: 2951-2958.

26. Hong JP, Jung HD, Kim YW (2006) Recombinant human epidermal growth factor (EGF) to enhance healing for diabetic foot ulcers. Ann Plast Surg 56: 394-398.

27. Tsang MW, Wong WK, Hung CS, Lai KM, Tang W, et al. (2003) Human epidermal growth factor enhances healing of diabetic foot ulcers. Diabetes Care 26: 1856-1861.

28. Fernandez-Montequin JI, Valenzuela-Silva CM, Diaz OG, Savigne W, Sancho-Soutelo N, et al. (2009) Intra-lesional injections of recombinant human epidermal growth factor promote granulation and healing in advanced diabetic foot ulcers: multicenter, randomised, placebo-controlled, double-blind study. Int Wound J 6: 432-443.

29. Acosta JB, Savigne W, Valdez C, Franco N, Alba JS, et al. (2006) Epidermal growth factor intralesional infiltrations can prevent amputation in patients with advanced diabetic foot wounds. Int Wound J 3: 232-239.

30. Grotendorst GR, Hessinger DA (1999) Purification and partial characterization of the phospholipase A2 and co-lytic factor from sea anemone (Aiptasia pallida) nematocyst venom. Toxicon 37: 1779-1796.

31. Grayson LS, Hansbrough JF, Zapata-Sirvent RL, Dore CA, Morgan JL, et al. (1993) Quantitation of cytokine levels in skin graft donor site wound fluid. Burns 19: 401-405.

32. Grose R, Werner S (2003) Wound healing studies in transgenic and knockout mice. A review. Methods Mol Med 78: 191-216.

33. Werner S, Grose R (2003) Regulation of wound healing by growth factors and cytokines. Physiol Rev 83: 835-870.

34. Ono I, Gunji H, Zhang JZ, Maruyama K, Kaneko F (1995) Studies on cytokines related to wound healing in donor site wound fluid. J Dermatol Sci 10: 241-245.

35. Ono I, Gunji H, Zhang JZ, Maruyama K, Kaneko F (1995) A study of cytokines in burn blister fluid related to wound healing. Burns 21: 352-355.

36. Ellis IR, Schor AM, Schor SL (2007) EGF AND TGF-alpha motogenic activities are mediated by the EGF receptor via distinct matrix-dependent mechanisms. Exp Cell Res 313: 732-41.

37. Simpson RM, Wells A, Thomas D, Stephens P, Steadman R, et al. (2010) Aging fibroblasts resist phenotypic maturation because of impaired hyaluronan-dependent CD44/epidermal growth factor receptor signaling. Am J Pathol 176: 1215-1228.

38. Pastore S, Mascia F, Mariani V, Girolomoni G (2008) The epidermal growth factor Rrceptor system in skin repair and inflammation. J Invest Dermatol 128: 1365-1374.

39. Heidaran MA, Fleming TP, Bottaro DP, Bell GI, Di Fiore PP, et al. (1990) Transformation of NIH3T3 fibroblasts by an expression vector for the human epidermal growth factor precursor. Oncogene 5: 1265-1270.

40. Di Fiore PP, Pierce JH, Fleming TP, Hazan R, Ullrich A, et al. (1987) Overexpression of the human EGF receptor confers an EGF-dependent transformed phenotype to NIH 3T3 cells. Cell 51: 1063-70.

41. Massoglia S, Gray A, Dull TJ, Munemitsu S, Kun HJ, et al. (1990) Epidermal growth factor receptor cytoplasmic domain mutations trigger ligand-independent transformation. Mol Cell Biol 10: 3048-3055.

42. Kao CY, Oakley CS, Welsch CW, Chang CC (1997) Growth requirements and neoplastic transformation of two types of normal human breast epithelial cells derived from reduction mammoplasty. In Vitro Cell Dev Biol Anim 33: 282-288.

43. Boerner JL, Danielsen A, Maihle NJ (2003) Ligand-independent oncogenic signaling by the epidermal growth factor receptor: v-ErbB as a paradigm. Exp Cell Res 284: 111-121.

44. Hynes NE (2005) Receptor tyrosine kinases as therapeutic targets in cancer. Discov Med 5: 483-488.

45. Gazdar AF (2009) Personalized medicine and inhibition of EGFR signaling in lung cancer. N Engl J Med 361: 1018-1020.

46. Gilmore JL, Gallo RM, and Riese DJ 2nd (2006) The epidermal growth factor receptor (EGFR)-S442F mutant displays increased affinity for neuregulin-2beta and agonist-independent coupling with downstream signalling events. Biochem J 396: 79-88.

47. Riese DJ 2nd, Gallo RM, Settleman J (2007) Mutational activation of ErbB family receptor tyrosine kinases: insights into mechanisms of signal transduction and tumorigenesis. Bioessays 29: 558-65.

48. Lund KA, Lazar CS, Chen WS, Walsh BJ, Welsh JB, et al. (1990) Phosphorylation of the epidermal growth factor receptor at threonine 654 inhibits ligand-induced internalization and down-regulation. J Biol Chem 265: 20517-20523.

49. Xia L, Yuan YZ, Xu CD, Zhang YP, Qiao MM, et al. (2002) Effects of epidermal growth factor on the growth of human gastric cancer cell and the implanted tumor of nude mice. World J Gastroenterol 8: 455-458.

50. Gong L, Debruyne PR, Witek M, Nielsen K, Snook A, et al. (2009) Bile acids initiate lineage-addicted gastroesophageal tumorigenesis by suppressing the EGF receptor-AKT axis. Clin Transl Sci 2: 286-293.

51. Moran AE, Hunt DH, Javid SH, Redston M, Carothers AM, et al. (2004) Apc deficiency is associated with increased Egfr activity in the intestinal enterocytes and adenomas of C57BL/6J-Min/+ mice. J Biol Chem 279: 43261-43272.

52. Bashir O, Fitzgerald AJ, Berlanga-Acosta J, Playford RJ, Goodlad RA (2003) Effect of epidermal growth factor administration on intestinal cell proliferation, crypt fission and polyp formation in multiple intestinal neoplasia (Min) mice. Clin Sci (Lond) 105: 323-330.

53. Wilson KJ, Gilmore JL, Foley J, Lemmon MA, Riese DJ 2nd (2009) Functional selectivity of EGF family peptide growth factors: implications for cancer. Pharmacol Ther 122: 1-8.

54. Krakowski ML, Kritzik MR, Jones EM, Krahl T, Lee J, et al. (1999) Transgenic expression of epidermal growth factor and keratinocyte growth factor in beta-cells results in substantial morphological changes. J Endocrinol 162: 167-175.

55. Wong RW, Kwan RW, Mak PH, Mak KK, Sham MH, et al. (2000) Overexpression of epidermal growth factor induced hypospermatogenesis in transgenic mice. J Biol Chem 275: 18297-18301.

56. Kullenberg B, Jansen C, Fredang N, Ohlsson B, Axelson J (2000) Transforming growth factor alpha (TGF-alpha) increases cell number in a human pancreatic cancer cell line but not in normal mouse pancreas. Int J Pancreatol 28: 199-205.

57. Sandgren EP, Luetteke NC, Qiu TH, Palmiter RD, Brinster RL, et al. (1993) Transforming growth factor alpha dramatically enhances oncogene-induced carcinogenesis in transgenic mouse pancreas and liver. Mol Cell Biol 13: 320-330.

58. Rose-Hellekant TA, Sandgren EP (2000) Transforming growth factor alpha- and c-myc-induced mammary carcinogenesis in transgenic mice. Oncogene 19: 1092-1096.

59. Thorgeirsson SS, Santoni-Rugiu E, Davis CD, Snyderwine EG (1997) Hepatic tumor induction in c-myc mono-transgenic and TGF-alpha/c-myc double-transgenic mice. Arch Toxicol Suppl 19: 359-366.

60. Calvisi DF, Factor VM, Loi R, Thorgeirsson SS (2001) Activation of beta-catenin during hepatocarcinogenesis in transgenic mouse models: relationship to phenotype and tumor grade. Cancer Res 61: 2085-2091.

61. Tamano S, Merlino GT, Ward JM (1994) Rapid development of hepatic tumors in transforming growth factor alpha transgenic mice associated with increased cell proliferation in precancerous hepatocellular lesions initiated by N-nitrosodiethylamine and promoted by phenobarbital. Carcinogenesis 15: 1791-1798.

62. Cook PW, Brown JR, Cornell KA, Pittelkow MR (2004) Suprabasal expression of human amphiregulin in the epidermis of transgenic mice induces a severe, early-onset, psoriasis-like skin pathology: expression of amphiregulin in the basal epidermis is also associated with synovitis. Exp Dermatol 13: 347-56.

63. Cheng J, Huang H, Zhang ZT, Shapiro E, Pellicer A, et al. (2002) Overexpression of epidermal growth factor receptor in urothelium elicits urothelial hyperplasia and promotes bladder tumor growth. Cancer Res 62: 4157-4163.

64. Wei Q, Clarke L, Scheidenhelm DK, Qian B, Tong A, et al. (2006) High-grade glioma formation results from postnatal pten loss or mutant epidermal growth factor receptor expression in a transgenic mouse glioma model. Cancer Res 66: 7429-7437.

65. Prenzel N, Fischer OM, Streit S, Hart S, Ullrich A (2001) The epidermal growth factor receptor family as a central element for cellular signal transduction and diversification. Endocr Relat Cancer 8: 11-31.

66. Wang F, Liu R, Lee SW, Sloss CM, Couget J, et al. (2007) Heparin-binding EGF-like growth factor is an early response gene to chemotherapy and contributes to chemotherapy resistance. Oncogene 26: 2006-2016.

67. Revillion F, Lhotellier V, Hornez L, Bonneterre J, Peyrat JP (2008) ErbB/HER ligands in human breast cancer, and relationships with their receptors, the bio-pathological features and prognosis. Ann Oncol 19: 73-80.

68. Lee S, Medina D, Tsimelzon A, Mohsin SK, Mao S, et al. (2007) Alterations of gene expression in the development of early hyperplastic precursors of breast cancer. Am J Pathol 171: 252-262.

69. Lemos-Gonzalez Y, Rodriguez-Berrocal FJ, Cordero OJ, Gomez C, Paez de la Cadena M (2007) Alteration of the serum levels of the epidermal growth factor receptor and its ligands in patients with non-small cell lung cancer and head and neck carcinoma. Br J Cancer 96: 1569-1578.

70. Kakiuchi S, Daigo Y, Ishikawa N, Furukawa C, Tsunoda T, et al. (2004) Prediction of sensitivity of advanced non-small cell lung cancers to gefitinib (Iressa, ZD1839). Hum Mol Genet 13: 3029-43.

71. Shibata MA, Ward JM, Green JE, Merlino G (1997) Enhanced sensitivity to tumor growth and development in multistage skin carcinogenesis by transforming growth factor-alpha-induced epidermal growth factor receptor activation but not p53 inactivation. Mol Carcinog 18: 160-170.

72. Molinolo AA, Amornphimoltham P, Squarize CH, Castilho RM, Patel V, et al. (2009) Dysregulated molecular networks in head and neck carcinogenesis. Oral Oncol 45: 324-334.

73. Hellberg C, Ostman A, Heldin CH (2010) PDGF and vessel maturation. Recent Results Cancer Res 180: 103-114.

74. Andrae J, Gallini R, Betsholtz C (2008) Role of platelet-derived growth factors in physiology and medicine. Genes Dev 22: 1276-1312.

75. Doolittle RF, Hunkapiller MW, Hood LE, Devare SG, Robbins KC, et al. (1983) Simian sarcoma virus onc gene, v-sis, is derived from the gene (or genes) encoding a platelet-derived growth factor. Science 221: 275-277.

76. Tallquist M, Kazlauskas A (2004) PDGF signaling in cells and mice. Cytokine Growth Factor Rev 15: 205-213.

77. Leveen P, Pekny M, Gebre-Medhin S, Swolin B, Larsson E, et al. (1994) Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. Genes Dev 8: 1875-1887.

78. Heldin CH, Westermark B (1999) Mechanism of action and in vivo role of platelet-derived growth factor. Physiol Rev 79: 1283-1316.

79. Yoeli-Lerner M, Toker A (2006) Akt/PKB signaling in cancer: a function in cell motility and invasion. Cell Cycle 5: 603-605.

80. Gotzmann J, Fischer AN, Zojer M, Mikula M, Proell V, et al. (2006) A crucial function of PDGF in TGF-beta-mediated cancer progression of hepatocytes. Oncogene 25: 3170-3185.

81. Leask A (2010) Towards an anti-fibrotic therapy for scleroderma: targeting myofibroblast differentiation and recruitment. Fibrogenesis Tissue Repair 3: 8.

82. Hoch RV, Soriano P (2003) Roles of PDGF in animal development. Development 130: 4769-4784.

83. Liu Y, Wen XM, Lui EL, Friedman SL, Cui W, et al. (2009) Therapeutic targeting of the PDGF and TGF-beta-signaling pathways in hepatic stellate cells by PTK787/ZK22258. Lab Invest 89: 1152-1160.

84. Rubin K, Terracio L, Ronnstrand L, Heldin CH, Klareskog L (1988) Expression of platelet-derived growth factor receptors is induced on connective tissue cells during chronic synovial inflammation. Scand J Immunol 27: 285-294.

85. Heldin CH, Ostman A, Ronnstrand L (1998) Signal transduction via platelet-derived growth factor receptors. Biochim Biophys Acta 1378: F79-113.

86. Pahara J, Shi H, Chen X, Wang Z (2010) Dimerization drives PDGF receptor endocytosis through a C-terminal hydrophobic motif shared by EGF receptor. Exp Cell Res 316: 2237-2250.

87. Wardega P, Heldin CH, Lennartsson J (2010) Mutation of tyrosine residue 857 in the PDGF beta-receptor affects cell proliferation but not migration. Cell Signal 22: 1363-1368.

88. Heldin CH (2004) Development and possible clinical use of antagonists for PDGF and TGF-beta. Ups J Med Sci 109: 165-178.

89. Lennartsson J, Burovic F, Witek B, Jurek A, Heldin CH (2010) Erk 5 is necessary for sustained PDGF-induced Akt phosphorylation and inhibition of apoptosis. Cell Signal 22: 955-960.

90. Zheng L, Ishii Y, Tokunaga A, Hamashima T, Shen J, et al. (2010) Neuroprotective effects of PDGF against oxidative stress and the signaling pathway involved. J Neurosci Res 88: 1273-1284.

91. Wilhelm SM, Adnane L, Newell P, Villanueva A, Llovet JM, et al. (2008) Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. Mol Cancer Ther 7: 3129-3140.

92. Papanas N, Liakopoulos V, Maltezos E, Stefanidis I (2007) The diabetic foot in end stage renal disease. Ren Fail 29: 519-528.

93. Pierce GF, Mustoe TA, Senior RM, Reed J, Griffin GL, et al. (1988) In vivo incisional wound healing augmented by platelet-derived growth factor and recombinant c-sis gene homodimeric proteins. J Exp Med 167: 974-987.

94. Pierce GF, Mustoe TA, Lingelbach J, Masakowski VR, Griffin GL, et al. (1989) Platelet-derived growth factor and transforming growth factor-beta enhance tissue repair activities by unique mechanisms. J Cell Biol 109: 429-440.

95. Pierce GF, Vande BJ, Rudolph R, Tarpley J, Mustoe TA (1991) Platelet-derived growth factor-BB and transforming growth factor beta 1 selectively modulate glycosaminoglycans, collagen, and myofibroblasts in excisional wounds. Am J Pathol 138: 629-646.

96. Watterson KR, Lanning DA, Diegelmann RF, Spiegel S (2007) Regulation of fibroblast functions by lysophospholipid mediators: potential roles in wound healing. Wound Repair Regen 15: 607-616.

97. Jiang H, Rhee S, Ho CH, Grinnell F (2008) Distinguishing fibroblast promigratory and procontractile growth factor environments in 3-D collagen matrices. FASEB J 22: 2151-2160.

98. Rajkumar VS, Shiwen X, Bostrom M, Leoni P, Muddle J, et al. (2006) Platelet-derived growth factor-beta receptor activation is essential for fibroblast and pericyte recruitment during cutaneous wound healing. Am J Pathol 169: 2254-2265.

99. Ansel JC, Tiesman JP, Olerud JE, Krueger JG, Krane JF, et al. (1993) Human keratinocytes are a major source of cutaneous platelet-derived growth factor. J Clin Invest 92: 671-678.

100. Antoniades HN, Galanopoulos T, Neville-Golden J, Kiritsy CP, Lynch SE (1991) Injury induces in vivo expression of platelet-derived growth factor (PDGF) and PDGF receptor mRNAs in skin epithelial cells and PDGF mRNA in connective tissue fibroblasts. Proc Natl Acad Sci U S A 88: 565-569.

101. Braiman-Wiksman L, Solomonik I, Spira R, Tennenbaum T (2007) Novel insights into wound healing sequence of events. Toxicol Pathol 35: 767-779.

102. Battegay EJ, Rupp J, Iruela-Arispe L, Sage EH, Pech M (1994) PDGF-BB modulates endothelial proliferation and angiogenesis in vitro via PDGF beta-receptors. J Cell Biol 125: 917-928.

103. Lindblom P, Gerhardt H, Liebner S, Abramsson A, Enge M, et al. (2003) Endothelial PDGF-B retention is required for proper investment of pericytes in the microvessel wall. Genes Dev 17: 1835-1840.

104. Appelmann I, Liersch R, Kessler T, Mesters RM, Berdel WE (2010) Angiogenesis inhibition in cancer therapy: platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) and their receptors: biological functions and role in malignancy. Recent Results Cancer Res 180: 51-81.

105. Hellström M, Kaln M, Lindahl P, Abramsson A, Betsholtz C (1999) Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. Development 126: 3047-55.

106. Gao Z, Sasaoka T, Fujimori T, Oya T, Ishii Y, et al. (2005) Deletion of the PDGFR-beta Gene affects key fibroblast functions important for wound healing. J Biol Chem 280: 9375-9389.

107. Greenhalgh DG, Sprugel KH, Murray MJ, Ross R (1990) PDGF and FGF stimulate wound healing in the genetically diabetic mouse. Am J Pathol 136: 1235-1246.

108. Beer HD, Longaker MT, Werner S (1997) Reduced expression of PDGF and PDGF receptors during impaired wound healing. J Invest Dermatol 109: 132-138.

109. Ashcroft GS, Horan MA, Ferguson MW (1997) The effects of ageing on wound healing: immunolocalisation of growth factors and their receptors in a murine incisional model. J Anat 190: 351-365.

110. Cao Y, Hong A, Schulten H, Post MJ (2005) Update on therapeutic neovascularization. Cardiovasc Res 65: 639-648.

111. Agren MS, Steenfos HH, Dabelsteen S, Hansen JB, Dabelsteen E (1999) Proliferation and mitogenic response to PDGF-BB of fibroblasts isolated from chronic venous leg ulcers is ulcer-age dependent. J Invest Dermatol 112: 463-469.

112. Pierce GF, Tarpley JE, Tseng J, Bready J, Chang D, et al. (1995) Detection of platelet-derived growth factor (PDGF)-AA in actively healing human wounds treated with recombinant PDGF-BB and absence of PDGF in chronic nonhealing wounds. J Clin Invest 96: 1336-1350.

113. Hitoshi Y, Harris BT, Liu H, Popko B, Israel MA (2008) Spinal Glioma: Platelet-Derived Growth Factor B-Mediated Oncogenesis in the Spinal Cord. Cancer Res 68: 8507-8515.

114. Waterfield MD, Scrace GT, Whittle N, Stroobant P, Johnsson A, et al. (1983) Platelet-derived growth factor is structurally related to the putative transforming protein p28sis of simian sarcoma virus. Nature 304: 35-39.

115. Clarke MF, Westin E, Schmidt D, Josephs SF, Ratner L, et al. (1984) Transformation of NIH 3T3 cells by a human c-sis cDNA clone. Nature 308: 464-467.

116. Garrett JS, Coughlin SR, Niman HL, Tremble PM, Giels GM, et al. (1984) Blockade of autocrine stimulation in simian sarcoma virus-transformed cells reverses down-regulation of platelet-derived growth factor receptors. Proc Natl Acad Sci U S A 81: 7466-7470.

117. Leal F, Williams LT, Robbins KC, Aaronson SA (1985) Evidence that the v-sis gene product transforms by interaction with the receptor for platelet-derived growth factor. Science 230: 327-330.

118. Yeh HJ, Pierce GF, Deuel TF (1987) Ultrastructural localization of a platelet-derived growth factor/v-sis-related protein(s) in cytoplasm and nucleus of simian sarcoma virus-transformed cells. Proc Natl Acad Sci U S A 84: 2317-2321.

119. Johnsson A, Betsholtz C, Heldin CH, Westermark B (1985) Antibodies against platelet-derived growth factor inhibit acute transformation by simian sarcoma virus. Nature 317: 438-440.

120. Pech M, Gazit A, Arnstein P, Aaronson SA (1989) Generation of fibrosarcomas in vivo by a retrovirus that expresses the normal B chain of platelet-derived growth factor and mimics the alternative splice pattern of the v-sis oncogene. Proc Natl Acad Sci U S A 86: 2693-2697.

121. Uhrbom L, Hesselager G, Nister M, Westermark B (1998) Induction of brain tumors in mice using a recombinant platelet-derived growth factor B-chain retrovirus. Cancer Res 58: 5275-5279.

122. Dai C, Celestino JC, Okada Y, Louis DN, Fuller GN, et al. (2001) PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes in vivo. Genes Dev 15: 1913-1925.

123. Shih AH, Dai C, Hu X, Rosenblum MK, Koutcher JA, et al. (2004) Dose-dependent effects of platelet-derived growth factor-B on glial tumorigenesis. Cancer Res 64: 4783-4789.

124. Govindarajan B, Shah A, Cohen C, Arnold RS, Schechner J, et al. (2005) Malignant transformation of human cells by constitutive expression of platelet-derived growth factor-BB. J Biol Chem 280: 13936-13943.

125. Assanah M, Lochhead R, Ogden A, Bruce J, Goldman J, et al. (2006) Glial progenitors in adult white matter are driven to form malignant gliomas by platelet-derived growth factor-expressing retroviruses. J Neurosci 26: 6781-6790.

126. Appolloni I, Calzolari F, Tutucci E, Caviglia S, Terrile M, et al. (2009) PDGF-B induces a homogeneous class of oligodendrogliomas from embryonic neural progenitors. Int J Cancer 124: 2251-2259.

127. Effendi K, Sakamoto M (2010) Molecular pathology in early hepatocarcinogenesis. Oncology 78: 157-160.

128. Maass T, Thieringer FR, Mann A, Longerich T, Schirmacher P, et al. (2011) Liver specific overexpression of platelet-derived growth factor-B accelerates liver cancer development in chemically induced liver carcinogenesis. Int J Cancer 128: 1259-1268.

129. Lederle W, Stark HJ, Skobe M, Fusenig NE, Mueller MM (2006) Platelet-derived growth factor-BB controls epithelial tumor phenotype by differential growth factor regulation in stromal cells. Am J Pathol 169: 1767-1783.

130. Alvarez RH, Kantarjian HM, Cortes JE (2006) Biology of Platelet-Derived Growth Factor and Its Involvement in Disease. Mayo Clin Proc 81: 1241-1257.

131. Holdhoff M, Supko JG, Gallia GL Hann CL, Bonekamp D et al. (2009) Intratumoral concentrations of imatinib after oral administration in patients with glioblastoma multiforme. J Neurooncol 97: 241-245.

132. Brennan C, Momota H, Hambardzumyan D, Ozawa T, Tandon A et al. (2009) Glioblastoma subclasses can be defined by activity among signal transduction pathways and associated genomic alterations. PLoS One 4: e7752.

133. Li G, Mitra S, Wong AJ (2010) The epidermal growth factor variant III peptide vaccine for treatment of malignant gliomas. Neurosurg Clin N Am 21: 87-93.

134. Tomasson MH, Williams IR, Hasserjian R, Udomsakdi C, McGrath SM, et al. (1999) TEL/PDGFbetaR induces hematologic malignancies in mice that respond to a specific tyrosine kinase inhibitor. Blood 93: 1707-1714.

135. Stover EH, Chen J, Lee BH, Cools J, McDowell E, et al. (2005) The small molecule tyrosine kinase inhibitor AMN107 inhibits TEL-PDGFRbeta and FIP1L1-PDGFRalpha in vitro and in vivo. Blood 106: 3206-3213.

136. Langley RR, Fidler IJ (2007) Tumor cell-organ microenvironment interactions in the pathogenesis of cancer metastasis. Endocr Rev 28: 297-321.

137. Forsberg K, Valyi-Nagy I, Heldin CH, Herlyn M, Westermark B (1993) Platelet-derived growth factor (PDGF) in oncogenesis: development of a vascular connective tissue stroma in xenotransplanted human melanoma producing PDGF-BB. Proc Natl Acad Sci USA 90: 393-397.

138. Calorini L, Bianchini F (2010) Environmental control of invasiveness and metastatic dissemination of tumor cells: the role of tumor cell-host cell interactions. Cell Commun Signal 8: 24.

139. Wyckoff J, Wang W, Lin EY, Wang Y, Pixley F, et al. (2004) A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. Cancer Res 64: 7022-7029.

140. Mancino A, Lawrence T (2010) Nuclear factor-kappaB and tumor-associated macrophages. Clin Cancer Res 16: 784-789.

141. Matei D, Emerson RE, Lai YC, Baldridge LA, Rao J, et al. (2006) Autocrine activation of PDGFRalpha promotes the progression of ovarian cancer. Oncogene 25: 2060-2069.

142. Nakamura T, Matsumoto K, Kiritoshi A, Tano Y, Nakamura T (1997) Induction of hepatocyte growth factor in fibroblasts by tumor-derived factors affects invasive growth of tumor cells: in vitro analysis of tumor-stromal interactions. Cancer Res 57: 3305-3313.

143. Kodama M, Kitadai Y, Sumida T, Ohnishi M, Ohara E, et al. (2010) Expression of platelet-derived growth factor (PDGF)-B and PDGF-receptor β is associated with lymphatic metastasis in human gastric carcinoma. Cancer Sci 101: 1984-1989.