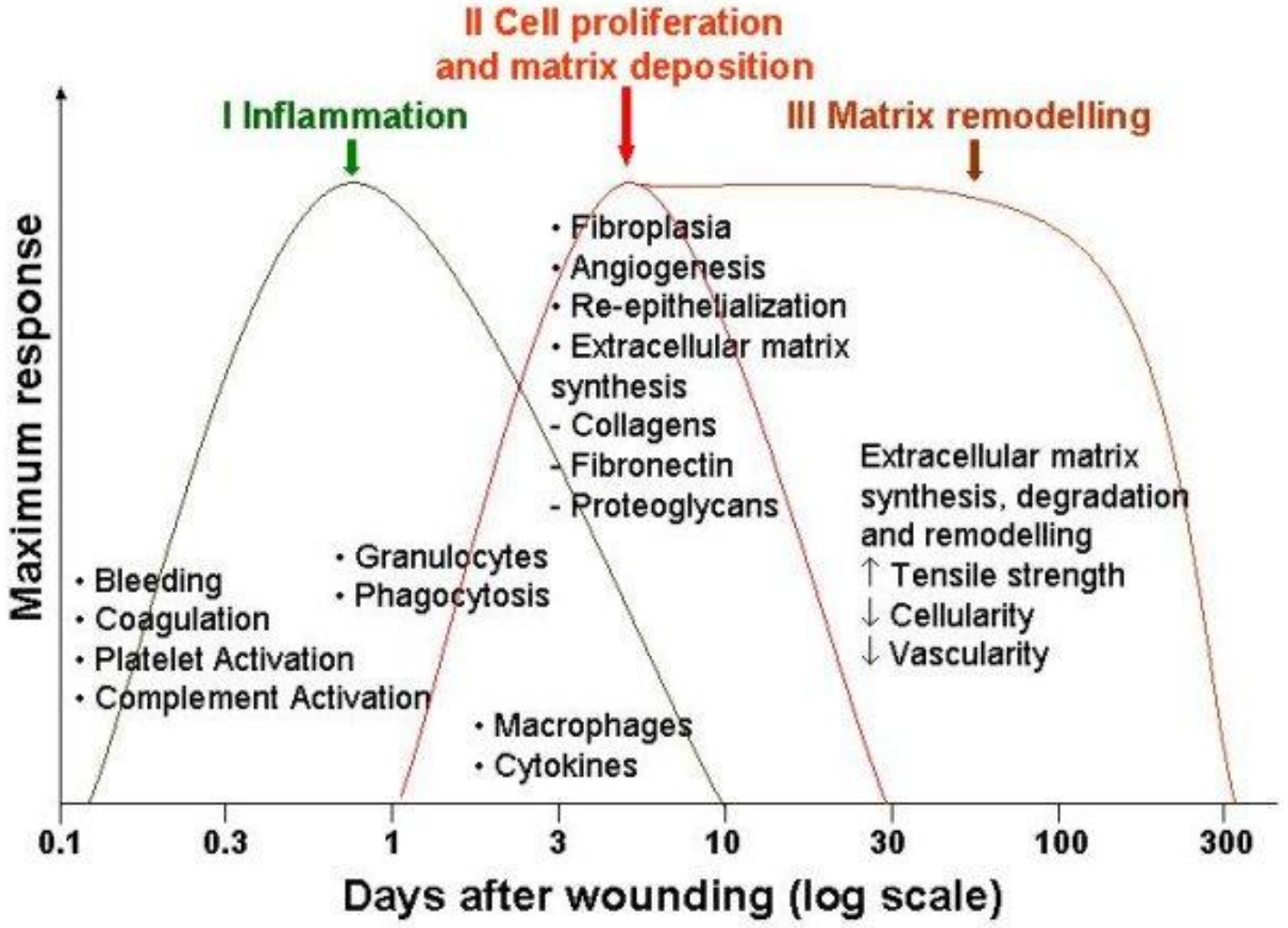
Биофармацевтика, занятие 6
Факторы роста – ЭФР, тромбоцитарный фактор роста, ФРФ, ТРФ, нейротропные ростовые факторы

Иван И. Воробьев, к.х.н.
ptichman@gmail.com

МГУ, ФФМ

23.04.13

Заживление ран

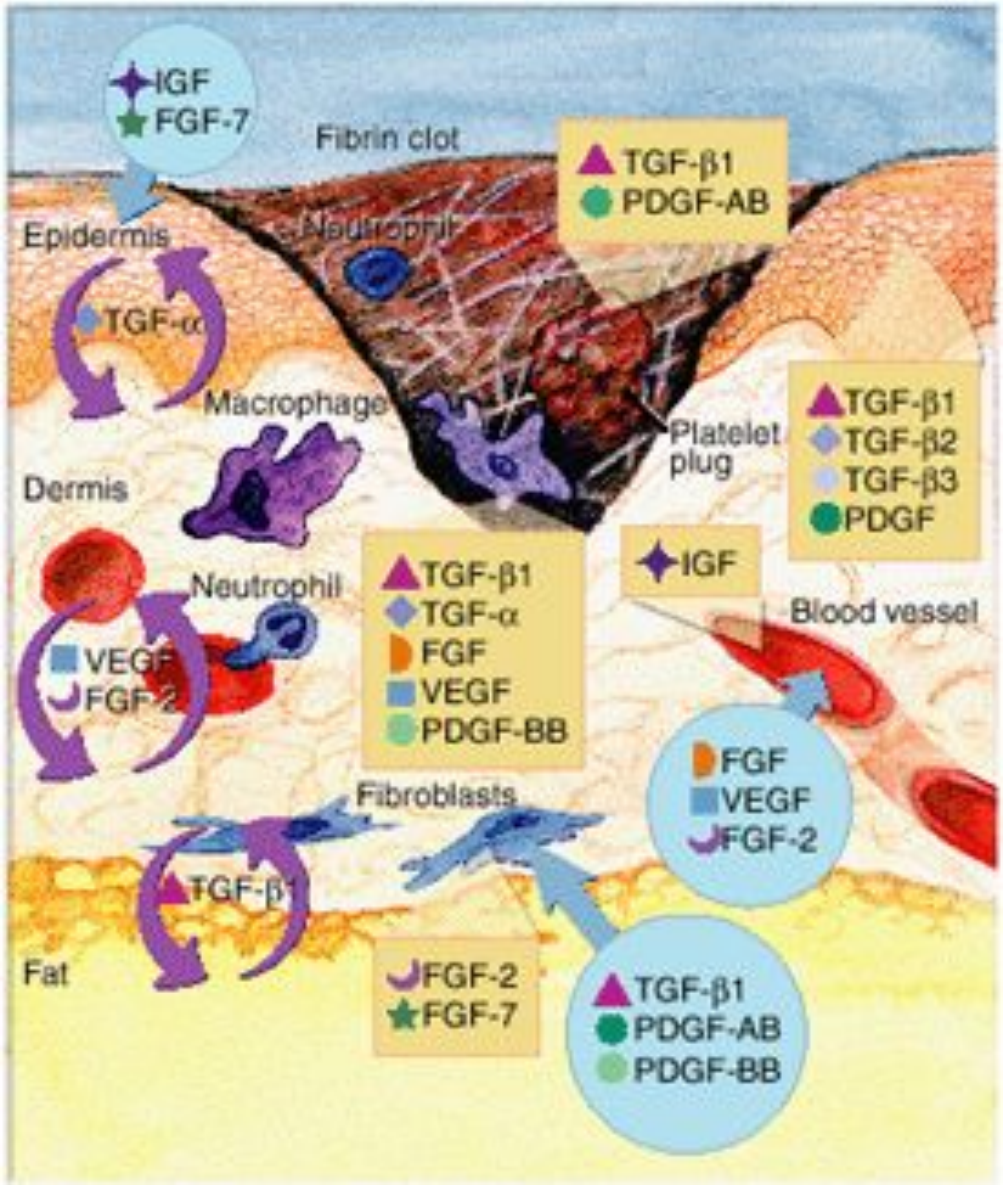


Stuart Enoch, Patricia Price. Cellular, molecular and biochemical differences in the pathophysiology of healing between acute wounds, chronic wounds and wounds in the aged // World Wide Wounds, online Aug 2004

Заживление ран

- **Haemostasis** he alpha granules of the platelets - PDGF, IGF-1, EGF, TGF-β. These proteins initiate the wound healing cascade by attracting and activating fibroblasts, endothelial cells and macrophages.
- **Early inflammatory phase** activation of complement, infiltration of the wound with granulocytes or polymorphonuclear leucocytes (PMNLs). attracted by C5a, platelets, TGF-β. cleared away by extrusion to the wound surface
- **Late inflammatory phase** Blood monocytes to tissue macrophages. Monocytes attracted by complement, clotting components, IgG fragments, collagen and elastin breakdown products, leukotriene B4, platelet factor IV, PDGF and TGF-β. release further cytokines and growth factors into the wound, recruiting fibroblasts, keratinocytes and endothelial cells. release collagenase, secrete TGF-α, heparin-binding epidermal growth factor (HB-EGF) bFGF. Lymphocyte (>72 h) attracted by IL-1, IgG and complement. IL-1 regulates collagenase, thus remodeling extracellular matrix (ECM).
- **Proliferative phase** Granulation tissue.
  - **Fibroblast migration**: Fb produce the matrix proteins fibronectin, hyaluronan (HA) and later collagen and proteoglycans. Attracted by PDGF and TGF-β, construct the new ECM.
  - **Collagen synthesis**: By Fb - types I and III collagen to form the new matrix.
  - **Angiogenesis**: By releasing of TGF-β and PDGF by platelets, attract macrophages and granulocytes. Mp releases tumour necrosis factor-α; and bFGF.
  - **Granulation tissue formation**:
  - **Epithelialisation**: EGF for mitogenesis and chemotaxis, bFGF and KGF- epithelial proliferation.
- **Remodelling phase** Collagen degradation by MMPs, produced by Fb, granulocytes, Mp. Cut by TIMPs. Mediated by TGF-β. Contraction by Fb and ECM

Заживление ран – ростовые факторы



Cytokines in Wound Healing in *R&D Systems' 2002 catalog*

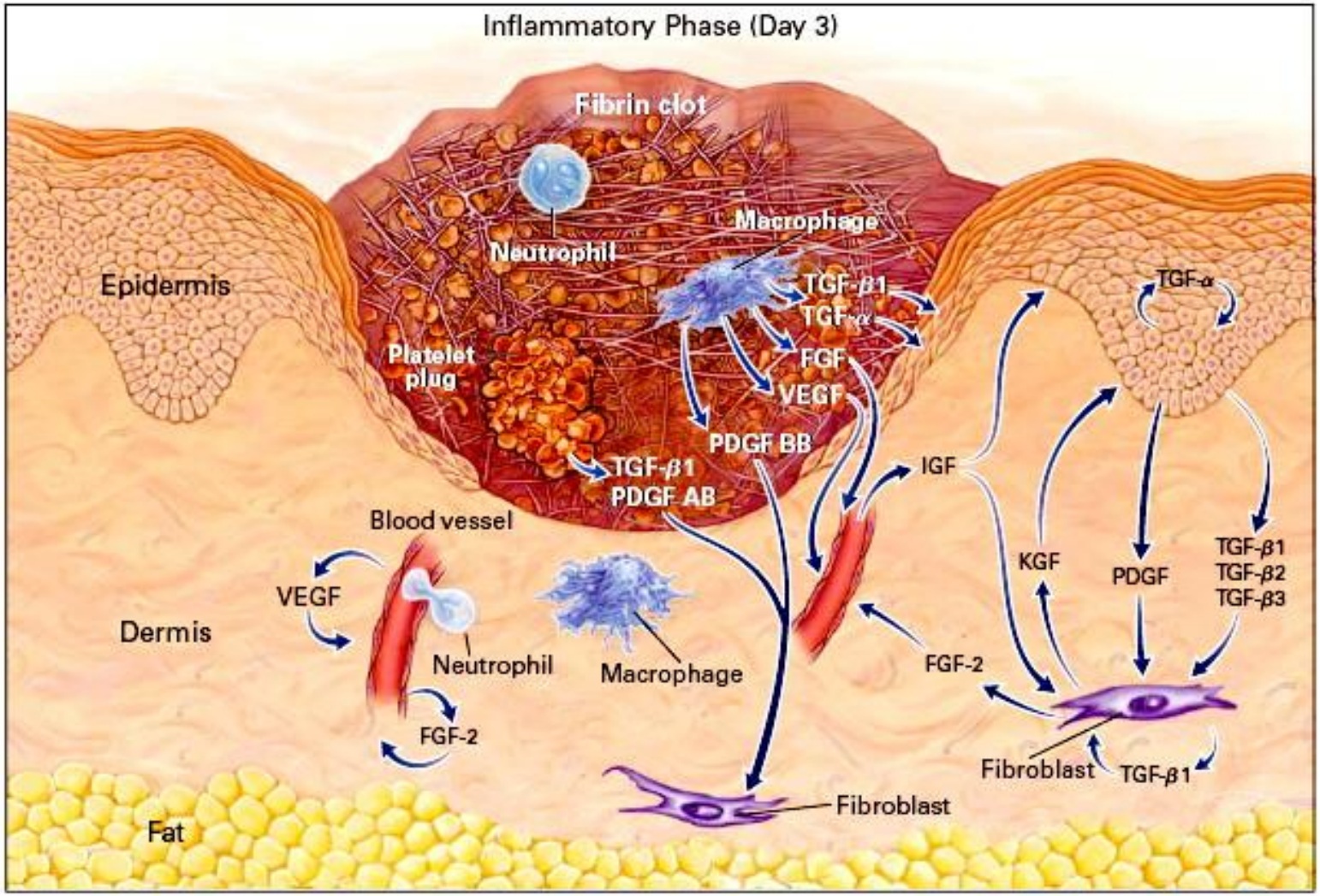


Figure 1. A cutaneous wound three days after injury. Growth factors thought to be necessary for cell movement into the wound are shown: TGF*β* 1, TGF*β* 2, and TGF*β* 3: transforming growth factor *β* 1, *β* 2, and *β*3, respectively
TGFα: transforming growth factor α, FGF: fibroblast growth factor, VEGF: vascular endothelial growth factor
PDGF, PDGF AB, and PDGF BB: platelet-derived growth factor, platelet-derived growth factor AB, and platelet-derived growth factor BB, respectively, IGF: insulin-like growth factor, KGF: keratinocyte growth factor.

Singer AJ, Clark RA. **Cutaneous wound healing**. N Engl J Med 341:738-746, 1999.

Singer & Clark. N Engl J Med 341:738-746, 1999.

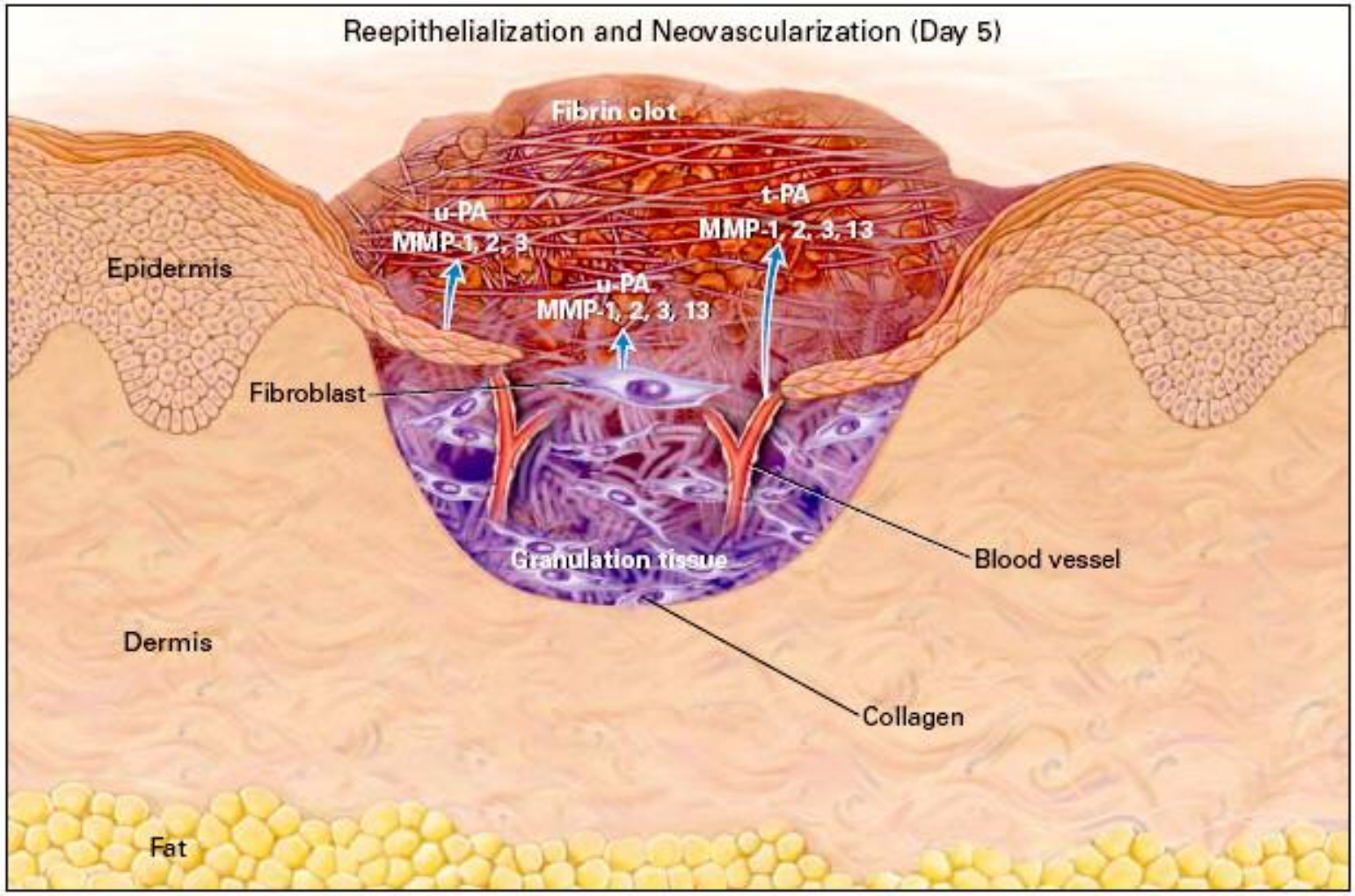
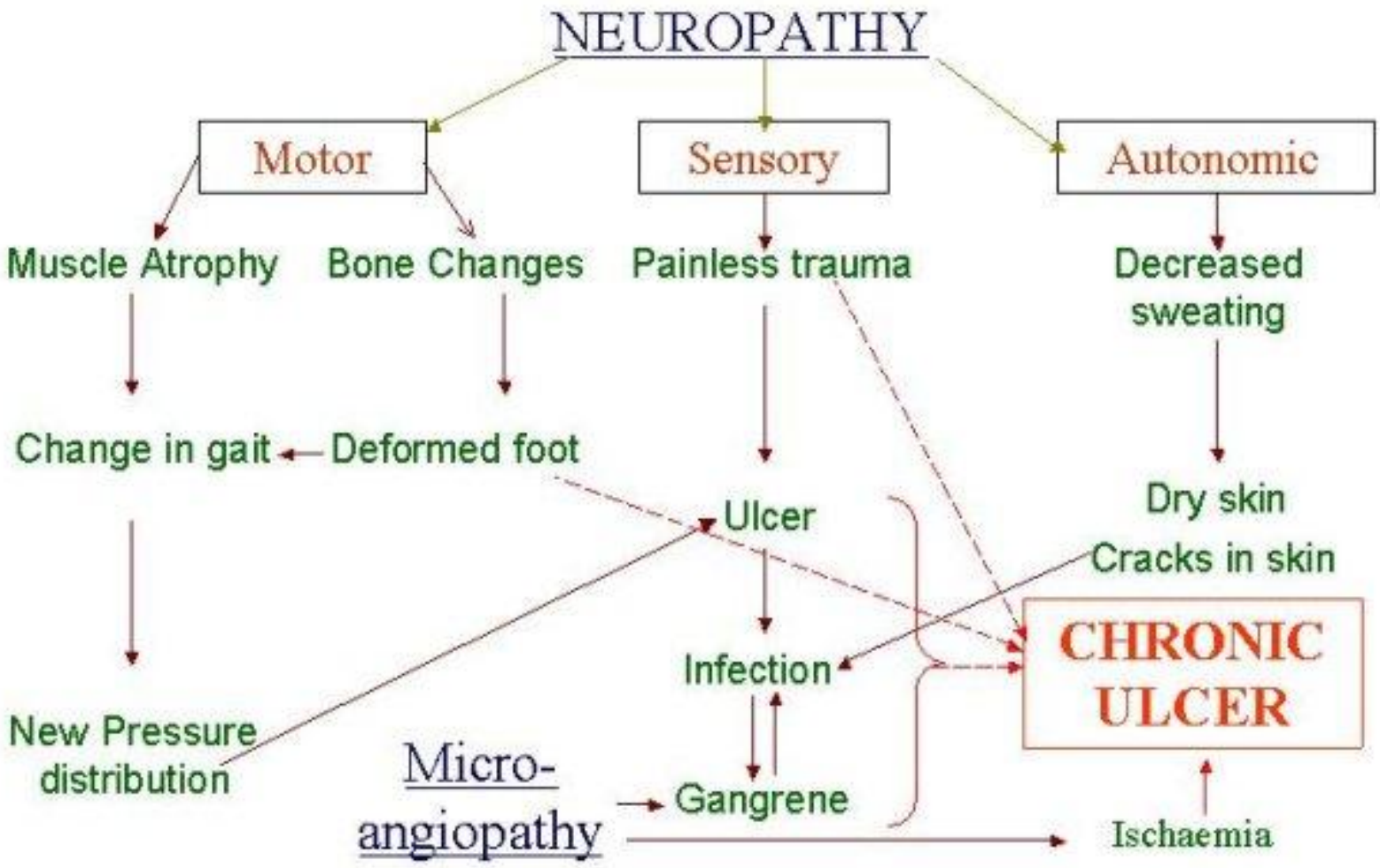


Figure 2. **A cutaneous wound five days after injury**. Blood vessels are seen sprouting into the fibrin clot as epidermal cells resurface the wound. Proteinases thought to be necessary for cell movement are shown. u-PA: urokinase-type plasminogen activator; MMP-1, 2, 3, and 13: matrix metalloproteinases 1, 2, 3, and 13 (collagenase 1, gelatinase A, stromelysin 1, and collagenase 3, respectively); t-PA: tissue plasminogen activator.

Синдром диабетической стопы



Stuart Enoch, Patricia Price. Cellular, molecular and biochemical differences in the pathophysiology of healing between acute wounds, chronic wounds and wounds in the aged // World Wide Wounds, online Aug 2004

TABLE 1. CYTOKINES THAT AFFECT WOUND HEALING.

CYTOKINE	MAJOR SOURCE	TARGET CELLS AND MAJOR EFFECTS
Epidermal growth factor family		Epidermal and mesenchymal regeneration
Epidermal growth factor	Platelets	Pleiotropic-cell motility and proliferation
Transforming growth factor α	Macrophages, epidermal cells	Pleiotropic-cell motility and proliferation
Heparin-binding epidermal growth factor	Macrophages	Pleiotropic-cell motility and proliferation
Fibroblast growth factor family		Wound vascularization
Basic fibroblast growth factor	Macrophages, endothelial cells	Angiogenesis and fibroblast proliferation
Acidic fibroblast growth factor	Macrophages, endothelial cells	Angiogenesis and fibroblast proliferation
Keratinocyte growth factor	Fibroblasts	Epidermal-cell motility and proliferation
Transforming growth factor β family		Fibrosis and increased tensile strength
Transforming growth factors β1 and β2	Platelets, macrophages	Epidermal-cell motility, chemotaxis of macrophages and fibroblasts, extracellular-matrix synthesis and remodeling
Transforming growth factor β3	Macrophages	Antiscarring effects
Other		
Platelet-derived growth factor	Platelets, macrophages, epidermal cells	Fibroblast proliferation and chemoattraction, macrophage chemoattraction and activation
Vascular endothelial growth factor	Epidermal cells, macrophages	Angiogenesis and increased vascular permeability
Tumor necrosis factor α	Neutrophils	Pleiotropic expression of growth factors
Interleukin-1	Neutrophils	Pleiotropic expression of growth factors
Insulin-like growth factor I	Fibroblasts, epidermal cells	Reepithelialization and granulation-tissue formation
Colony-stimulating factor 1	Multiple cells	Macrophage activation and granulation-tissue formation

Эпидермальный фактор роста - ЭФР

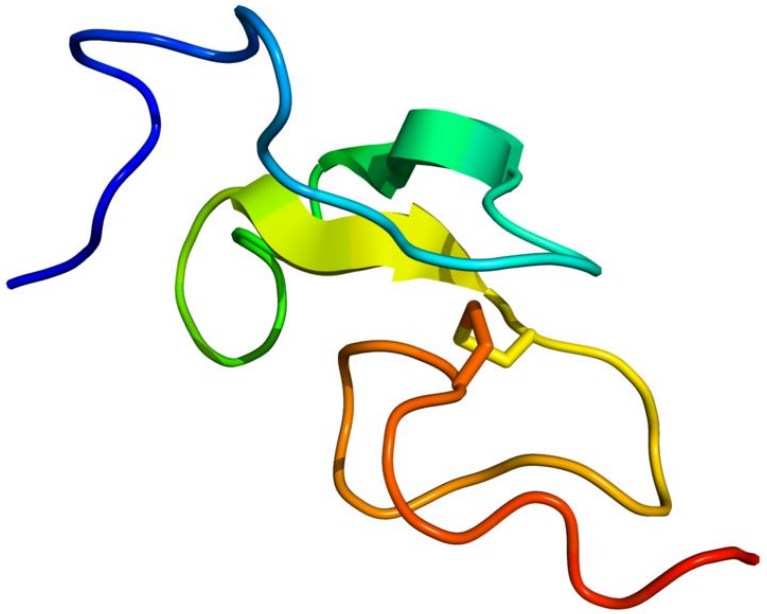
Фактор роста фибробластов - ФРФ

Трансформирующий фактор роста - ТФР

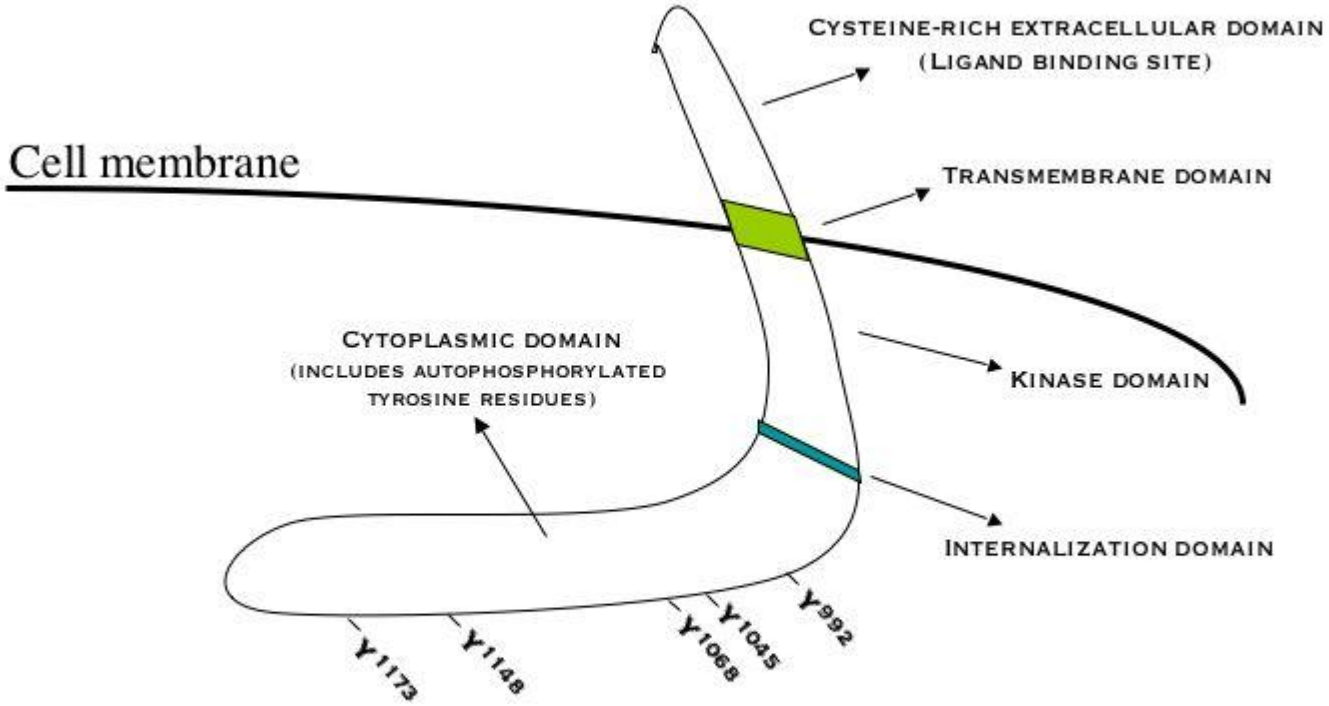
Тромбоцитарный фактор роста - ТцФР

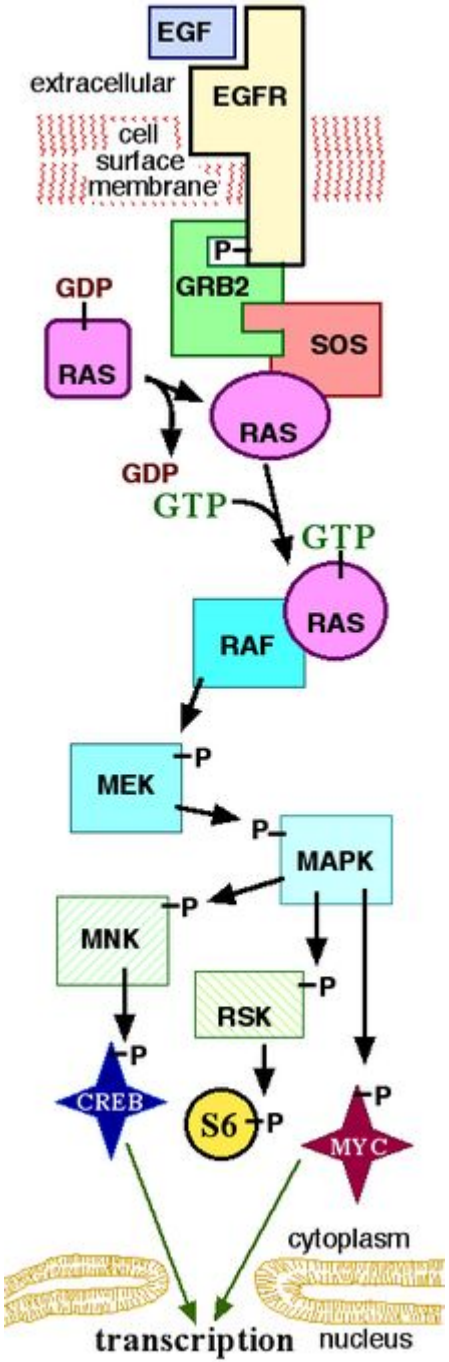
Эпидермальный фактор роста

- ЭФР, 6 кДа, 53 а.к., негликозилирован, 3 дисульфидных связи
- Воздействует в первую очередь на эндотелий, эпителий, фибробласты. Основная затрагиваемая ткань – кожа.



Рецептор ЭФР





Путь передачи сигнала ЭФР

- Комплекс с рецептором 2:2
- MAPK/ERK pathway
- MAPK- Mitogen-activated protein kinases, originally called ERK - Extracellular signal-regulated kinases
- Основной ответ клетки – прохождение клеточного цикла
- Уровень рецептора ЭФР повышен у клеток некоторых солидных опухолей

Путь передачи сигнала ЭФР

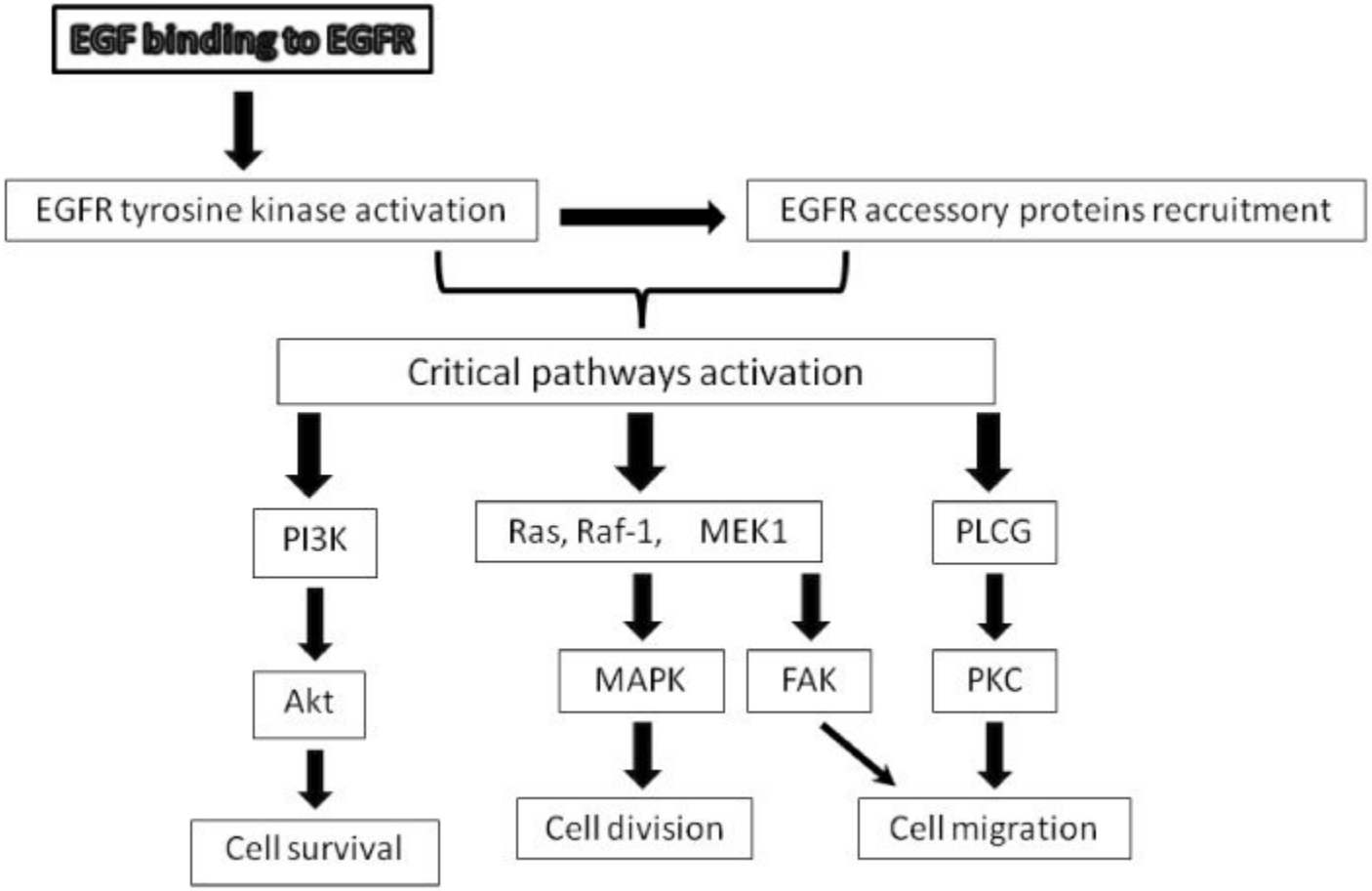


Figure 1: Main EGFR signaling pathways in wound healing and cancer. EGFR occupation by EGF or other cognate agonistic ligand triggers a conformational change within the receptor's topography leading to carboxy-terminal tyrosines phosphorylation and accessory proteins recruitment. Three major signaling 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 Akt or PKB on serine 473. Consequently Akt 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 downregulation and protein kinase activity by phosphorylating the yuxtamembrane residue of threonine 654. This results in a temporary inhibition of the tyrosine kinase activity.

Лекарственная форма ЭФР

- Торговое название REGEN-D, мазь, разрешена для клинического применения только в Индии, 150 мкг/г ЭФР.
- Показания – язва при синдроме диабетической стопы
- Эффективность определена как уменьшение времени заживления язвы против плацебо
- Нет данных о наблюдениях за канцерогенезом

Экспрессия рецептора ЭФР клетками опухолей

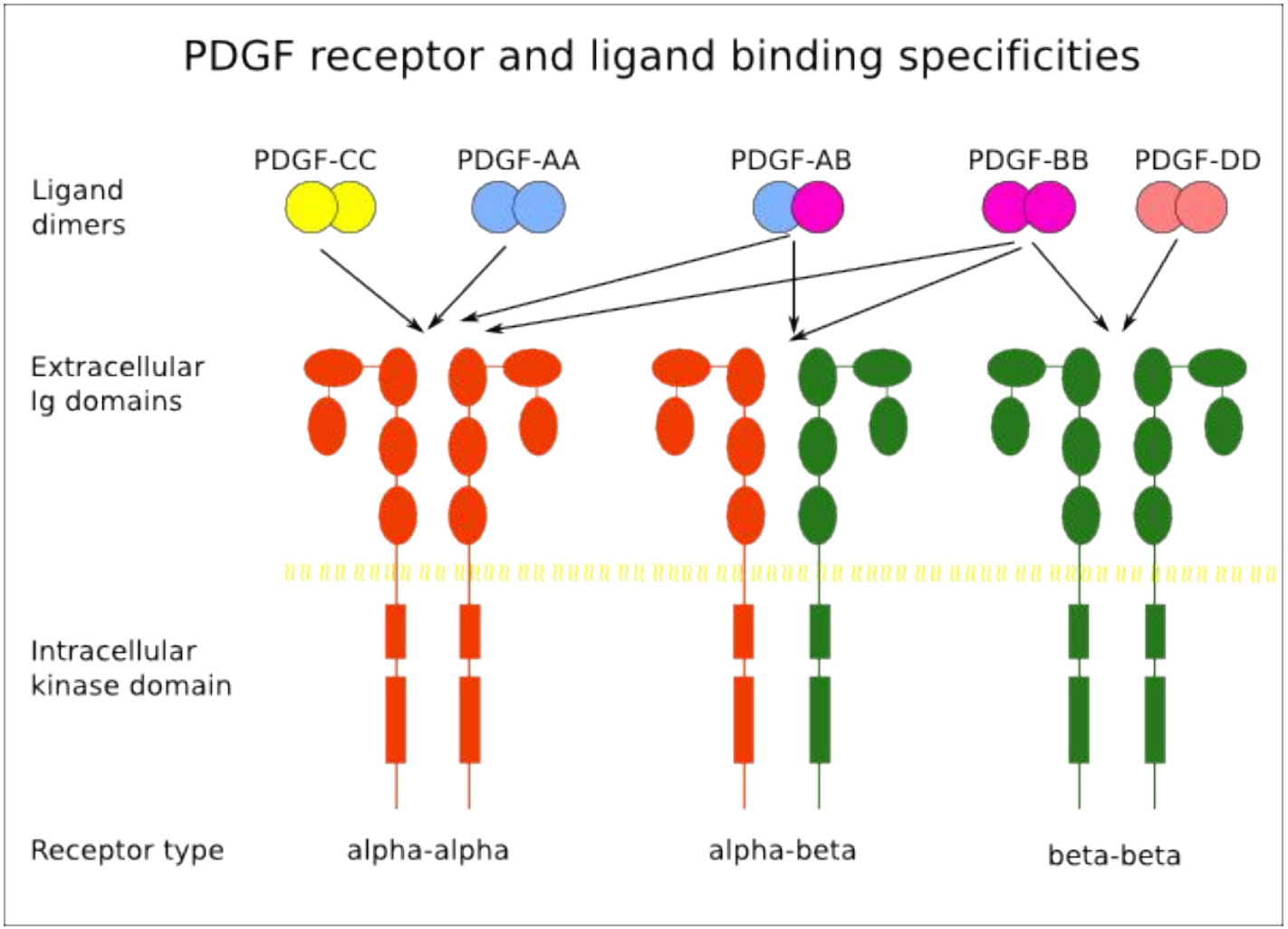
Tissue / Organ	Expression %
Lung	40-80
Breast	14-91
Stomach	33-74
Colon	25-77
Pancreas	30-50
Prostate	40-80
Kidney	50-90
Ovary	35-70
Head and neck	36-100

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.

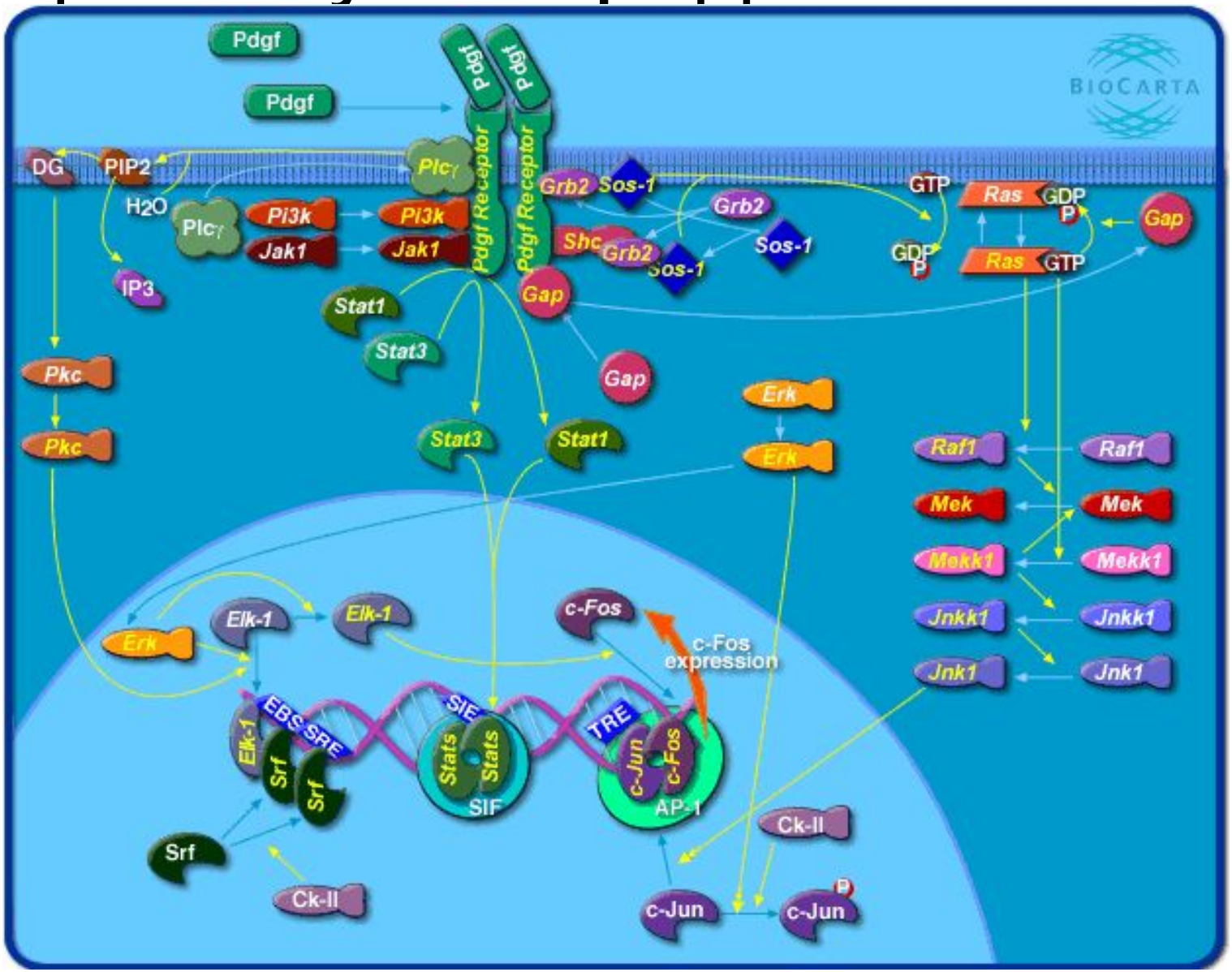
Тромбоцитарный фактор роста

- 5 гомологов, 4 гомодимера PDGF-A, -B … -D, гетеродимер PDGF-A-B
- Для PDGF-A 1 сайт гликозилирования, 3 дисульфидных связи. 110 а.к., 125 а.к.
- Синтезируются мегакариоцитами, хранятся в альфа-гранулах тромбоцитов
- Основные мишени – фибробласты, гладкая мускулатура, глиальные клетки
- Основное действие - паракринное

Рецептор ТцФР



ТцФР - путь передачи сигнала



ТцФР при заживлении ран

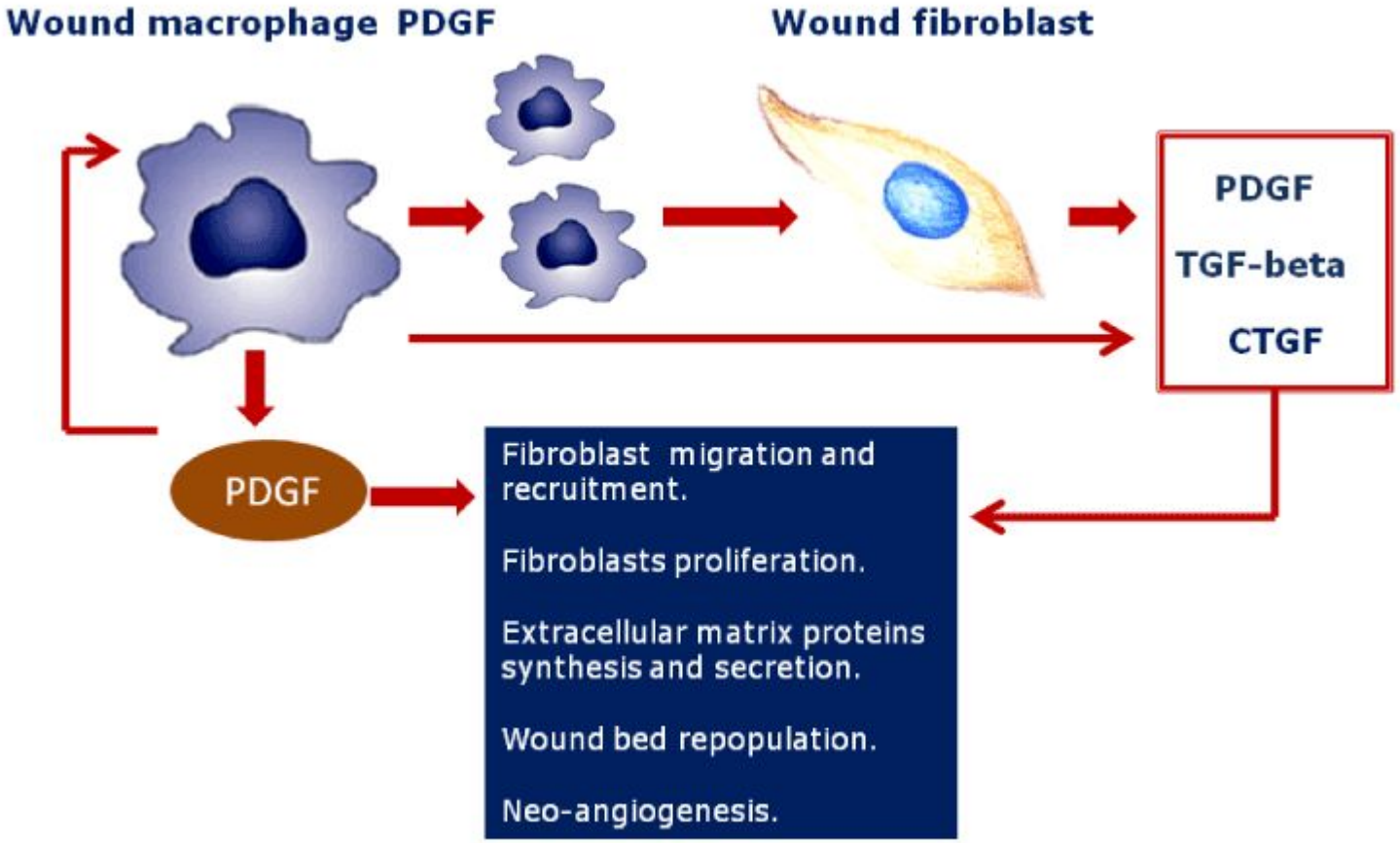


Figure 2: PDGF signal amplification through infiltrated macrophages. As described elsewhere for tumors, infiltrated macrophages plays an important role in wound healing. PDGF is one of the few growth factors distinguished by the ability to recruit monocytes/macrophages to the wound bed. PDGF protein 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.

Лекарственная форма ТцФР

- Торговое название **Регнарекс гель**, МНН **Becaplermin**
- Гель, действующее вещество – гомодимер ТцФР-В
- Продуцент – дрожжи S.cerevisae, гликозилированный продукт секретируется в ростовую среду, мономеры связаны двумя дисульфидными связями
- Нестерильные тубы по 15 г геля, 100 мкг/г субстанции, носитель – 0,01% карбоксиметилцеллюлозы, вспомогательные вещества – NaCl, NaOAc, HOAc, метилпарабен, пропилпарабен, м-крезол, L-лизин
- Стабильность белка в лекарственной форме обеспечивается его иммобилизацией на отрицательно заряженных нитях КМЦ, восстанавливающие группы на конце полимера КМЦ нейтрализованы свободными аминокислотами – лизином. (патент США 5,457,093)
- Оказывает воздействие только на толщину грануляционной ткани, показания – язвы при диабете, курс лечения ~20 недель.

Клиническая эффективность

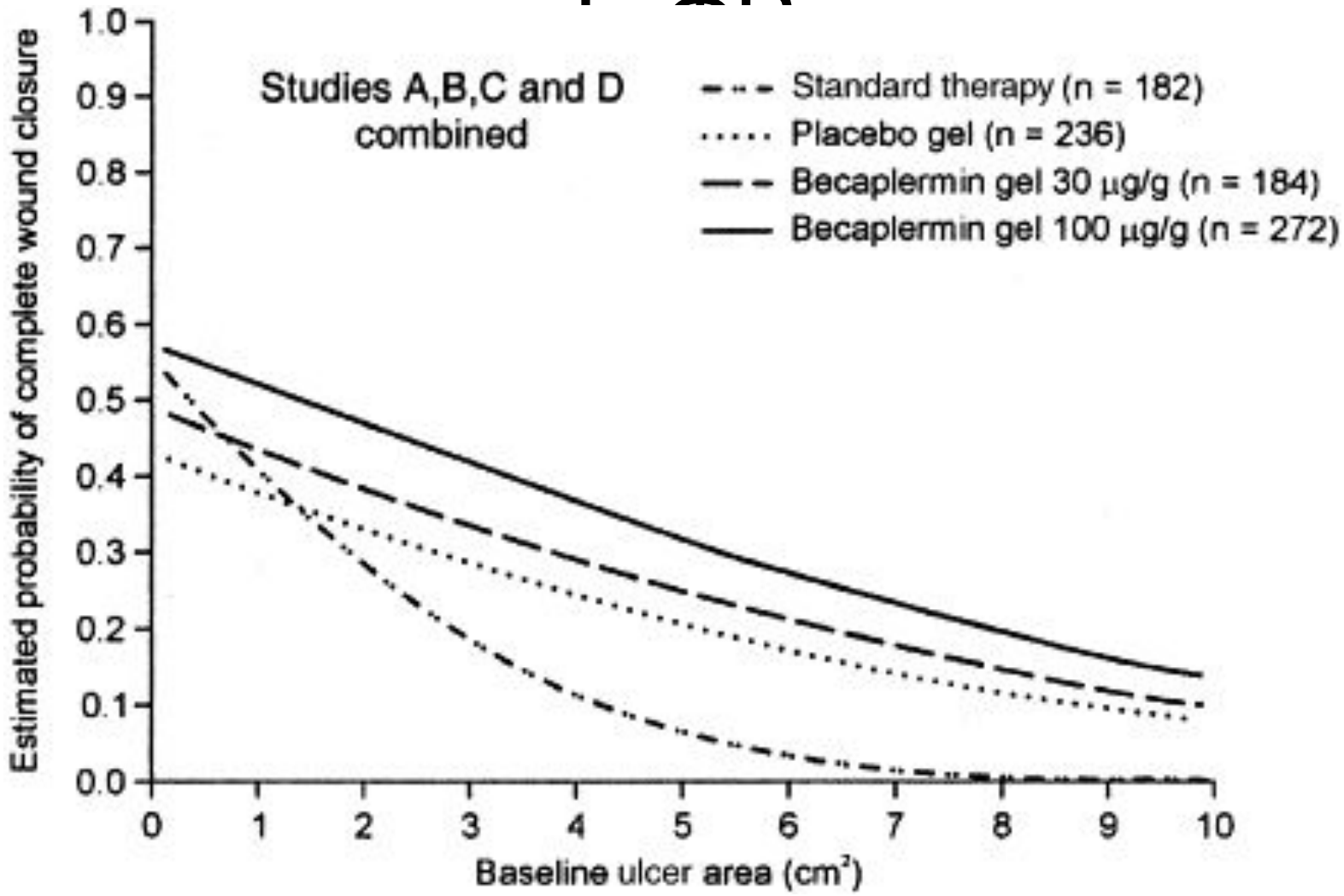


Fig. 2. Estimated probability of complete ulcer healing based upon baseline ulcer area (intent-to-treat patients, baseline ulcer are ≤10 cm^2).

Perry BH, Sampson AR, Schwab BH, Karim MR, Smiell JM. // Control Clin Trials. 2002 Aug;23(4):389-408. PMID: 12161082

Канцерогенез, вызываемый ТцФР

Adv Skin Wound Care. 2011 Jan;24(1):31-9. doi: 10.1097/01.ASW.0000392922.30229.b3.

A matched cohort study of the risk of cancer in users of becaplermin.

Ziyadeh N, Fife D, Walker AM, Wilkinson GS, Seeger JD.

Abstract

BACKGROUND:

Becaplermin is recombinant human platelet-derived growth factor for topical administration that might plausibly be related to cancer risk. Extended follow-up of patients from clinical trials of becaplermin compared with placebo identified a relative risk of cancer of 2.8 (95% confidence interval [CI], 0.6-12.8). The authors aimed to further investigate any association between becaplermin use and the occurrence of cancer by following a large cohort of patients in a clinical practice setting.

METHODS:

In a cohort of insured people, becaplermin initiators were matched to similar people who did not initiatebecaplermin and were followed for up to 6 years for cancer incidence (up to 9 years for cancer mortality). Cancerincidence was identified from health insurance claims and validated by review of medical records. Cancer mortality was identified through linkage to the National Death Index.

RESULTS:

Among 1622 becaplermin initiators, there were 28 confirmed cancers and 9 cancer deaths, and among the 2809 matched comparators, there were 43 confirmed cancers and 16 cancer deaths. There was no increased risk of cancer with becaplermin (hazard ratio, 1.2; 95% CI, 0.7-1.9). Cancer mortality through 2003 was increased (rate ratio [RR] = 5.2; 95% CI, 1.7-17.6) among subjects with 3 or more dispensings. Additional follow-up through 2006 indicated no elevated cancer mortality risk overall (RR, 1.0; 95% CI, 0.5-2.3) and no statistically significant increase in the subgroup with more than 3 dispensings (RR, 2.4; 95% CI, 0.8-7.4).

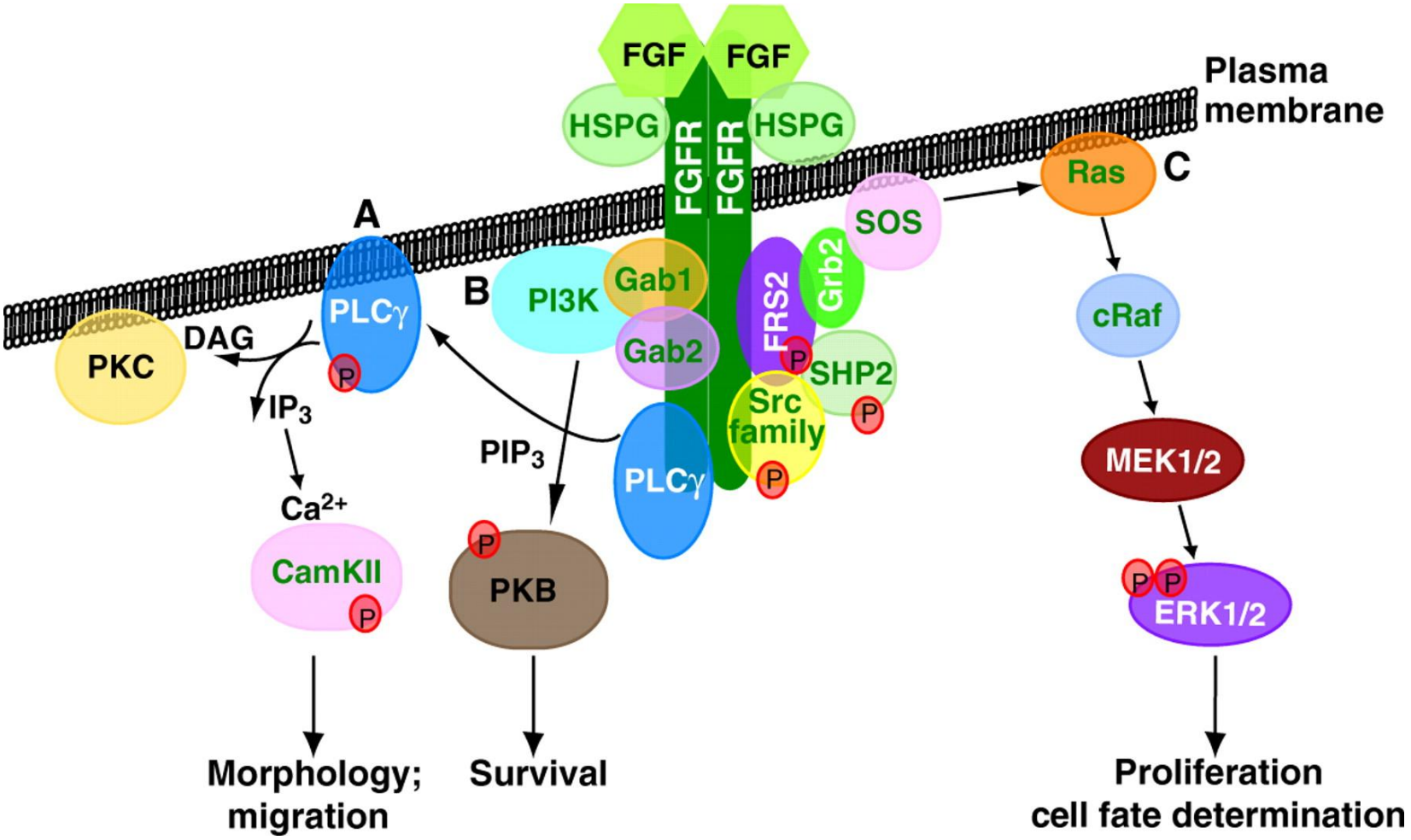
CONCLUSIONS:

Becaplermin does not appear to increase the risk of cancer or cancer mortality.

Семейство факторов роста фибробластов

- Около 20 членов, ФРФ-1 … ФРФ-20, 18-28 кДа
- Обладают общим доменом размером около 140 а.к.
- Плотно связывают гепарин и гепарин-подобные глюкозоаминогликаны, т.е. связаны с внеклеточным матриксом
- Некоторые члены семейства не воздействуют на фибробласты

An overview of FGF signalling.



Dorey K , and Amaya E Development 2010;137:3731-3742

Палифермин (ФГФ-7) и его рецептор.

- Фактор роста кератиноцитов (KGF, FGF-7) воздействует только на эпителиальные клетки, обладающие рецептором FGFRIIIb
- Природный ФГФ-7 содержит 163 а.к. и сайт N-гликозилирования в N-концевой области
- Делеция 23 а.к. на N-конце зрелого полипептида ФГФ-7 приводит к потере сайта N-гликозилирования и двукратному увеличению митогенной

Лекарственный препарат ФГФ-7

- Торговое название Kepivance, МНН Palifermin
- Показания – риск развития тяжелого орального мукозита вследствие высокодозной химиотерапии или лучевой терапии с последующей пересадкой гематопоэтических стволовых клеток
- Клиническая эффективность – 20% больных с мукозитом уровня 4 против 62% в группе плацебо.
- Курс – 6 дней по одной инъекции в/в 60 мкг/кг/д
- Метод получения – E.coli, растворимый продукт в цитоплазме, окисление дисульфидной связи

Трансформирующие ростовые факторы

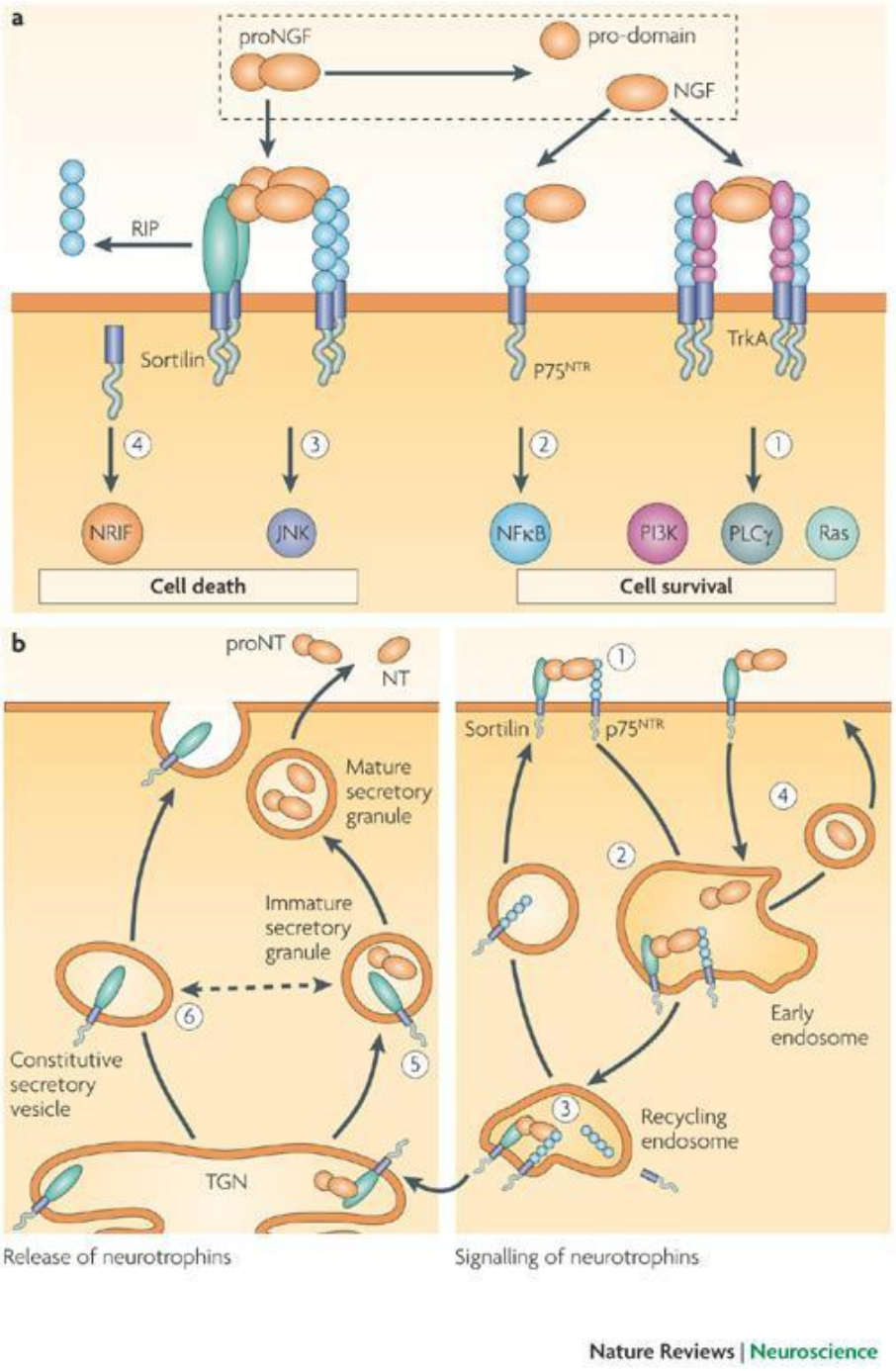
- Два члена семейства – TGF-α и три варианта TGF-β
- TGF-α связан с мембраной, частично отделяется от мембраны при протеолизе, связывается с рецептором ЭФР.
- TGF-β – гомодимеры, 2x112 а.к., синтезируются большинством типов клеток, плейотропны, ингибируют деление эпителиальных и гемопоэтических клеток
- TGF-β стимулируют рост клеток соединительной ткани, костей и хрящей, индуцируют секрецию внеклеточного матрикса и модулируют экспрессию матриксных металлопротеаз (MMP)

Нейротрофные ростовые факторы

Target	Neurotrophin	Cellular actions	Suggested mechanisms
Presynaptic neurotransmitter release	BDNF, NT3, NT4	Increased neurotransmitter release	• RAS–MAPK mediated phosphorylation of synapsin[156] • PLCγ-dependent increase of Ca^{2+} (REF. 206) • Increased release sites via the myosin VI–adapter protein GIPC complex[206] • Increased expression of RAB3A[157] • CDC42-dependent increase in actin polymerization[122]
Neurotransmitter receptors	BDNF	Increased postsynaptic response	Postsynaptic tyrosine kinase-dependent mechanism[150]
		Increased NMDA receptor conductance	Tyrosine kinase-mediated phosphorylation of NR1 (REF. 159) and NR2B[160]
		Increased surface expression of AMPA receptors	• CaMKII and PKC phosphorylation at Ser831 in GluR1 (REF. 161) • PKC-mediated interaction between GluR2 and NSF[207]
		Increased synthesis of AMPA receptor subunit	TRKB-dependent protein synthesis[161]
		Decreased surface expression of $GABA_AR$	Reduced phosphorylation of $GABA_AR$ subunit by phosphatase[164]
Ion channels	BDNF	Increased TRPC channel activity	Activation via PLCγ–IP_3 pathway-dependent Ca^{2+} mobilization from the store[208]
	NGF, BDNF, NT4	Increased Na_V channel conductance	Increased MAPK activation and CREB phosphorylation[209,210]
	BDNF	Decreased $Na_V1.2$ channel conductance	FYN tyrosine kinase-mediated phosphorylation[211]
	BDNF	• Decreased $K_V1.3$ channel current (acute) • Increased $K_V1.3$ channel current and accelerated inactivation kinetics (chronic)	TRKB-mediated tyrosine phosphorylation of $K_V1.3$ channel (REF. 212)
	NGF, BDNF, NT4	Increased VGCC conductance	Increased phosphorylation of MAPK and CREB[209,210,213]
Gene expression	BDNF, NT3, NGF	Increased transcription	Increased CREB phosphorylation via CaMKIV and MAPK pathways[177,214–216]
	BDNF	Increased translation	PI3K–AKT–mTOR-mediated phosphorylation of translation-regulating factors[217]
Morphology	BDNF	• Increased dendritic growth and spine density • Altered spine morphology	• Activation of RAS–MAPK pathway[193] • Increased in Ca^{2+} via TRPC activation[194] • Activation of PI3K–AKT–mTOR pathway[195] • Increased actin polymerization[196] • Increased tubulin polymerization[199]

BDNF, brain-derived neurotrophic factor; CaMK, Ca^{2+}/calmodulin kinase; CDC42, cell division cycle 42 (GTP-binding protein, 25kDa); CREB, cyclic AMP response element (CRE)-binding protein; $GABA_AR$, type A GABA receptor; GluR, glutamate receptor (subunit of AMPA receptors); IP_3, inositol trisphosphate; K_V, voltage-gated K^+ channel; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; Na_V, voltage-gated Na^+ channel; NGF, nerve growth factor; NR, NMDA receptor subunit; NSF, *N*-ethylmaleimide-sensitive fusion protein; NT, neurotrophin; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PLCγ, phospholipase Cγ; TRKB, tropomyosin-related kinase B; TRPC, transient receptor-potential cation channel subfamily C; VGCC, voltage-gated Ca^{2+} channel.

Рецептор NGF



a | On binding of nerve growth factor (NGF) to the receptor tyrosine kinase TrkA, receptor dimerization and transphosphorylation stimulate three major signalling pathways: the phosphatidylinositol 3-kinase (PI3K)–protein kinase B pathway, the phospholipase C (PLC) pathway and the Ras–mitogen activated protein kinase pathway (1). Stimulation of TrkA is promoted by TrkA forming a complex with $p75^{NTR}$. $p75^{NTR}$ also facilitates the binding of brain-derived neurotrophic factor (BDNF) and neurotrophin 4 (NT4)/NT5 to TrkB and that of NT3 to TrkC (not shown). $p75^{NTR}$ also activates nuclear factor- в (NF в) pathways, independent of Trk (2). Whereas all of the above pathways promote cell survival, binding of proNGF to sortilin–$p75^{NTR}$ complexes activates cell death through the stimulation of Jun N-terminal kinase (JNK) (3) or through ligand-dependent regulated intramembrane proteolysis (RIP) of $p75^{NTR}$. Such stimulation of RIP results in release of the cytoplasmic tail of the receptor followed by nuclear translocation of the $p75^{NTR}$ adaptor NRIF (neurotrophin receptor interacting factor) (4). **b** | Sortilin promotes proNGF binding to $p75^{NTR}$ (step 1), enabling signalling from the cell surface (such as JNK activation). Sortilin also mediates internalization (step 2) and delivery of proNGF (probably in complex with $p75^{NTR}$) to so-called recycling endosomes, where RIP initiates NRIF-dependent stimulation of cell death (step 3). Whereas sortilin moves to the *trans*-Golgi network (TGN), non-cleaved $p75^{NTR}$ molecules recycle back to the cell surface or are transported retrogradely back to the soma (not shown). In addition, sortilin might be involved in internalization of proNGF, followed by furin-mediated processing in endosomal compartments and release of mature NGF (which stimulates TrkA at the cell surface) (step 4). This model explains the ability of proNGF to indirectly promote cell survival through TrkA. Finally, sortilin could affect secretion of proBDNF and BDNF by delivering growth factors to immature secretory granules (ISGs). ISGs form mature secretory granules, which are responsible for regulated release of BDNF and proBDNF following neuronal stimulation (step 5). How sortilin exits ISGs is unclear, but it might involve routing of sortilin to the constitutive secretory pathway (step 6).

Роль нейротрофных ростовых факторов при нейродегенеративных заболеваниях

PLoS One. 2010 Dec 28;5(12):e14433. doi: 10.1371/journal.pone.0014433.

Pharmacological treatment of painful HIV-associated sensory neuropathy: a systematic review and meta-analysis of randomised controlled trials.

Phillips TJ, Cherry CL, Cox S, Marshall SJ, Rice AS.

BACKGROUND:
Significant pain from HIV-associated sensory neuropathy (HIV-SN) affects ~40% of HIV infected individuals treated … There is an urgent need to develop effective pain management strategies for this condition.
METHOD AND FINDINGS:
…
REVIEW METHODS:
Four authors assessed the eligibility of articles for inclusion. Agreement of inclusion was reached by consensus and arbitration. Two authors conducted data extraction and analysis. Dichotomous outcome measures (≥ 30% and ≥ 50% pain reduction) were sought from RCTs reporting interventions with statistically significant efficacies greater than placebo. These data were used to calculate RR and NNT values.
RESULTS:
Of 44 studies identified, 19 were RCTs. Of these, 14 fulfilled the inclusion criteria. Interventions demonstrating greater efficacy than placebo were smoked cannabis NNT 3.38 95%CI(1.38 to 4.10), topical capsaicin 8%, and recombinant human nerve growth factor (rhNGF). No superiority over placebo was reported in RCTs that examined amitriptyline (100mg/day), gabapentin (2.4 g/day), pregabalin (1200 mg/day), prosaptide (16 mg/day), peptide-T (6 mg/day), acetyl-L-carnitine (1g/day), mexilitine (600 mg/day), lamotrigine (600 mg/day) and topical capsaicin (0.075% q.d.s.).
CONCLUSIONS:
Evidence of efficacy exists only for capsaicin 8%, smoked cannabis and rhNGF. However, rhNGFis clinically unavailable and smoked cannabis cannot be recommended as routine therapy. Evaluation of novel management strategies for painful HIV-SN is urgently needed.

Следующее занятие 7

Полипептидные гормоны. Инсулин, глюкагон, соматотропин, гонадотропины

- Инсулин в диабете I типа, структура молекулы инсулина, рецептор инсулина и пути передачи сигнала, история производства инсулина, варианты лекарственных форм инсулина, модифицированные варианты, глюкагон
- Гормон роста человека (соматотропин), рилизинг-факторы и антагонисты, рецептор, биологическая активность, метаболические эффекты, терапевтическое применение
- Инсулин-подобный фактор роста
- Семейство гонадотропных гормонов – фолликулостимулирующий гормон, лютеинизирующий гормон, хорионический гонадотропин, влияние структуры гликанов на биологическую активность и фармакокинетику фоликулостимулирующего гормона.