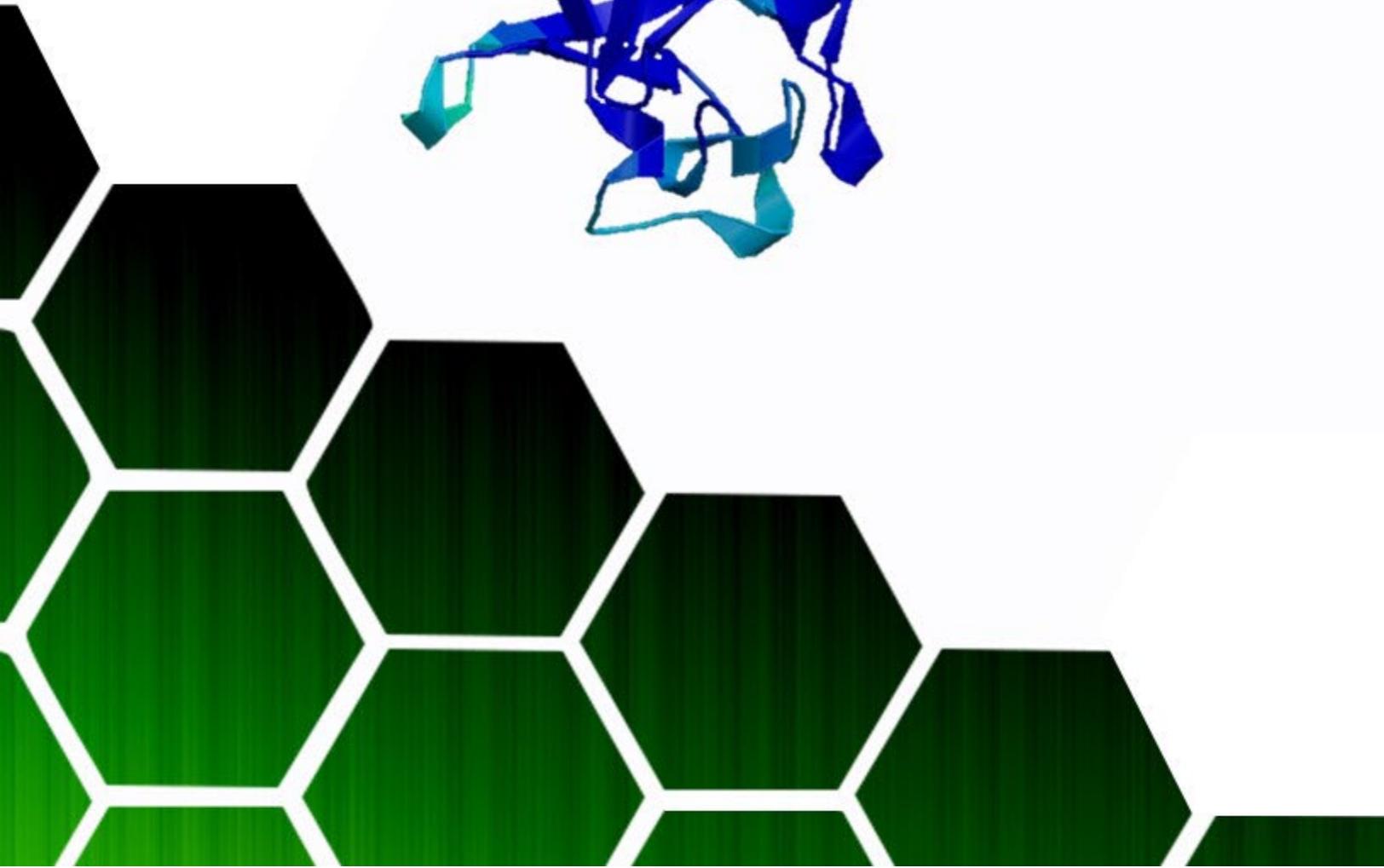




Growth Factor Handbook



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Growth Factor Handbook

Handbook for use with Gold Biotechnology Growth Factors

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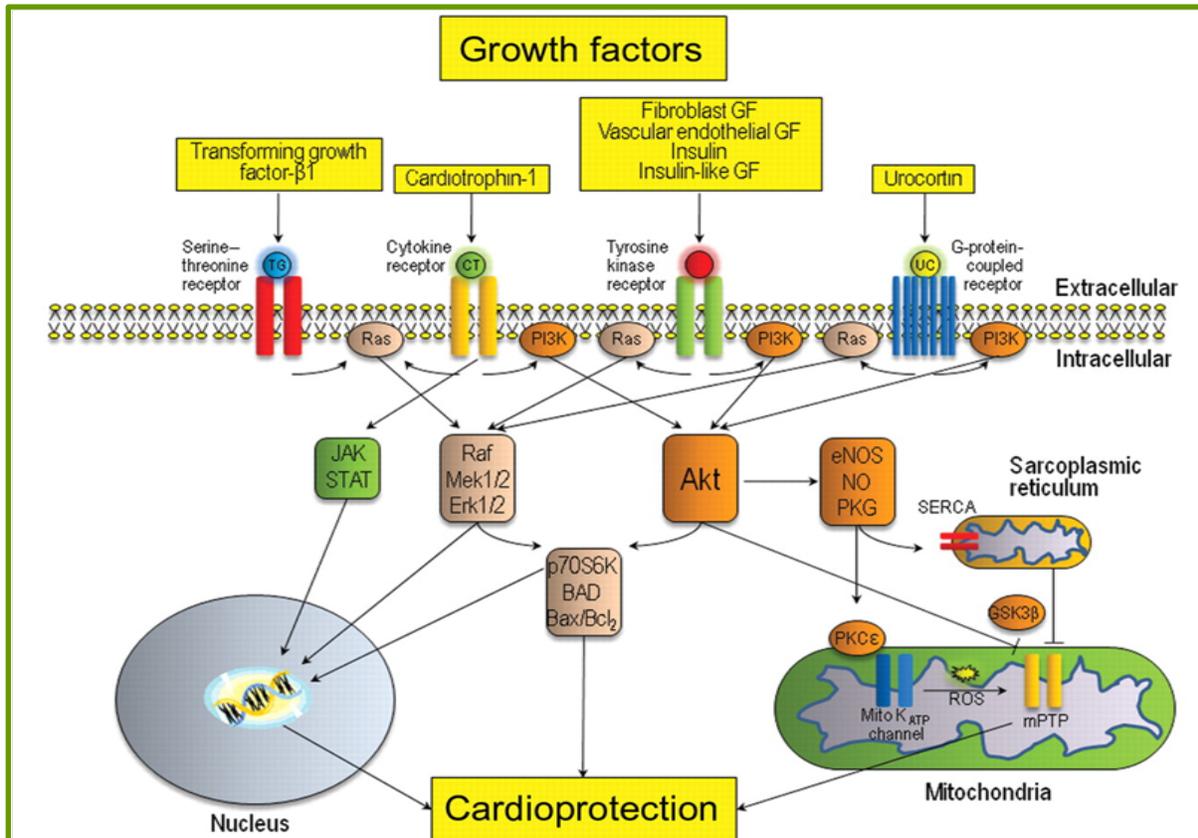
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Editor - C. Menne (12/23/2022)

Introduction

The purpose of this handbook is to provide a useful, accurate, easy-to-find resource for Gold Biotechnology growth factors and proteins. Over the last 5 decades, growth factors, ligands and their receptors have become one of the most prominent fields of study; including but not limited to medicinal and therapeutic research, developmental science, basic stem cell research and aging research.



This schematic provides a simplified overview of the intracellular transduction pathways underlying cardioprotection elicited by the growth factors: transforming growth factor- β 1 (TGF- β 1), cardiotrophin-1 (CT-1), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), insulin, insulin-like growth factor (IGF), and urocortin. Ligand binding to their respective cell-surface receptors on the cardiomyocyte activates intracellular signalling kinase cascades including Raf-Ras-Mek1/2-Erk1/2 and PI3K-Akt of the reperfusion injury salvage kinase (RISK) pathway, the JAK-STAT pathway, and various anti-apoptotic mechanisms (including the phosphorylation and inhibition of Bax and BAD as well as the inhibition of cytochrome C release).

Credit: Hausenloy, D. J., & Yellon, D. M. (2009). Cardioprotective growth factors. *Cardiovascular research*, 83(2), 179-194.

Fibroblast Growth Factor (FGF) Family

The Fibroblast Growth Factor (FGF) family of proteins is one of the largest polypeptide growth factor families in the animal kingdom, found in organisms ranging from nematodes to humans. FGFs are heparin-binding proteins and their interaction with heparan sulfate is necessary for optimal FGF receptor activation and signal transduction. There are 18 signaling FGFs, categorized numerically from FGF1-FGF23 (FGF11-14 are evolutionarily, but not functionally, related to the conventional FGFs and human FGF19 is homologous to mouse FGF15). There are 5 types of Fibroblast Growth Factor receptors (FGFRs) found in mouse and humans. Four of these FGFRs are signaling receptor tyrosine kinase molecules. These growth factors and their receptors interact with heparan sulfate to form FGF·HS·FGFR signaling complexes (Wu, 2003). Heparin protects FGFs from heat inactivation and heparan sulfate may also modulate the binding specificity of FGFs towards different FGF receptors (Gospodarowicz, 1986; Wu, 2003). FGFs and FGFRs are involved with embryonic development, angiogenesis, wound healing, metabolic regulation and cancer and play critical roles in the control of many fundamental cellular processes, such as cell proliferation, differentiation, migration and metabolic activity.

The coding regions of the *Fgf* genes range from under 5 kb to over 100 kb. The prototypical *Fgf* gene contains three coding exons with exon 1 containing the initiation methionine. The *Fgf* genes are found scattered over 12 chromosomes in the human genome (Ornitz 2001):

- 3q (FGF12)
- 4q (FGF2, 5)
- 5q & 5p (FGF1, 10, 18)
- 8p (FGF17, 20)
- 10q (FGF8)
- 11q (FGF3, 4, 19)
- 12p (FGF6, 23)
- 13q (FGF9, 14)
- 15q (FGF7)
- 17p (FGF11)
- 19p & 19q (FGF21, 22)
- Xq (FGF13, 16)

FGFs have a homologous core region that consists of 120–130 amino acids ordered into 12 anti-parallel β -strands (β 1– β 12) flanked by divergent amino and carboxyl termini. In general, the primary sequence variation of the FGF N- and C-terminal tails account for the varied biology of the ligands (Beenken 2009).

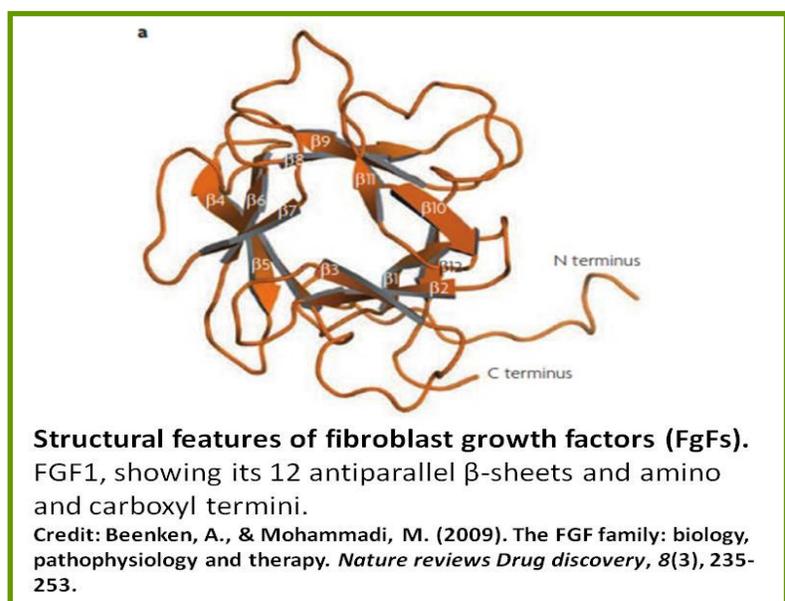
Fibroblast Growth Factor receptors (FGFR)

FGFRs are part of a group of receptor tyrosine kinases and are activated by FGFs and HS induced dimerization (Schlessinger, 2000). The unspliced form of FGFR contains an intracellular tyrosine kinase domain, a trans-membrane region, an extracellular region containing three Ig domains, a string of acidic residues between the first and second Ig domains (Givol, 1992), and an HS binding site in the second Ig domain (Kan, 1993). The Ig domain I has been shown to be dispensable, and receptor variants containing only the Ig domain II and III (β form) have been found to exhibit an equivalent degree of binding to FGFs as the variants containing all three Ig domains (α form). Ig domain III can undergo differential splicing and thereby exhibits IIIb and IIIc splice variants (Givol, 1992). The IIIa splice variant of the FGFR terminates within Ig-like domain III to yield a secreted extracellular FGF-binding protein with no known signaling capability (Ornitz, 1996). All receptors bind to more than one FGF. In many cases, some FGFs themselves can also activate more than one receptor (i.e., FGF1, which binds to all seven principal FGFRs: FGFR1c, FGFR1b, FGFR2c, FGFR2b, FGFR3c, FGFR3b, and FGFR4) (Ornitz, 1996).

FGF1

Fibroblast Growth Factor 1 (FGF1) is a single-chain polypeptide, heparin binding growth factor. FGF1 has been shown to induce the proliferation and migration of endothelial, mesodermal and neuroectodermal cells and is also involved in angiogenesis, embryogenesis and tissue repair (Dikov, 1998). FGF1 was initially purified along with FGF2 by Gospodarowicz et al. from brain as a mitogenic factor of cultured fibroblasts (Gospodarowicz, 1975).

FGF1 is primarily found in neural tissue, though it is also present in kidney, smooth muscle cells and fibroblasts, suggesting that it is widely distributed and plays a necessary role in normal cellular physiology (Jaye, 1988). Additionally, FGF1 has also been found in numerous human glioma cell lines, appearing in numbers inversely correlated with the number of FGF1 receptors in those lines, suggesting a possible role in gliomic autocrine growth stimulation (Libermann, 1987).



FGF1 has 55% amino acid homology with FGF2. FGF1 lacks amino-terminal signal peptides and is not secreted from cells through classical secretory pathways, but it can still be found on the cell surface and within the extracellular matrix. It is possibly released from damaged cells or some exocytotic mechanism, independent of the ER-Golgi pathway (Ornitz, 2001).

FGF1 is located on human chromosome 5q31.3. Its mRNA structure and translation is the least complicated among FGF family members. Along with the absence of a signal sequence, its open reading frame (ORF) is flanked by termination codons (Jaye, 1986).

As a proliferative factor of human preadipocytes, FGF1 may be important for the regulation of human adipogenesis (Hutley, 2004; Jonker, 2012). FGF1 leads to microvascular branching in endothelial cells (Uriel, 2006) and has been shown to repair nerve injuries. It enabled functional regeneration of transected spinal cords in rats (Cheng, 1996) and restored some motor function to paralyzed limbs in a 6-month-old boy with brachial plexus avulsion (Lin, 2005).

FGF2

Fibroblast Growth Factor 2 (FGF2) is a single-chain polypeptide, heparin binding growth factor that is functionally important for cardiac response to injury, wound healing, cortical neurogenesis and angiogenesis. FGF2 is also commonly used in media required to support growth of human embryonic stem cells (hESC), induced pluripotent stem cells (iPSC), and other tissue-specific progenitor cells.

FGF2 was originally purified from bovine pituitary tissue (Gospodarowicz, 1975) and again from bovine brain tissue along with FGF1 (Gospodarowicz, 1978). FGF2 was later named basic fibroblast growth factor (bFGF) based on its chemical properties and to distinguish it from acidic fibroblast growth factor (aFGF, FGF1). FGF2 has a 55% amino acid homology with FGF1 and interacts with heparin sulfate with nanomolar affinity. Heparin protects FGFs from heat inactivation (Gospodarowicz, 1978). Exogenous heparin or endogenous heparan sulfate is also required for optimal receptor activation (Rapraegar, 1991; Ornitz, 1992) and heparan sulfate may also modulate the binding specificity towards different FGF receptors (Wu, 2003). Different translational start sites produce variant proteins ranging in molecular weight from 18-34 kDa, with the 18 kDa variant being the most abundant form. FGF2 is highly conserved and shows greater than 90% sequence identity across a wide range of vertebrate species. FGF2 is one of the most significant regulators of human embryonic stem cell (hESC) self-renewal. FGF2 is an essential component of most culture media used to grow human ES and iPS cells and maintain their pluripotency.

FGF2 lacks an amino-terminal signal peptide and is not secreted from cells, but can still be found on the cell surface and within the extracellular matrix. It is possible that it is released from damaged cells or some exocytotic mechanism, independent of the endoplasmic-reticulum-Golgi pathway (Ornitz, 2001).

FGF2 is a potent inducer of DNA synthesis in a variety of cell types. FGF2 activates mesenchymal splice variants of FGF receptors (c splice variants) but also can activate FGF receptor 1b. FGF2 has little activity towards FGFR2b and FGFR3b (Ornitz, 1996).

Epidermal Growth Factor

Stanley Cohen discovered Epidermal Growth Factor (EGF) in 1962 and also co-discovered the Nerve Growth Factor (NGF) with Rita Levi-Montalcini, for which they won the Nobel Prize in Physiology and Medicine in 1986. Cohen isolated EGF from the salivary gland extract of mice and was able to show that it accelerated the healing of corneal wounds. EGF has since proven to be a general growth factor which acts on a large variety of cells, including epithelial cells. As with most of these types of growth factors, EGFs require the presence of specific binding sites, termed receptors, on the surface of the target cells (Lindsten, 1993).

Since the discovery of the founding member of this family, additional EGF family members have also been found, including:

- HB-EGF (Heparin Binding EGF-like growth factor)
- TGF-alpha (transforming growth factor)
- AR (amphiregulin)
- EPR (epiregulin)
- Epigen
- BTC (betacullulin)
- NRG1 (neuregulin-1)
- NRG2 (neuregulin-2)
- NRG3 (neuregulin-3)
- NRG4 (neuregulin-4)
- Tomoregulin
- Neuroglycan C

EGF family members can be characterized by their association with the EGF receptor (EGFR). Additionally, all family members contain one or more repeats of the Cysteine rich conserved amino acid sequence:

CX₇CX₄₋₅CX₁₀₋₁₃CXCX₈GXRC,

where “X” represents any amino acid (Dreux, 2006). This consensus sequence is known as the EGF motif and is crucial for binding members of the HER receptor tyrosine kinase family (Harris, 2003).

Epidermal Growth Factor receptors (EGFR)

The Epidermal Growth Factor receptor (EGFR) is a type of tyrosine kinase style cell-surface receptor for members of the EGF family of growth factors. There are 4 closely related receptors in the EGFR (ErbB) family: EGFR (ErbB-1), HER2/c-neu (ErbB-2), Her3 (ErbB-3) and Her-4 (ErbB-4). ErbB receptors are made up of a cysteine-rich, extracellular, N-terminal binding region (the ectodomain) containing approximately 620 amino acids, a hydrophobic transmembrane-spanning region, and an intracellular, highly conserved, C-terminal cytoplasmic tyrosine kinase domain (including several phosphorylation sites) (Seshacharyulu, 2012). The extracellular region of each family member is made up of four subdomains, L1, CR1, L2, and CR2, where "L" signifies a leucine-rich repeat domain and "CR" a cysteine-rich region.

There are two distinct conformations for the subdomains for ErbB-1, ErbB-3 and ErbB-4: an open, active conformation and a closed, inactive conformation. In the closed conformation, the CR1 and CR2 subdomains form intermolecular links, preventing L1 and L2 from binding to ligands (Cho, 2002; Garrett, 2002). The closed conformation is the preferred state of these receptors in the absence of ligands. In the presence of one of the EGF ligands, CR1 and CR2 unlink from each other, opening up the ligand binding pockets of L1 and L2, and allow ligand binding to proceed. This process also extends the dimerization arm of CR1 to interact with an identical arm of another receptor molecule, forming a homodimer (Ferguson, 2003; Ogiso, 2002). The ligand binding shifts the equilibrium, stabilizing the open conformation; the CR1 homodimers accumulate and maintains active receptor signaling (Bouyain, 2005; Dawson, 2005). A characteristic feature of ErbB2 is its inability to bind to any of the EGF ligands. However it is activated as a consequence of a heterodimer formation with one of the other EGF receptors (Seshacharyulu, 2012).

Mutations, amplifications or misregulations of EGFR or family members are implicated in about 30% of all epithelial cancers. Mutations involving EGFR often lead to its constitutive activation, which could result in uncontrolled cell division – a predisposition for cancer (Lynch, 2004).

Many therapeutic approaches are aimed at the EGFR. Cetuximab and Panitumumab are examples of monoclonal antibody inhibitors. Another method in use are small molecules which inhibit the EGFR tyrosine kinase (called TKI), which is on the cytoplasmic side of the receptor. Without kinase activity, EGFR is unable to activate itself, which is a prerequisite for binding of downstream adaptor proteins. Ostensibly by halting the signaling cascade in cells that rely on this pathway for growth, tumor proliferation and migration is diminished. TKIs are either reversible or irreversible. Reversible inhibitors compete with ATP molecules while Irreversible inhibitors covalently bind to the kinase active sites.

Table 1: EGF Family vs. EGFR Specificity (Linggi, 2006; Kinugasa 2004; Giordano, 2009)

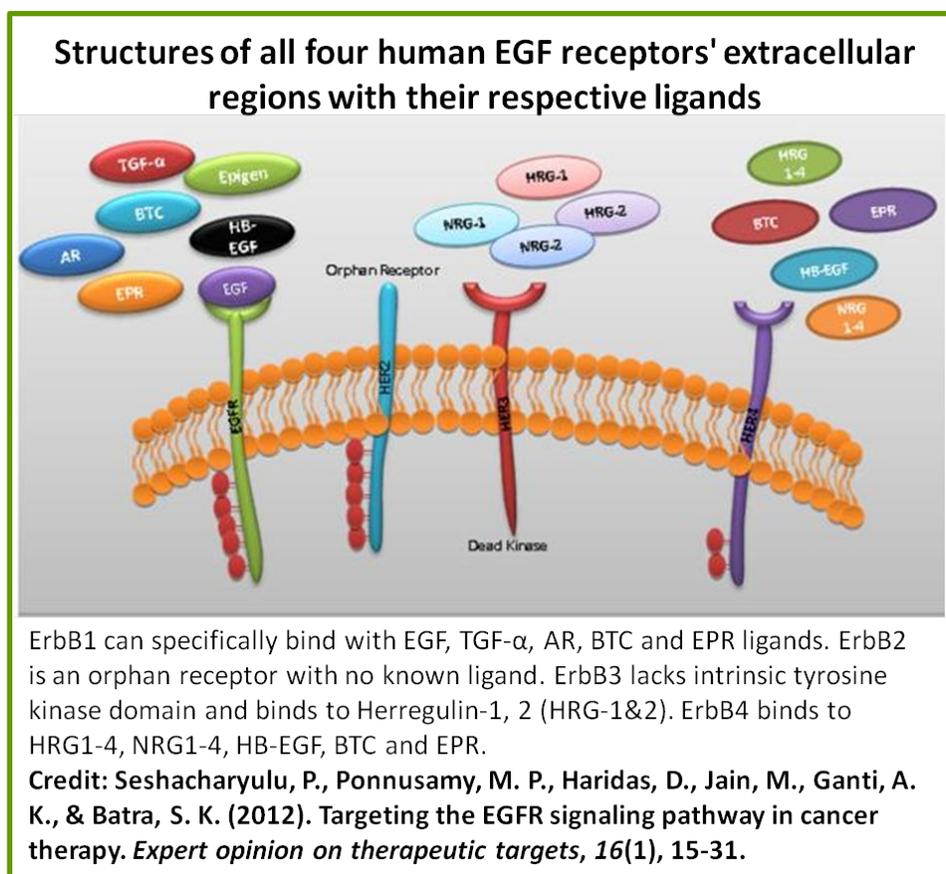
Ligand	Receptor			
	ErbB-1	ErbB-2	ErbB-3	ErbB-4
EGF	+	-	-	-
TGF- α	+	-	-	-
HB-EGF	+	-	-	+
Amphiregulin	+	-	-	-
Betacellulin	+	-	-	+
Epigen	+	-	-	-
Epiregulin	+	-	-	+
Neuregulin 1	-	-	+	+
Neuregulin 2	-	-	+	+
Neuregulin 3	-	-	-	+
Neuregulin 4	-	-	-	+
Tomoregulin	-	-	-	+
Neuroglycan C	-	-	+	-

EGF

EGF is a small mitogenic growth factor that stimulates cell growth, proliferation and differentiation, oncogenesis and wound healing. Human EGF is a small, ~6 kDa protein (Harris, 2003) with 53 amino acids and three intramolecular disulfide bonds (Carpenter, 1990). EGF is typically synthesized as a very long preproprotein of 1207 amino acids from which the growth factor (position 970-1023) is released by proteolytic cleavage.

EGF is essential for cellular proliferation, differentiation, and survival (Herbst, 2004). Since EGF's discovery from the mouse submandibular gland, it has been found in many human tissues including submandibular glands and parotid glands. It has also been found in human platelets, macrophages, urine, saliva, milk, and plasma (Kumar, 2009).

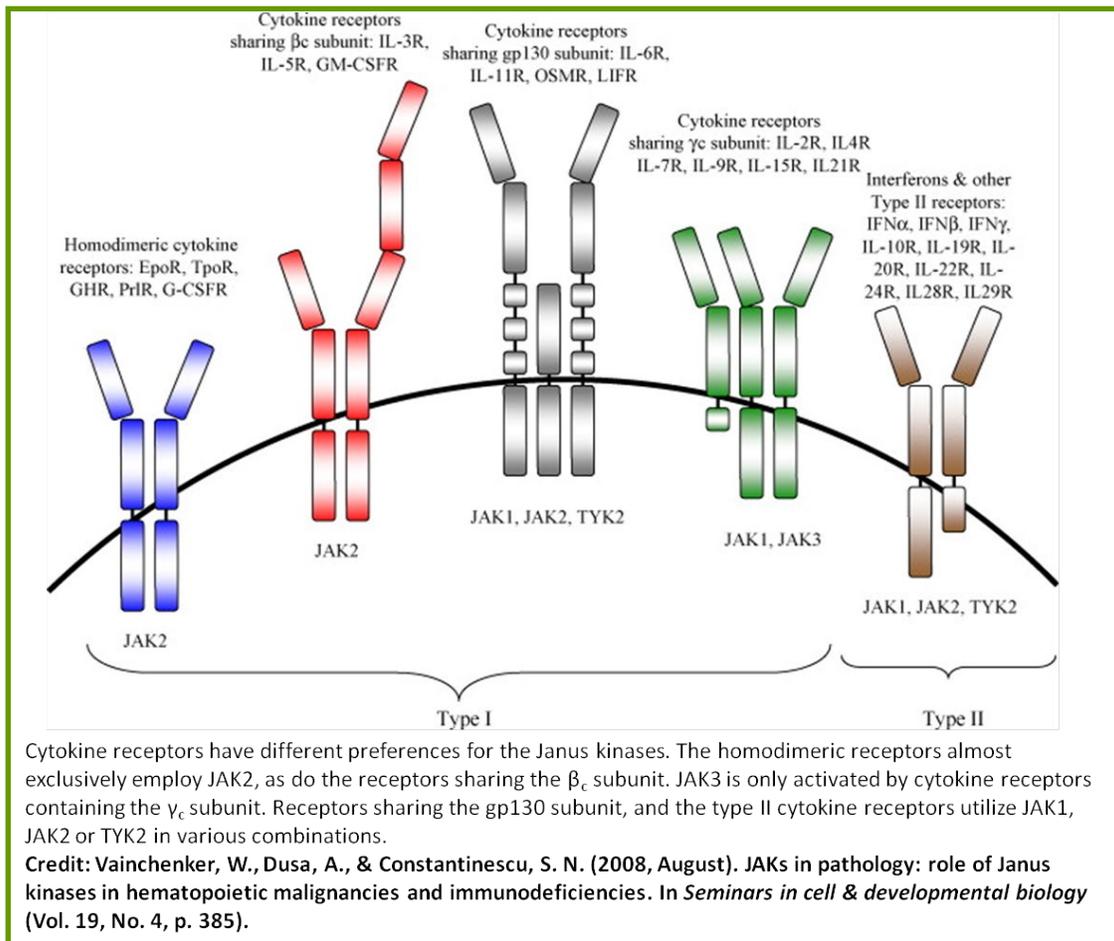
The biological effects of salivary EGF include the healing of oral and gastroesophageal ulcers, inhibition of gastric acid secretion, stimulation of DNA synthesis as well as mucosal protection from intraluminal injurious factors such as gastric acid, bile acids, pepsin, and trypsin and to physical, chemical and bacterial agents (Venturi, 2009). Human and Murine EGF have 70% homology at the amino acid level. EGF proteins are evolutionarily conserved across many species. EGF has been shown to increase the risk of cancer in patients, and EGF receptor inhibition has been shown to decrease that cancer risk (Herbst, 2004). Pharmaceutical drugs developed for this purpose of inhibiting EGF receptors include Gefitinib and Erlotinib for lung cancer and Cetuximab for colon cancer.



Interleukins

Interleukins are a large family of cytokines which are soluble hormone-like mediators of the immune system. They were first seen to be expressed by white blood cells (leukocytes), but have subsequently been found to be produced by a wide variety of body cells. The first interleukin (IL2) was discovered simultaneously in the 1960's by Kasakura and Lowenstein (Kasakura, 1965) and Gordon and MacLean (Gordon, 1965) at McGill University. IL2 was discovered first, but it was designated IL2 because their data indicated that IL1 facilitated IL2's production by T-cells. Smith *et al.* later discovered that IL2 is mediated via a specific IL2 receptor (Robb, 1981). There are currently 37 interleukin families.

Interleukins are involved in processes of cell activation, cell differentiation, proliferation, and cell-to-cell interactions. The expression of interleukins is usually strictly regulated, i.e., the factors are often not secreted constitutively. Interleukins are most often synthesized after cell activation as a consequence of a physiological or non-physiological stimulus. The majority are synthesized by helper CD4 T lymphocytes (as well as through monocytes, macrophages and endothelial cells). There are also some interleukins which act in an autocrine manner and regulate their own synthesis or the expression of their own receptors.



Interleukin Receptors

There are two classes of receptors for interleukins; Type 1 cytokine receptors and Type 2 cytokine receptors. Type 1 cytokine receptors include interleukin receptors (for IL2-7, 9, 11-13, 15, 21, 23 and 27), colony stimulating factor receptors (for Epo, CSF2 and CSF3), as well as some other cytokine receptors (including Oncostatin M and LIF).

Type I cytokine receptors are transmembrane receptors which are expressed on the cell surface. They are also called hemopoietin receptors, and share a common amino acid motif. These receptors recognize and respond to cytokines which have a typical, four α -helical structure. Some members of the type I cytokine receptor family are involved in ligand/cytokine interaction while others are involved in signal transduction.

Like Type I receptors, Type II cytokine receptors are also receptors which are expressed on the surface of certain cells. However, these receptors do not possess the signature sequence “WSXWS” motif that is characteristic of Type I receptors. Typically, Type II cytokine receptors are heterodimers or multimers with a high and a low affinity component. Currently no complete structure of the extracellular domains of a type II cytokine receptor is available.

Type II cytokine receptors include those that bind type I and type II interferons, and those that bind members of the IL10 family (IL10, IL20 and IL22) (Dumoutier, 2003 and Xu, 2001).

IL3

IL3 is a popular cytokine used for a variety of cell cultures (i.e. mast cells or basophils) providing the cytokinetic connection between the immune and hematopoietic systems. IL3 is capable of inducing the growth and differentiation of multi-potential hematopoietic stem cells, neutrophils, eosinophils, megakaryocytes, macrophages, lymphoid and erythroid cells. Haig, *et al.* (1994) recently showed a synergistic affect between IL3 and another growth factor, KITLG (sometimes called SCF or Stem Cell Factor), on both bone marrow-derived mast cells (BMMC) and serosal/connective-tissue mast cells (CTMC).

The human IL3 protein is about 17 kDa (152 amino acids), while the murine IL3 is about 18.5 kDa (160 amino acids). The human IL3 has a length of approximately 2.6 kb and contains five exons (Ensemble: ENSG00000164399). The gene maps to human chromosome 5q31. The murine IL3 gene maps to chromosome 11. While murine and human IL3 genes structurally resemble each other, their sequences are evolutionarily less well conserved. Human and murine IL3 have approximately 29% homology at the protein level and there is no cross-species reactivity between them.

The human IL3 gene is located in close vicinity to other cytokine genes, including those encoding GM-CSF, M-CSF, IL4 and IL5. The distance between the IL3 and the GM-CSF gene is

approximately 9 kb with the IL3 gene on the 5' side of the GM-CSF gene (Yang, 1988). IL3 is widely used in the inducing of growth and differentiation of multi-potential hematopoietic stem cells (hSC), neutrophils, eosinophils, megakaryocytes, macrophages, lymphoid and erythroid cells. It has also been used to support the proliferation of murine cell lines with multi-potential progenitors, immature myeloid cells as well as T or pre-B lymphoid cells (Reddy, 2000).

IL3 receptors are expressed on macrophages, mast cells, eosinophils, megakaryocytes, basophils, bone marrow progenitor cells, and various myeloid leukemia cells. When IL3 binds to its receptor, it causes a phosphorylation of the receptor and also activates protein kinase C in IL3 dependent cell lines. The receptor for IL3 consist of a ligand-specific α subunit and a common β subunit (which are also shared by IL5 and CSF2). Both subunits belong to the Type I cytokine receptor and both are required to transduce a signal across the membrane (Mui, 1995). IL3 is an increasingly valuable substance; both from an academic standpoint in which it is often used in culture and in its ability to stimulate the proliferation of very immature hematopoietic progenitor cells, exploited in the generation of stem cells, allowing stem cell separation and re-infusion into patients undergoing procedures such as intensive chemotherapy.

Hedgehog Signaling Pathway

The hedgehog signaling pathway is one of the key regulators of animal development and is present in all bilateral animals (Ingham, 2011). The pathway is named for the polypeptide, intercellular signaling molecule called Hedgehog (Hh) which was found in *Drosophila* fruit flies. The Hedgehog pathway was discovered in the late 1970's during research to look at specific genes which affected *Drosophila* segmentation development (Nüsslein-Volhard, 1980). The knockout larvae tended to have lawns of denticles, which appeared “stubby” and “hairy” and inspired the name: hedgehog. The *Hh* gene is involved in establishing the fly's body plan and remains important during later stage embryogenesis and metamorphosis.

In cells, Hedgehog binds to a cell-surface transmembrane protein called Patched (PTCH), which allows the expression of the receptor, Smoothed (SMO) (Chen, 2004). The accumulation of SMO inhibits the cleavage of the Cubitus interruptus (Ci) protein (Alcedo, 2000). Intact Ci protein accumulation allows for the transcription of genes such as decapentaplegic (*dpp*), which is the fruit fly homolog for human BMP. The Hedgehog signaling pathway has been shown to play a crucial role in T cell development and may also control the function of mature T lymphocytes (Michel, 2013).

There are 3 hedgehog homologues, Desert Hedgehog (DHH) and Indian Hedgehog (IHH), which were discovered in mammals, and Sonic Hedgehog (SHH), which were discovered in chickens. The three *Hh* genes have a high degree of homology between species. The Hedgehog pathway is vitally important during vertebrate embryonic development, including brain, skeletal, musculature, gastrointestinal and lung development. SHH acts to establish cell fate in the developing limb, somites, and neural tubes, IHH is involved in chondrocyte development (Vortkamp, 1996) and osteogenesis, and DHH plays a key role in germ cell development (Bitgood, 1996). The expression patterns of the Hedgehog family members do not generally overlap, with the exception of the gut, in which both IHH and SHH are expressed (Bitgood, 1995).

The most researched of the three is SHH, which was named by Riddle et al. (1993) for the video game character (Sonic) in 1993. Of the three *Hh* gene homologues, *Shh* appears to be the most critical in development, involved in patterning many systems, including the limb (Riddle, 1993) and midline structures in the brain (Herzog, 2003), spinal cord (Lewis, 2001), the thalamus by the *zona limitans intrathalamica* (Scholpp, 2006) and the teeth (Dassule, 2000).

Hedgehog Receptors

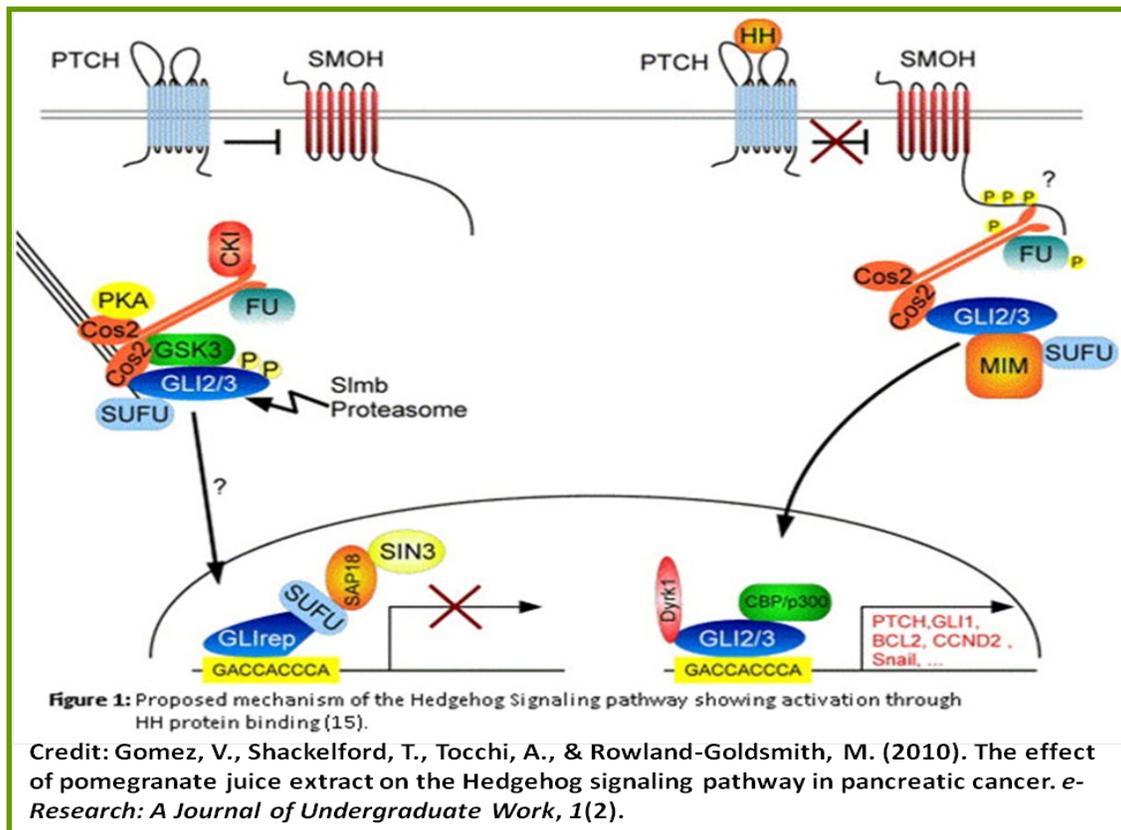
The receptors for the hedgehog signaling pathway are multitransmembrane proteins called Patched (PTCH1 and PTCH2). There is a 57% amino acid homology between the human PTCH1 and PTCH2, with a 96% amino acid homology between human and mouse PTCH1 and a 91% homology between human and mouse PTCH2. The PTCH2 protein has a truncated C-terminal

cytoplasmic domain compared with PTCH1 as well as only one of the two glycosylation sites present in PTCH1 (Carpenter, 1998). When PTCH is activated by Hedgehog, the signal protein Smoothed (SMO) is inhibited and causes a sequence of downstream events leading to the activation of the Gli transcription factors Gli2 and Gli3 which then eventually lead to the expression of Hh target genes (Michel, 2013).

Mutations of the PTCH genes have been associated with nevoid basal cell carcinoma syndrome, esophageal squamous cell carcinoma, trichoepitheliomas, transitional cell carcinomas of the bladder and holoprosencephaly (HPE) (Ming, 2002).

Sonic Hedgehog (SHH)

Sonic hedgehog (SHH) is one of the most studied protein ligands in the vertebrate pathway. SHH begins as a ~45 kDa protein that is processed to a 20 kDa N-terminal signaling domain (SHH-N) and a 25 kDa C-terminal domain (which does not appear to have any signaling properties). SHH signals in a secreted, (typically) autocrine protein, mediating the signaling of notochords and floor plates (Patten, 2000) and plays a key role in vertebrate organogenesis, in the growth of digits on limbs and the organization of the brain. It remains important in adults, controlling cell division in adult stem cells and has also been implicated in the development of various cancers. SHH has also been shown to act in a paracrine manner on villus formation during embryonic intestinal development (Walton, 2012).



The SHH protein is secreted at the zone of polarizing activity (ZPA), located on posterior side of a limb bud in an embryo. SHH has also been demonstrated to attract commissural axons at the ventral midline of the developing spinal cord, possibly acting as an axonal guidance cue (Charron, 2003). Kolpak (2005) showed that SHH acts as a morphogen, attracting retinal ganglion cell (RGC) axons at low concentrations, but repelling them at higher concentrations.

SHH is able to inhibit local cellular proliferation when in high concentrations (Wilson, 2005), causing the floor plate to become thin compared to the lateral walls of the neural tube. At lower concentrations, SHH induces cellular proliferation as well as the induction of various ventral neural cell types (Ericson, 1996).

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FGF

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EGF

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