MEDIVET
VETERINARY SUPPLIES

A Historical Commentary & background to

StemVet Fractionated Ovine Placental Extract.

INTRODUCTION

The oral use of dried placental extract has been used in traditional Chinese Medicine for over 2000 years. In traditional Chinese Medicine, the indications for its use have ranged from Emaciaton, hectic fever, night sweating, cough, breath and anorexia in consumptive diseases; impotence, seminal emission; infertility; and lack of lactation.

The Western medical uses and dermatological benefits of placental extracts have been reported in the medical literature since 1954. More recently, benefits of topical use placental extracts on chronic, non-healing wounds, and vitiligo has also been reported. The pharmacological mechanism of action of placental extracts ranges from:

1. anti-inflammatory and the wound healing properties and regenerative effects properties of transforming growth factors (TGF),
2. fibroblast growth factor (FGF),
3. hepatocyte growth factor (HGF),
4. fibronectin like peptide,
5. keratinocyte growth factor,
6. epidermal growth factor,
7. growth hormone releasing factor,
8. stem cell factor and
9. parathyroid hormone-related hormone (PTHrP).

These cytokine growth factors and extracellular matrix molecules are known to activate and be released by multipotent stem cells to promote the proliferation of a variety of cell types cells.

Sterile placental extracts have also been used by oral, intramuscular and intravenous administration in which benefits were seen in humans and animals with:

1. autonomic dysfunction,
2. neuronal regeneration,
3. liver regeneration,
4. alopecia,
5. atrophic rhinitis,
6. myopia and senile chorio-retinal dystrophies,
7. peripheral analgesia mediated by an opioid mechanism,
8. protection against infection,
9. possible treatment of hyperuricemia and gout patients, and
10. anti-aging effect as assessed by increased IGF-1 serum levels...

Medivet Pty Ltd.

Reg Office: 6 Raschella St. #2, Hope Valley S.A. 5090.
P: 1800 356 505 F: +612 9920 0798 E: info@medivet.net.au
© All rights reserved.

IN GENERAL

What is Placenta?

The placenta is the membraneous vascular organ that develops in female mammals during pregnancy, lining the uterine wall and partially enveloping the fetus, to which it is attached by the umbilical cord. The organ proliferates cells from merely one single ovum to over 60 trillion cells to form a complete fetus in 10 months. Interestingly, the placenta itself is also generated from an oval cell - placenta, and fetus share the same origin. In proliferating fetal cells, the placenta synthesises and secretes various growth factors and cytokines, indispensable to fetal growth, through the course of gestation.

What are Growth Factors?

Medical studies have proven that Growth Factors can

- help stimulate normal growth,
- regenerate and accelerate the repair of aged or injured muscle, skin collagen, bone cartilage and nerve tissues

to aid total rejuvenation of the body.

As an example, it stimulates the liver regeneration and increases the level of blood protein.

Growth Factors also provide your body with the natural resources required to

- produce new cells,
- help your body to absorb nutrients taken orally,
- maintain blood glucose levels,
- repair scars from accidents and surgery, and
- help to improve brain and mental acuity.

It also

- helps stimulate the body to burn fat for fuel instead of the body's own muscle,
- helps your body to produce natural collagen to reduce wrinkles and improve complexion noticeably,
- increases stamina and endurance, build lean muscles and
- balances blood sugar.

In addition, Growth Factors

- help increase serotonin levels to brighten moods, and
- rejuvenate internal organs such as kidneys, liver and pancreas.
- They also aid in the prevention of heart problems, including cholesterol and heart failure.

Deficiency in Growth Factors and Cytokines causes aging. An infant's body continues to generate huge quantities of Growth Factors and Cytokines after birth. It needs them to grow and to build up a post-natal immune system.

However, as one grows and gets older, the necessity of these substances lessens drastically, and the body naturally ceases the synthesis of these mitogenic substances. This process is called aging. Without these elements, the body starts to get old; skin colour darkens, age spots appear, wrinkles deepen, complexion worsens and ailments and sickness would ensue.

What are Cytokines?

The human body's immune system has many different types of cells acting together to take care of unwanted infections and altered cells (cancerous cells).

Cytokines are produced by the immune system to communicate and orchestrate the attack against diseases including AIDS, bacterial infections, cancer, influenza, rheumatoid arthritis and body degeneration.

Who should you use Placenta Extract?

Everybody. That is, if you are concerned about your health.

- It is especially useful for pregnant women who have abstained from the regular drugs for cough, cold and/or influenza.
- In cases of Agalactorrhea, placenta extract can stimulate milk production in as short a time as hours.
- It also strengthens the immune system, which would make you less susceptible to illnesses.
- Patients who have undergone surgery or suffered major injuries and sickness could turn to placenta extracts for a speedy recovery.

Placental therapy will unquestionably improve health, and it will also enhance beauty.
Men and women use placenta extracts to slow down the aging process; users, including celebrities, will testify to its astounding effect and value in the greatest reverence. The result is great skin that's firmer and visibly void of wrinkles, blemishes or undesirable pigmentation, not achievable through any topical application of cream and gel. Placental Extract is the ideal dietary supplement to healthy living and the perfect concomitant of a beautiful life.

Growth factors and Spermatogenesis

Although the gonadotropins and testosterone are required for normal spermatogenesis, it is believed that local control factors regulate spermatogenesis.

For many years these regulatory factors had not been identified. Over the past five years, a number of growth factors have been identified in testis or isolated testicular cell types or secretions.

Growth factors are key regulatory molecules which affect cell proliferation, meiosis, and differentiated function. These factors usually act in an autocrine (acting upon the cell which secreted it) or paracine (affecting another cell) manner and thus are involved in intercellular communications.

Growth factor secretion by testicular cell types or testis tissue has been analyzed using a variety of assays measuring cell proliferation in vitro, as well as assays using immunocytochemicals.

Growth factor gene expression in testis has been analyzed by Northern blot analysis and *in situ* hybridization, which gives information concerning the stage and cell specific expression of the gene. Inbred strains of mice with mutations or deletions in a growth factor gene have been used to suggest the function of two specific factors in testicular development and growth. Among the growth factors expressed or secreted by testicular cell types, most are common to some other cell types in the body, such as

- transforming growth factors alpha and beta,
- epidermal growth factor,

- fibroblast-like growth factors,
- insulin-like growth factors,
- interleukins,
- endorphins,
- inhibin and
- activin,

while others may be more testis specific such as

- mullerian inhibiting substance (anti-mullerian hormone) and
- Sertoli cell secreted growth factor.

A variety of proto-oncogenes are expressed at discrete stages of spermatogenesis, as well as by the somatic cells of the testis. Many of these encode growth factors, receptors or other proteins involved in signal transduction. Eventually, this information will be used to develop specific therapies and diagnostic procedures for the infertile male.

INDIVIDUAL GROWTH FACTORS IN PLACENTAL EXTRACT

Transforming Growth Factor (TGF)

TGFalpha

Transforming growth factor alpha (TGFa) was originally isolated as one of two factors actively secreted from virally transformed fibroblasts. Subsequent exposure of normal fibroblasts to this factor elicited a reversible transformed or malignant phenotype.

Originally named sarcoma growth factor, TGFa was later identified, cloned, and placed in the epidermal growth factor (EGF) family of cytokines.

Analysis of the promotor region of the gene suggests that transcription is influenced by EGF, estrogen, glucocorticoids, protein kinase C (PKC), retinoic acid, and thyroid hormone. The human TGF- gene includes six exons which encode a 160 amino acid (aa) precursor protein. The precursor contains an amino-terminal, plasma membrane-targeting signal sequence, extracellular domain possessing N- and O-linked glycosylation sites, a single transmembrane-spanning region, and a cytoplasmic tail.

The mouse and rat homologues of human TGF- have since been cloned and are approximately 93% identical to the human sequence at the aa level, suggesting a high degree of evolutionary conservation.

The mature, soluble form of TGF- is a 50 aa section at the carboxy-terminal end of the extracellular domain. This region contains several highly conserved cysteine residues, which participate in intramolecular disulfide bonding to generate a series of loops. This three-dimensional structure, now known as the EGF-like motif, is critical to the achievement of high affinity binding to its receptor and is common to all members of the EGF family.

Shedding of TGF- from its integral membrane precursor is highly regulated. Adenosine triphosphate (ATP), calcium, EGF receptor (EGF R) activation, G-protein signaling, matrix metalloproteinases and PKC have all been implicated in the regulatory mechanisms of soluble TGF- release.

Evidence suggests that tumor necrosis factor alpha converting enzyme (TACE), a member of the disintegrin and metalloproteinase (ADAM) family, is the enzyme responsible for cleaving mature TGF- from its precursor (5). Once released, mature TGFa has been described as an approximately 5 - 30 kDa protein due to variable glycosylation.

Soluble TGF- acts in autocrine and paracrine fashions via high affinity binding to its receptor, EGF R. Receptor-ligand binding elicits EGF R dimerization, tyrosine autophosphorylation, and activation of signal transduction cascades involving proteins possessing the Src homology domain that eventually impact transcription.

TGFa expression is characterized by a relatively widespread distribution. While TGFa was first described in embryonic and neoplastic cells, it has since been described in the normal adult endocrine, hematopoietic, immune, integumentary, nervous, respiratory, and urinary systems. TGFa expression is exaggerated in transformed cells and tumor tissues of many types. Although TGFa is most often associated with its proliferation, differentiation, and transformation-promoting effects, it is also involved in angiogenesis, bone resorption, cell metabolism, cell migration and wound healing. Further, TGFa has been implicated not only in myriad forms of cancer, but also in several neurodegenerative disorders.

TGFb1

Transforming growth factor (TGFb1), a 'factor' that promoted the transformation of cultured fibroblasts into a tumor-like phenotype, was subsequently found to be more of a tumor suppressor than tumor promoter and to be a mixture of two proteins, TGF-b1 and TGF-b2.

These molecules are members of a superfamily that includes TGF-b1 through 5, bone morphogenic proteins, activins and inhibins. Human TGF-b1 is a 25 kDa, disulfide-linked, non-glycosylated homodimer. There is nearly 100% sequence conservation across mammalian species.

TGF-b1 is cleaved from the C-terminus of a disulfide-linked dimer of pro-TGF-b1 by a subtilisin-like pro-protein convertase protease. It is normally secreted as an inactive, or latent, complex.

There are two types of latent complexes. A small latent complex consists of TGF-b1 noncovalently bound to a disulfide-linked dimer of the N-terminal part of pro-TGF-b1, referred to as latency associated peptide (LAP). A large latent complex contains, in addition, latent TGF-b binding protein (LTBP) disulfide-linked to LAP. LTBP may facilitate secretion or targeting of latent TGF-b. The latency proteins also contribute stability.

Free TGF-b has a half life of about 2 minutes, whereas 'latent' TGF-b has a half life of 90 minutes. Biological activity requires release of TGF-b1 from the latent complex. This can be done in vitro by disruption of LAP (e.g. acidification). The physiological mechanism of release from latency, an important control for the regulation and localization of TGF-b activity, remains obscure, though proteolysis of LAP is probably part of the mechanism.

Two different receptor proteins are involved in TGF-b1 signalling. A dimer of 75 kDa ligand binding proteins (TGF-b RII) has a constitutively active intracellular serine-threonine kinase. With bound TGF-b1 (free of LAP), TGF-bRII recruits and phosphorylates a dimer of 53 kDa signal transducing proteins (TGF-bRI). Phosphorylation of TGF-b RI activates a kinase, initiating a downstream signal via an intracellular protein, SMADS.

TGF-b1 is synthesized, with only a few exceptions, by virtually all cells, and TGF receptors are expressed by all cells. TGF-b affects nearly every physiological process in some way; its systemic and cell-specific activities are too complicated to review here. There are, however, three fundamental activities: TGF-b1 modulates cell proliferation, generally as a supressor; TGF-b1 enhances the deposition of extracellular matrix through promotion of synthesis and inhibition of degradation; TGF-b1 is immunosuppressive through a variety of mechanisms. The specific action of TGF-b on a particular cell depends on the exact circumstances of that cell's environment.

TGFb2

TGF-b2 is synthesized as a preprocytokine with a 19 amino acid (aa) signal sequence, a 283 aa proregion, and a 112 aa mature segment. It dimerizes with formation of disulfide bonds between the 'pro' regions and disulfide bonds between the 'mature' regions. The mature region is 71% and 80% identical with human TGF-b1 and TGF-b3 and 97% identical with the corresponding mouse protein. After proteolytic cleavage of the disulfide-linked mature region, it remains hydrogen-bonded to the disulfide-linked prosegments (LAP or latency-associated protein). If secreted in this form, LAP keeps TGF-b2 in an inactive state until dissociation, caused by proteases, glycosidases, or extreme pH.

In many types of cells, an additional protein, latent TGF-b binding protein (LTBP), is covalently linked to the LAP homodimer prior to secretion. LTBP, a 130 kDa cysteine-rich glycoprotein, creates a 235 kDa large latent complex that is secreted, most likely binding to the extracellular matrix).

The latency components are believed to act as natural antagonists of TGF-b activity, to target TGF-b to distinct tissues, and to maintain a reservoir of TGF-b. On release from latency, active homodimeric TGF-b can bind to cell-surface receptors or to other proteins, such as b2-macroglobulin.

The signal transducing receptor for TGF-b2 is a heterotetrameric complex of two type I signal-transducing receptors (53 kDa; TGF-b RI) and two type II ligand-binding receptors (75 - 85 kDa; TGF-b RII). The binding of TGF-b2 appears to initially involve a type III TGF-b receptor, either 300 kDa betaglycan or 180 kDa endoglin, which then "hands off" to TGF-b RII.

TGF-b2 is expressed by a variety of cells, including osteoclasts, thymic epithelium, keratinocytes, hepatocytes, chief cells of the stomach, satellite cells, skeletal muscle cells, prostatic epithelium, bronchial epithelium, neurons and astrocytes, fibroblasts and visceral smooth muscle, and macrophages.

TGF-b2 has marked cross-species bioactivity (e.g., human TGF-b2 is active on mouse cells, while porcine TGF-b2 is active on rabbit cells).

TGF-b2 has four fundamental activities:

1. it is a growth inhibitor for most types of cells;
2. it enhances the deposition of extracellular matrix;
3. it is immunosuppressive, suppressing APC expression of both IL-12 and CD40L while upregulating IL-10 secretion; and
4. during fetal development, it is expressed in discrete areas, such as epithelium, myocardium, cartilage and bone of extremities and in the nervous system, suggesting specific functions.

EPIDERMAL GROWTH FACTOR

Epidermal growth factor (EGF) was discovered in crude preparations of nerve growth factor prepared from mouse submaxillary glands as an activity that induced early eyelid opening,incisor eruption, hair growth inhibition, and stunting of growth when injected into newborn mice.

EGF is a member of a family of growth factors that bind to the same 170 kDa receptor, including TGF-b, vaccinia growth factor and amphiregulin. EGF is initially synthesized as a large (130 kDa) precursor molecule in which the mature, soluble EGF sequence (6 kDa) is located. The precursor, which functions as a source for soluble EGF, may also have a role in mediation of intercellular communication between cells displaying pro-EGF on their surfaces

and cells with EGF receptors. This “juxtacrine” activity is also shown by a number of other unrelated factors.

Although EGF has been detected in nearly all bodily fluids, the concentration of EGF in tissues is generally low (4). EGF has been detected in secretory cells of eccrine sweatglands. An EGF-like activity is stored in platelets and released on degranulation. In fluids such as saliva, mammary fluids and secretions, prostatic and seminal fluids, and urine, EGF concentrations are rather high.

A wide variety of in vitro and in vivo biological effects have been attributed to EGF. In vitro, EGF is a mitogen for fibroblasts and endothelial cells and promotes colony formation of epidermal cells in culture. In vivo, EGF induces epithelial development, promotes angiogenesis, and inhibits gastric acid secretion. EGF has been shown to be effective in accelerating wound healing.

FIBROBLAST GROWTH FACTOR

FGF basic, also called FGF-2 or heparin-binding growth factor 2 (HBGF-2), is the most extensively studied member of a family of related factors that currently consists of seven members showing an overall sequence homology of 30 - 50% at the amino acid level.

FGF basic has been isolated from a variety of sources, including neural tissue, pituitary, adrenal cortex, corpus luteum, and placenta. When isolated from natural sources, FGF basic usually has an apparent molecular mass of about 18 kDa. Several reports indicate that a variety of larger forms of FGF basic, with molecular masses up to about 24 kDa, also exist as a result of amino-terminal extensions of the protein produced by initiation of translation at non-AUG start sites.

Results suggest that the presence of these amino-terminal extensions results in localization of FGF basic to the cell nucleus rather than to the cytoplasm. Although FGF basic lacks a typical hydrophobic signal peptide sequence, evidence indicates that this factor can be secreted by a non-classical pathway.

FGF basic has been shown to stimulate the proliferation of cells of mesodermal and neuroectodermal origin, including fibroblasts, endothelial cells, astrocytes, oligodendrocytes, neuroblasts, keratinocytes, bovine lens epithelial cells, osteoblasts, smooth muscle cells, and melanocytes.

FGF basic is chemotactic and mitogenic for endothelial cells in vitro, inducing endothelial cell production of factors involved in the breakdown of the basement membrane and the migration of capillary endothelial cells into collagen matrices to form capillary-like tub.

FGF basic induces neuron differentiation, survival and regeneration and rescues neurons in vitro and in vivo from lesion-induced death. FGF basic stimulates the non-mitogenic functions of glial cells, such as migration, and has been located in areas of the hippocampus, brain stem, peripheral ganglia, and the isocortex.

FGF has been implicated as a mesoderm-inducing factor in Xenopus and FGF basic expression has been detected in a variety of tissues in mouse embryos, suggesting a role for FGF in modulating embryonic development and differentiation. These and other in vitro observations suggest that FGFs play a role in vivo in the modulation of such normal processes as angiogenesis, wound healing and tissue repair, embryonic development and differentiation, and neuronal function and neural degeneration.

These observations, as well as the observations that inappropriate expression of FGF basic and other members of the FGF family can result in tumor production, also suggest that FGFs

may participate in the production of a variety of pathological conditions resulting from uncontrolled cell proliferation and uncontrolled angiogenesis.

HEPATOCYTE GROWTH FACTOR

Hepatocyte growth factor (HGF) is a pleiotropic growth factor originally isolated from rat platelets. This factor has also been called scatter factor, hepatopoietin A (hepatopoietin B is likely a fragment of A) and mammary growth factor. Mature HGF is an 82 kDa, 674 amino acid residue heterodimeric glycoprotein that is part of a small family of factors that also includes an HGF-like factor known as macrophage stimulating protein (MSP). These factors lack significant homology with other known growth factors. HGF has marked and varied effects on hepatocytes and other epithelial cells, as well as on endothelial, mesenchymal and hematopoietic cell types, including mitogenic, morphogenic, and motogenic activities. HGF demonstrates marked species cross-reactivity.

HGF is synthesized as a single chain, 728 amino acid residue pre-pro-peptide with a 29 amino acid signal sequence and a 25 amino acid pro-sequence. Mature HGF is formed by the activity of a unique serine protease that extracellularly cleaves a pro-sequence Arg-Val linkage, an activity that itself is activated by another protease in response to tissue damage.

The resulting molecule consists of a disulfide-linked 69 kDa -chain 440 amino acids in length with a predicted molecular weight of 50,800 and a 34 kDa -chain 234 amino acids in length with a predicted molecular weight of 26,000. Glycosylation presumably accounts for the differences between the predicted and observed molecular sizes of the two subunits. Each subunit has two potential N-linked glycosylation sites and an O-linked carbohydrate has been detected on the a-chain.

The biological actions of HGF are mediated by a high affinity membrane-bound receptor identified as the c-met proto-oncogene product. The c-met proto-oncogene is a 1408 amino acid glycoprotein. This receptor is part of a family that also includes the products of the Ron (the receptor for MSP) and Sea genes.

These receptors exhibit no significant homology to any other known growth factor receptors. The most common (190 kDa) isoform of the receptor undergoes considerable post-translational modification of the extracellular region, including glycosylation on one or more of eleven potential N-linked extracellular glycosylation sites, followed by proteolytic cleavage into disulfide-linked 50 kDa - and 145 kDa - chains.

Glycosylation of these sites is considered a prerequisite for subsequent proteolytic cleavage. The resultant heterodimer consists of an N-terminal 283 amino acid a-chain that is strictly extracellular and a C-terminal 1101 amino acid b-chain that contains the remaining extracellular, transmembrane and cytoplasmic domains.

At least two additional isoforms of the receptor have been reported. Both are possibly functional based on their ability to be phosphorylated in an in vitro kinase assay and both are suggested to arise via alternative splicing events. Although the first isoform gives rise to both a- and b- chains, an 18 amino acid segment is missing from the extracellular portion of the b-subunit. The second isoform, although expressed at the cell surface, never undergoes cleavage with heterodimer formation. The significance of these variant receptor forms is unknown.

The presence of HGF mRNA or HGF has been demonstrated in a variety of tissue types. The highest levels of HGF mRNA are found in adult lung, liver, skin and spleen. Detectable mRNA levels have also been found in blood cells, brain, bone marrow, kidney, and the placenta. Cells shown to express HGF mRNA include megakaryocytes of various tissues, monocytes, platelets, fibroblasts, smooth muscle cells, mast cells, and endothelial cells, but not epithelial cells. Expression of the HGF receptor, on the other hand, is found mainly in

epithelial cells, suggesting that HGF acts in a paracrine fashion to mediate interactions between epithelial and stromal cells during development and in normal tissue maintenance. Elevated levels of HGF reportedly have been found in the serum of individuals with a variety of liver disorders and with various types of leukemias or lymphomas.

HGF is pleiotropic in its activities which include mitogenesis, motogenesis, morphogenesis, and growth inhibition. For various types of epithelial and endothelial cells, HGF is a potent mitogen. In particular, HGF is known to stimulate the growth and DNA synthesis of normal epidermal melanocytes, keratinocytes, renal tubular epithelium, gastric epithelium, biliary epithelium, vascular endothelium and normal liver hepatocytes.

HGF also stimulates proliferation and proteoglycan synthesis for some mesenchymal cells, such as chondrocytes. It has been indicated that HGF can also stimulate the proliferation and differentiation of hematopoietic progenitor cells. HGF synergizes with erythropoietin to induce the formation of colonies of the erythroid lineage (BFU-E) in vitro. In the presence of erythropoietin plus stem cell factor, HGF stimulates the in vitro formation of multipotent (CFU-GEMM) colonies.

The ability of HGF to promote hepatocyte proliferation is of particular interest because any hepatocyte mitogen has potential clinical application in reversing compromised hepatic function in vivo. In addition to a normal role in endothelial cell maintenance, high levels of HGF are now suspected to be responsible for the initial development of Kaposi's sarcoma tumor cells, a process facilitated at a later stage by other cytokines.

HGF has also demonstrated motogenic activity towards epithelial, endothelial, and mesenchymal cells. Normal human keratinocytes, Madin-Darby canine kidney (MDCK) epithelial cells, bovine pulmonary artery endothelial cells, human omental microvascular endothelium, and chondrocytes all show marked migration in vitro in response to HGF.

HGF also induces MDCK cells to form branching epithelial tubules in collagen matrix.

Activities such as these are suggested to complement mitogenesis in the repair and/or regeneration of damaged tissues. Less desirably, HGF has been shown to promote the scattering and migration of human MKN-74 gastric adenocarcinoma cells, suggesting a role as a promoter of metastasis.

In contrast to its stimulatory activities, HGF also demonstrates inhibitory activity. In select human small (or oat) cell lung carcinoma cell lines, HGF has been noted to inhibit their in vitro growth rate by approximately half. In addition, HGF has been shown to strongly inhibit the growth of HepG2 hepatocellular carcinoma (HCC) cells, B6/F1 melanoma cells, and KB squamous carcinoma cells in culture via a cytostatic rather than cytolytic mechanism.

KERATINOCYTE GROWTH FACTOR

Human Keratinocyte Growth Factor (KGF) is a single chain, heparin-binding, 28 kDa glycoprotein that was originally isolated from media conditioned by the growth of human embryonic lung fibroblasts. Also known as FGF-7 (or fibroblast growth factor 7), it is part of the rapidly-expanding fibroblast growth factor family that currently includes 14 members.

Mature KGF is 163 amino acid (aa) residues in length, and contains five cysteines, which are not necessary for mitogenic activity, but do contribute to heparin binding. Within the FGF family, KGF shows 29% aa sequence identity to FGF-2 and 38% aa sequence identity to FGF-3. Cells reported to express KGF are fibroblasts, embryonic mesenchymal cells, and smooth muscle cells.

The receptor for KGF (KGF R) is a restricted-expression splice variant of the bek (bacterially-expressed kinase) gene product, a cell surface receptor with tyrosine kinase activity, also

designated FGF R2 (FGF Receptor 2). FGF R2 as a full-length, unspliced (or standard) receptor is a 135 kDa, type I (extracelluar N-terminus) transmembrane glycoprotein with an extracellular domain containing three Ig-like domains plus a heparin-binding motif in the interdomain sequence that connects the N-terminal (D1) and middle (D2) Ig-domains.

This standard receptor form is expressed ubiquitously in connective tissue cells and binds FGF-1, FGF-2, and FGF-4 with high affinity (Kd ~ 100 pM). FGF-5 and FGF-9 also bind, but with lower affinity (Kd ~2 nM). The KGF R splice variant differs from the standard receptor only within a 49 aa residue sequence found in the third (or membrane proximal) Ig-like domain (D3). Although this change has little effect on FGF-1 binding (Kd = 600 pM), its presence decreases FGF-2 binding (Kd = 3 nM) and allows for KGF binding (Kd = 200 pM).

As with KGF, the number of cells expressing KGF R are few and limited to epithelial cell types such as keratinocytes, transitional epithelium (but not umbrella cells), gastric columnar epithelial cells, embryonic lung epithelium, mammary epithelium, and hepatocytes. In addition to the KGF R, KGF also binds to heparan sulfate proteoglycans (HSPG).

In general the role that HSPGs play in the mediation of the biological activities of FGFs is unclear, although they are thought to facilitate binding of FGFs to their high-affinity tyrosine kinase receptors. It has been suggested that HSPGs may hold two FGF molecules in close proximity, thus allowing two individual FGF-FGF R complexes to dimerize or, alternatively, form one FGF-HSPG complex that can actively bind two separate FGF receptors. For KGF in particular, however, the HSPGs have been found to have either no effect or an inhibitory effect on KGF activity. Thus no concensus exists concerning the importance of heparan sulfate for KGF binding and biological activity.

Functionally, KGF has been suggested to be a paracine effector for a number of different epithelial cell types. Synthesized by dermal or lamina propria fibroblasts, it is proposed to act locally on the overlying epithelial sheet. In addition to its ability to induce cell proliferation, it may also promote epithelial differentiation.

During wound healing, KGF's role as a re-epithelializing agent is complemented by the presence of proinflammatory molecules which appear as a result of tissue damage. Cytokines such as IL-1(and) and IL-6 not only activate local connective tissue cells, resulting in foreign body clearance and tissue remodeling, but also stimulate the production of fibroblast KGF which contributes to re-epithelialization and wound closure.

STEM CELL FACTOR

Mice carrying gene mutations in either the dominant white spotting locus (W) or the steel locus (Sl) develop similar phenotypic abnormalities, including deficiencies in hematopoiesis, melanogenesis, and gametogenesis. A transmembrane tyrosine kinase proto-oncogene, designated c-kit was mapped to the W locus. The gene encoding a novel cytokine, the ligand for the c-kit tyrosine kinase receptor, was independently mapped by several groups of investigators to the Sl locus. Several names for this pleiotropic cytokine have been suggested, including stem cell factor (SCF), c-kit ligand (KL), mast cell growth factor (MGF), or steel-factor (SLF). For reviews on SCF, see references.

Initially, cDNA sequences for human and mouse SCF were discovered that encoded a transmembrane protein composed of a 25 amino acid residue signal peptide, a 189 amino acid extracellular domain, a 23 amino acid hydrophobic transmembrane span, and a 36 amino acid cytoplasmic segment. Subsequent analysis in the mouse revealed an alternative splice variant that deletes an entire exon, resulting in a transmembrane molecule 28 amino acids shorter in the extracellular domain. Human and mouse systems are now both known to contain the 248 amino acid and 220 amino acid alternatively spliced forms of SCF.

Both the larger 45 kDa form (also called KL-1) and the smaller 32 kDa form (also called KL-2) are cleaved to produce soluble factors. Cleavage of KL-1 gives rise to a 31 kDa soluble form. The splice variant KL-2 lacks the proteolytic cleavage site used to generate soluble KL-1, but uses a site that is cleaved with lower efficiency to generate a 23 kDa soluble molecule.

Cells identified as possible sources for SCF include fibroblasts, liver cells, Sertoli cells, endothelial cells, neurons, macrophages, oocytes, Schwann cells, cytotrophoblast cells, stratified squamous epithelium and numerous carcinoma cell lines.

Expression of KL-1 and KL-2 seems to be tissue-specific. KL-1 is associated with fibroblasts, brain and thymus while KL-2 is found in spleen, testis, placenta and cerebellum. Both the soluble and the transmembrane forms of SCF have growth factor activities.

However, since mouse mutations (Sld) capable of encoding only the soluble truncated form of SCF, lacking both the transmembrane and cytoplasmic domains, exhibit phenotypic defects almost identical to those mutations in which no SCF protein is produced, the membrane-bound form must be important in mediating cell-cell adhesion and interaction and must have a critical biological role in the intact organism. Native soluble SCF is a heavily N- and O-glycosylated protein that exists as a non-covalently associated dimer in solution. All four cysteine residues of SCF monomers are involved in intramolecular disulfide bonds.

Recombinant soluble SCF produced in E. coli is biologically active in in vitro bioassays, suggesting that glycosylation of the soluble form is not required for bioactivity in vitro. Mouse or rat soluble SCF is highly homologous to human soluble SCF (approximately 80% sequence identity). Whereas both rat and mouse SCF are active on human cells, the human protein is 800 fold less active on mouse or rat cells.

As predicted by the phenotypes of various Sl and W mutations, SCF has biological activities in three migratory cell lines including melanocytes, primordial germ cells, and hematopoietic progenitor cells during embryonal development and postnatal life. Although phenotypic defects in the nervous system have not been described for the Sl or W mutations to date, a complex pattern of SCF expression in the nervous system has been observed.

The multiple hematologic defects (macrocytic anemia, mast cell and bone marrow CFU-S deficiencies) observed for mice carrying Sl mutations and the results obtained from in vivo and in vitro experiments using purified SCF are consistent with the suggestion that SCF is a pleiotropic growth factor with diverse hematopoietic target cells, including early progenitor cells.

SCF can stimulate the proliferation of mature and immature mast cells in vitro and in vivo. In cultures of cord blood-derived immature mast cells, SCF stimulates the proliferation and differentiation of immature mast cells and their progenitors during the early stages of culture. At later stages the effect of SCF is merely to maintain the survival of immature mast cells.

SCF has also been demonstrated to facilitate the release of mast cell inflammatory mediators, either in the presence or absence of IgE-associated stimuli. On purified primitive hematopoietic precursors purified from mice or from humans, SCF acts in a synergistic manner with various growth factors, such as IL-3, IL-6, IL-11, GM-CSF, G-CSF and Epo to induce myeloid and erythroid lineage colony formation. In concert with IL-3, SCF has now been shown to serve as a growth and differentiation factor for the pluripotent CD34+/4-HCres (pre-CFU) stem cell.

SCF has been reported to protect human marrow progenitors from high radiation doses and to expand the primitive cellular components of bone marrow (pre-CFU-S, BFU-E and MK and CFU-MK, GEMM, and GM) following transplantation and thus accelerating recovery of bone marrow function. Other SCF activities not involving hematopoiesis or mast cell physiology include proliferative activity for type A (or primitive) spermatogonia, trophic and neurite-

inductive activity in a subpopulation of dorsal root ganglia neurons and, in conjunction with IL-7, a clonal expansive activity for CD45+ B cells.

FIBRONECTIN

Fibronectin is an extracellular matrix (ECM) component and is one of the primary cell adhesion molecules. It is composed of multiple homologous repeats and contains many functional domains. The occurrence of different isoforms is due to alternative mRNA splicing of the ED-A, ED-B and III-CS regions and subsequent post-translational modification. Although non-reactive with adhesion receptors in its soluble state, fibronectin is highly adhesive when on the surface. Polymerization of fibronectin into ECM must be tightly regulated to ensure appropriate adhesive proporties upon ECM formation. Because of its ability to interact with many ligands (*e.g.* cells, heparin, fibrin, collagen, DNA, immunoglobulin), fibronectin plays an important role in normal morphogenesis, including cell adhesion, migration, differentiation, and specific gene expression.

Fibronectin-like peptide from placental extract acts as wound healer

2005 JUN 5 -- Fibronectin type III-like peptide from human placental extracts acts as a wound healer. "A peptide of {{approx}}7.4 kDa has been purified from the aqueous extract of human placenta used as wound healer. Derived partial amino acid sequence from mass spectrometric analysis showed its homology with human fibronectin type III. Under nondenaturing condition, it formed aggregate, the elution pattern of which from reverse-phase HPLC was identical with that of fibronectin type III," scientists in India report.

"Immunoblot of the peptide with reference fibronectin type III-C showed strong cross reactivity," said Piyali Datta Chakraborty and Debasish Bhattacharyya at the Indian Institute of Chemical Biology. "Since fibronectin type III plays important roles in wound healing, similar peptide in the extract is likely to take part in the curing process." Chakraborty and Bhattacharyya published their study in the *Journal of Chromatography B - Analytical Technologies in the Biomedical and Life Sciences* (Isolation of fibronectin type III like peptide from human placental extract used as wound healer. J Chromatogr B, 2005;818(1 Sp. Iss.):67-73).

For additional information, contact Debasish Bhattacharyya, Department of Drug Design, Development and Molecular Modeling, Indian Institute of Chemical Biology, 4 Raja S.C. Mallick Road, Jadavpur, Calcutta, West Bengal 700032, India. E-mail: p_dattach@rediffmail.com.