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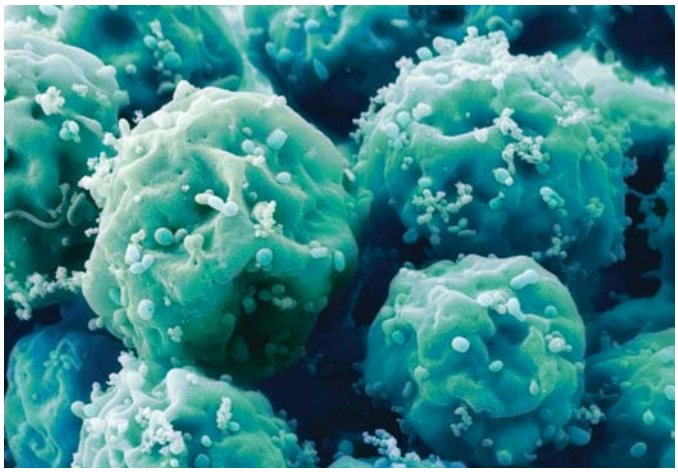
Stem Cells: Links to Human Cancer and Aging

The human body develops from a single diploid cell called a zygote and contains at adulthood an estimated 85 trillion cells, of which more than 150 billion turn over every day. All of these cells originate from a tiny population of so-called "embryonic" and "adult" stem cells which uniquely possess a long-term self-renewal capacity and have the potential to differentiate into a variety of cell lineages. "Embryonic Stem Cells (ESC)" is a term commonly used to refer to a distinct cluster of pluripotent stem cells found in the inner cell mass of mammalian blastocysts (early-stage embryos). Their primary function is to give rise to cell lineages of all three germ layers. On the other hand, "Adult Stem Cells (ASC)" is one of several terms used to describe a diverse group of multipotent stem cells clustered in various niches throughout the body, particularly in loci with high cell turnover such as bone marrow, skin, and intestine, but also in sites with low cell turn over such as brain and pancreas. ASC, also known as somatic or tissue-specific stem cells, serve as a renewable source of specialized cells for tissue development, maintenance, and repair. Depending upon the prevailing conditions in their microenvironment, individual stem cells express distinct cell-surface proteins and display differentiation patterns which normally suit the needs of the tissue or organ in which they reside. As discussed later, such stem-cell specialization is enabled by a battery of epigenetic regulatory factors which provide the means not only to arrest and maintain a particular stem-cell behavior, but also to modify it in response to changes in the cell's microenvironment. Therefore, although ESC and the seemingly various kinds of ASC display different gene expression and differentiation patterns, it remains unclear whether these dissimilarities reflect different cellular entities or different manifestations of the same cellular entity.

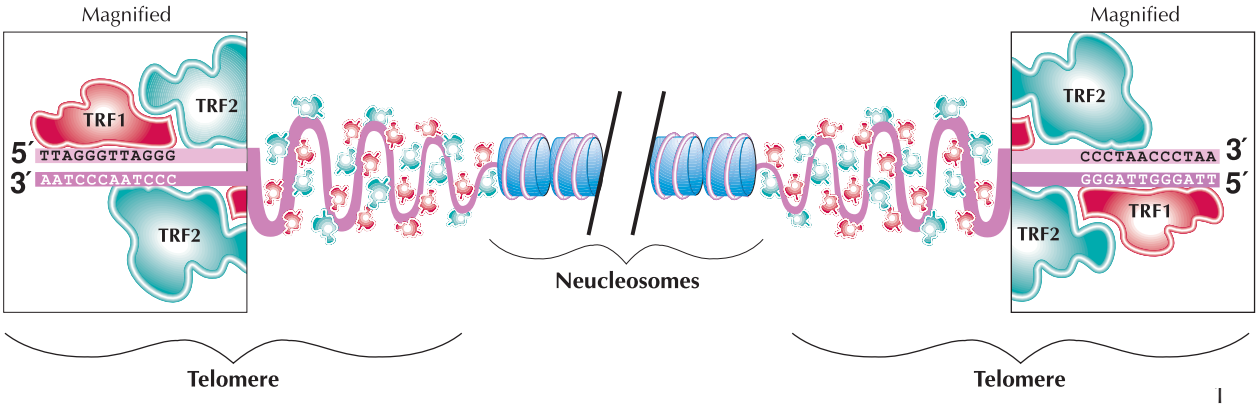
Stem cells divide infrequently and tend to form and stay within distinct-size clusters. Detachment of cells from the cluster (e.g. as a result of differentiation) triggers rapid replication of the remaining cells until the original cluster size is restored. Upon differentiation, stem cells give rise to rapidly propagating transient progeny, which then differentiate into immature tissue blastocytes. A successive series of proliferating progenitors, displaying steadily increasing lineage commitment, ultimately results in a large



number of different mature cells. The distinction between stem cells and their progeny is based on the longevity of their self-renewal, which is commonly assessed by the number of times that the cells can be sub-cultivated in culture conditions before turning senescent (i.e. remain viable but unable to divide). However, since these in-vitro assays may not reflect the in-vivo actuality, the term "stem/progenitor cells" is often used to refer to primary cells that could be expanded multiple times in culture while maintaining their multilineage potential.

The replicative lifespan of individual primary cells depends on the mitotic history of the cell and its ability to maintain functional telomeres at both ends of all chromosomes. Telomeres comprise the ends of each eukaryotic chromosome and consist of several kilobases of repetitive non-coding DNA sequence associated with specialized proteins called telomeric repeat binding factors (TRF1 and TRF2) (Figure 1A). The presence of sufficiently long telomeres at chromosome termini enables complete replication of coding sequences and confers chromosomal stability by reducing the vulnerability of linear DNA ends to nucleolytic degradation and non-homologous end joining. The conservation of the telomere sequence -TTAGGG-n in vertebrates, including bony fish, reptiles, amphibians, birds, and mammals, underscores the importance of telomere structure for genomic integrity and species survival.

Figure 1A - Schematic Illustration of a Eukaryotic Chromosome.



Stem Cells: Links to Human Cancer and Aging

Telomeres shorten with each round of cell division and if not re-elongated ultimately become too short to provide chromosome stability. The presence of such telomeres, called critically short telomeres, constitutes a signal for growth control mechanisms to elicit replicative senescence, a viable cellular state from which no further cell division can occur. Telomere-dependent cellular senescence, which shortens the replicative lifespan of hyper-proliferating cells, appears to be an evolutionary conserved tumor suppressor mechanism and a genetic program for organismal aging. The inherited telomere length in humans (5-15 kilobases) is a genetic variable that can significantly affect lifespan and the onset of age-related tissue degeneration. Homeostasis of telomere length, a hallmark of stemness and cancer, is usually accomplished through the action of telomerase, an enzyme that can counteract replication-induced telomere shortening by re-incorporating lost telomeric segments (50-150 bp/cell-cycling round). Telomerase is a multi-component enzyme with reverse transcriptase activity. The critical components sufficient for in-vitro expression of human telomerase activity are hTER (human telomerase encoded RNA) which serves as a template for TTAGGG synthesis, and hTERT (human telomere reverse transcriptase) which catalyzes DNA synthesis from the RNA template. In contrast to the constitutive expression of hTER in most somatic cells, significant expression of the catalytic subunit hTERT normally occurs only in germ cells during cell proliferation and in certain subsets of B and T lymphocytes following antigen activation. Down-regulation of telomerase activity through repression of hTERT expression appears to occur during early differentiation events associated with mature somatic-cell development. Re-activation or introduction of the hTERT gene is sufficient to bypass replicative senescence and to confer cell immortalization, which, if accompanied by inactivation of tumor suppressor genes and the activation of cellular oncogenes can result in neoplastic transformation. It should be noted, however, that although most tumors use telomerase to maintain telomeric DNA, sarcomas often use a telomerase-independent mechanism called Alternative Lengthening of Telomeres (ALT). The importance of telomerase for stem cell function is highlighted by a rare premature aging disorder called dyskeratosis congenita, an autosomal dominant disorder associated with premature death, typically from bone marrow failure and idiopathic pulmonary fibrosis **(1)**. Patients with this disorder have defective telomerase and very short telomeres as a result of germ-line mutations in the genes encoding hTER or hTERT, or as a consequence of dysfunctional DKC1 (dyskerin), a hTER stabilizing protein.

Age-related decline in the regenerative potential of tissue-specific stem cells, as a result of changes in their supporting niches, telomere shortening, and other genetic alterations, has been implicated in a number of aging syndromes including graying and loss of hair, osteoporosis, decreased spermatogenesis, fibrosis, and senility. Recent studies have demonstrated that age-dependent decline in the function of stem-cell compartments is associated with increased p16/INK4a-mediated replicative senescence, and that this mechanism is essential for preventing neoplastic transformation of genetically-altered and/or epigenetically-stressed primary cells. The execution of this mechanism is controlled by elaborate epigenetic machinery that tightly regulate transcriptional activation of the INK4a/ARF locus, a chromosomal site (9p21 in humans) comprising overlapping reading frames encoding two independent cyclin-dependent kinase (CDK) inhibitors, p16/INK4a (p16) and p14/ARF (ARF). The latter is a positive regulator of p53, a tumor suppressor protein that in addition to triggering apoptosis in response to DNA damage, can also elicit cell-cycle arrest in hyper-proliferating cells even in the absence of DNA damage. Interestingly, critically-short telomeres associated with TRF2 are resistant to p53-mediated apoptosis, whereas telomeres lacking TRF2 protection trigger length-independent apoptosis by both p53 and ATM (Ataxia Telagiectasia Mutated gene product) pathways **(2)**. The p53-dependent cell-cycle arrest is reversible upon subsequent inactivation of p53, while the replicative senescence induced by p16, either by itself or through activation of pRB, is permanent and appears to result from the irreversible formation of repressive heterochromatin at loci containing a number of cyclin and CDK genes. Cyclin-CDK complexes regulate progression through the cell cycle by inactivating, through phosphorylation, retinoblastoma proteins (pRB), which are key regulators of cell proliferation and differentiation during normal development and after genotoxic stress.

The expression of p16 is induced by a variety of stressful stimuli including expression of oncogenes, suboptimal culture conditions (or niche support), and loss of polycomb repressive complexes. Polycomb proteins are evolutionary conserved epigenetic regulatory factors that repress many target genes by forming complexes that function sequentially to block accessibility to promoter regions **(3)** (discussed further). Transcriptional repression by polycomb complexes is essential for the regulation of embryonic gene expression and maintenance of somatic stem cells. However, the presence of these complexes at the p16/ARF locus of stressed cells has been implicated in malignant melanoma and other types of cancer **(4)**. The importance of a functional p16/ARF locus for preventing tumor development is underscored by the high frequency of mutation of this site in human cancers. The relevance of this site for tissue aging has been suggested through a series of recent studies showing that single nucleotide polymorphisms near this locus are associated with age-related pathologies including frailty, Type 2 diabetes mellitus, and vascular heart disease **(reviewed in ref. 5)**.

The p16/ARF locus is epigenetically repressed in early life and then subjected to progressive activation, resulting in steadily increasing levels of p16 with age. The demonstration that p16 levels accumulate in stem cells of old mice suggests that these levels can constitute a good overall biomarker for aging **(6)**. Age-dependent expression of p16 in stem cell compartments is associated with widespread tissue degeneration, whereas deficiency of p16 expression increases tissue regenerative potential accompanied with tumorigenesis **(reviewed in ref. 6)**. These observations suggest that cancer prevention by p16-mediated cellular senescence might come at the expense of accelerated tissue aging. This notion is further supported by recent studies on the link between p16 and ID-1, a helix-loop-helix protein that can specifically inhibit p16 expression but not that of ARF.

ID-1 is a potent inhibitor of cell differentiation and plays an important role in the maintenance of many mammalian primary cells by coordinating cell division and differentiation. However, recent findings show that ID-1 can also promote cancer development by stimulating the proliferation, invasion, and survival of several types of human cancer cells. High expression of ID-1 has been observed in a large number of cancers including prostate, breast, ovary, thyroid, colorectal, liver, pancreas, and other tumors. Constitutive expression of ID-1 in cultured human primary melanocytes extends their lifespan in association with decreased expression of p16 but without notable changes in cellular growth, migration, or telomere length. In contrast, ID1-null primary mouse embryo fibroblasts undergo premature senescence associated with increased expression of p16 but not ARF (7).

Epigenetic Remodeling of Chromatin Structure

The versatility of individual stem cells to respond to diverse external cues by selecting and executing a suitable genetic program out of many permissible ones, or their epigenetics, enables a single and otherwise fixed genome to yield a plethora of cell types. Although the mechanisms underlying stem cell identity and plasticity have not been fully characterized, it has become clear that epigenetic remodeling of chromatin structure, particularly through covalent modification of genomic DNA and cognate histones, is pivotal in the establishment and maintenance of cellular identity, memory, and potentiality. The basic repeating unit of chromatin, termed nucleosome, consists of a 146 base-pair DNA chain wrapped around a histone octamer made of two each of the four core histones, H2A, H2B, H3, and H4. In higher-order chromatin structures, the nucleosomes are connected via DNA linkers of varying lengths coupled with histone H1 (Figure 1B). Core histones are evolutionary conserved, structurally-related proteins containing a highly positively charged, N-terminal tail of 25-40 residues that extends through the DNA coils and into the space surrounding the nucleosome. Nucleosomal histones, in particular their exposed tails, are subject to various post-translational modifications including methylation of K and R residues, acetylation and ubiquitination of K residues, and phosphorylation of serine (S) and threonine (T) residues (Figure 1C). Nucleosomal DNA is subject to specific epigenetic methylations, particularly of cytosine moieties within CpG dinucleotides. The functional relevance of the various covalent modifications of chromatin, which can be reversed through the action of specific nuclear enzymes, such as demethylases, deacetylases, and phosphatases, is a topic of current research and will be discussed here in general terms only.

Post-translational modifications of core histones trigger specific alterations in the spatial organization of chromatin, which in turn affect DNA-based processes including DNA repair, transcription, replication, and recombination. In the absence of histone modifications, the positively charged tails of core histones form stable salt bridges with negatively charged inter-nucleotide phosphate groups of adjacent DNA (Figure 1B). Such interactions can prevent, for example, interaction of RNA polymerase with promoter regions of genes whose expression is not needed by the cell. Modifications that reduce the net positive charge of histone tails, such as acetylation of K residues (neutralizes their positive charge) and phosphorylation of S and T residues (rendering them negatively charged), weaken tail-DNA interactions (Figure 1B) and are generally associated with transcriptionally active genes. Certain modifications, particularly methylation of specific K and R residues, generate docking sites for nuclear proteins that are involved in activation or repression of specific gene loci. For example, methylation of the K residue at position 4 of histone H3 produces binding site, H3K4Me*, for the chromatin remodeling protein Chd-1. Subsequent to its binding to H3K4Me*, Chd-1 recruits nucleosome remodeling enzymes such as acetylases and phosphokinases, whose action positively regulates DNA replication by disrupting H3 tail-DNA interactions.

Figure 1B - **Schematic Illustration of un-modified (a) and modified (b) nucleosomes.**

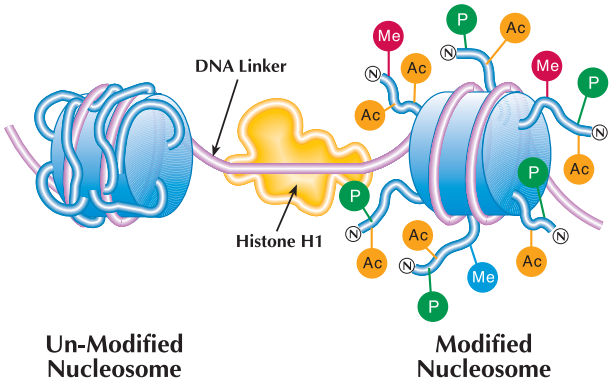


Figure 1D - **Schematic Illustration of euchromatin (left) and heterochromatin (right).**

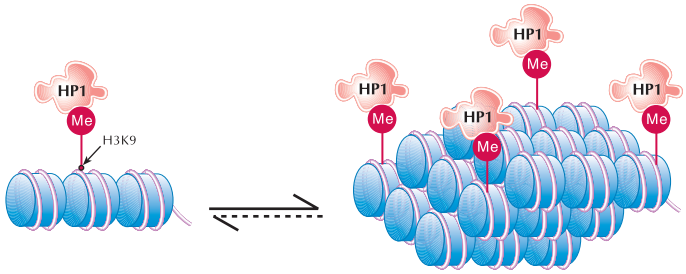
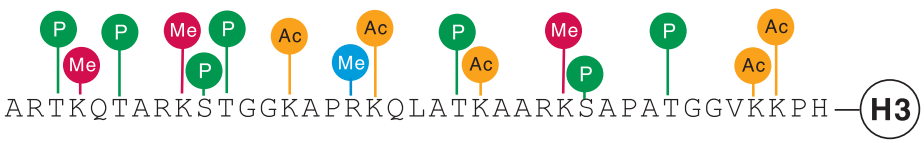


Figure 1C - **A heavily modified Histone H3 tail (an arbitrary example).**



The symbols P Me Ac ***represent Phoshate, Methyl, and Acetyl groups, respectively***

In contrast, the heterochromatin-associated protein HP-1 interacts with H3K9Me* and promotes formation and maintenance of replication-incompetent heterochromatin (Figure 1D). The symbol [Me*] denotes K residues that are either mono-, di-, or tri-methylated. Interestingly, high-resolution profiling of histone methylations in the human genome has revealed that tri-methylated H3K27 signals were higher at silent promoters than active promoters, whereas an opposite trend was associated with mono-methylated H3K27 **(8)**. Tri-methylation of this residue, which produces H3K27Me3, is catalyzed by the polycomb repressive complex (PRC)-2 which contains the histone methyltransferase EZH2. H3K27Me3 serves as a docking site for the bulky, BMI1-containing PRC-1, whose presence at this site blocks accessibility to many gene loci including the p16/ARF locus **(3)**. As already mentioned, sustained repression of the p16/ARF locus, which requires constitutive expression of PRC components such as EZH2 and BMI1 (an oncogenes), has been implicated in the development of various cancers. On the other hand, decreased expression of PRC components, particularly EZH2, in older or stressed cells has been suggested to be a major cause for the steady increase in p16 levels with age **(3)**. Also, since p16 is a phosphokinase inhibitor, it is possible that p16-mediated inhibition of histone phosphorylation at the promoter region of EZH2 or other PRC components indirectly contributes to its own expression. Likewise, P16-induced cellular senescence may result, in part, from p16 inhibition of histone phosphorylation at nucleosomes whose DNA transcription is required during mitosis and dependent upon such phosphorylation.

The Epigenome

Recent advances in the development of analytical methods for determining histone methylation profiles across large genomic sequences have enabled closer examinations of the so called “epigenetic signature” or “epigenome” of somatic cell types **(8, 9)**. The general picture emerging from these studies is that methylation of specific K and R residues of core histones is a fundamental mechanism for establishing and maintaining gene expression patterns that can carry epigenetic information through cell division. The epigenome of Embryonic Stem Cells (ESC) has been found to be enriched for chromatin structures displaying histone methylation marks of both transcriptionally active and silent promoters **(9)**. These chromatin structures, called “bivalent domains”, have been suggested to comprise transcriptionally repressed chromatin that is poised for selective activation by, for example, differentiation-inducing signaling pathway intermediates **(9)**. Global silencing of developmentally important genes that can be selectively activated in response to environmental cues appears to be controlled by a small group of transcription factors **(10-12)**. For example, it has been shown that retrovirus-mediated introduction of four transcription-factor genes, Oct4, Sox2, c-Myc, and Klf4, into adult fibroblasts selected for Nanog expression was sufficient to confer a pluripotent state upon the fibroblast genome **(11)**. Analysis of the reprogrammed epigenome of such induced pluripotent cells revealed that it was almost indistinguishable from that of ESC **(12)**. These results provide direct evidence that all chromatin modifications are reversible, and support the notion that targeted manipulation of the epigenome by agents that can induce reorganization of chromatin is a viable approach for the discovery of new therapeutic drugs for cancer treatment **(13)**. Likewise, it should be interesting to determine the effects on the epigenome of dietary and physical regiments which appear to prolong lifespan or reverse the course of age-related pathologies, such as caloric restriction and boxing workout by Parkinson’s patients, respectively.

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(2) Karlsender J. Science, Vol. 283, 1321-1325 (1999)
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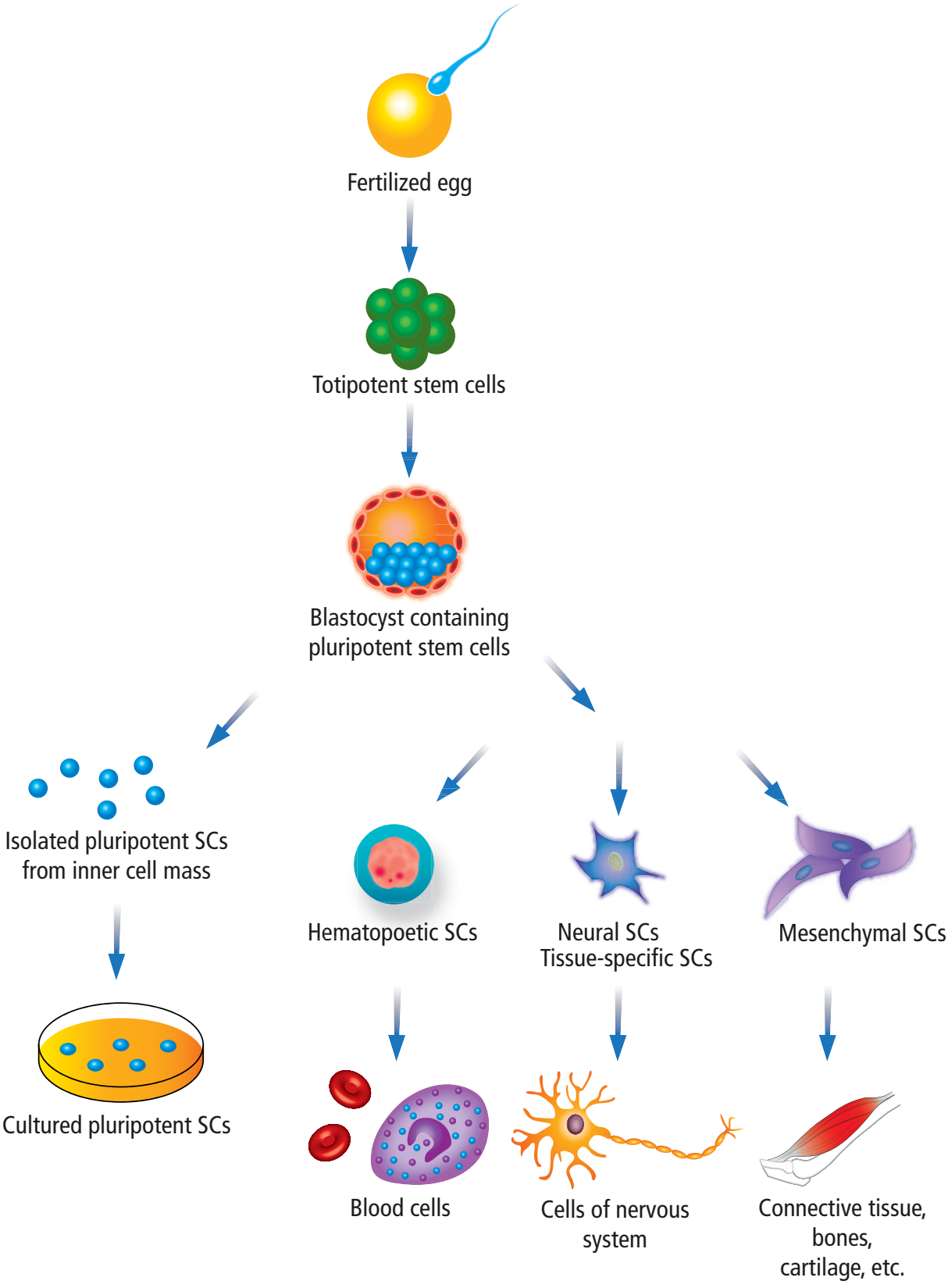
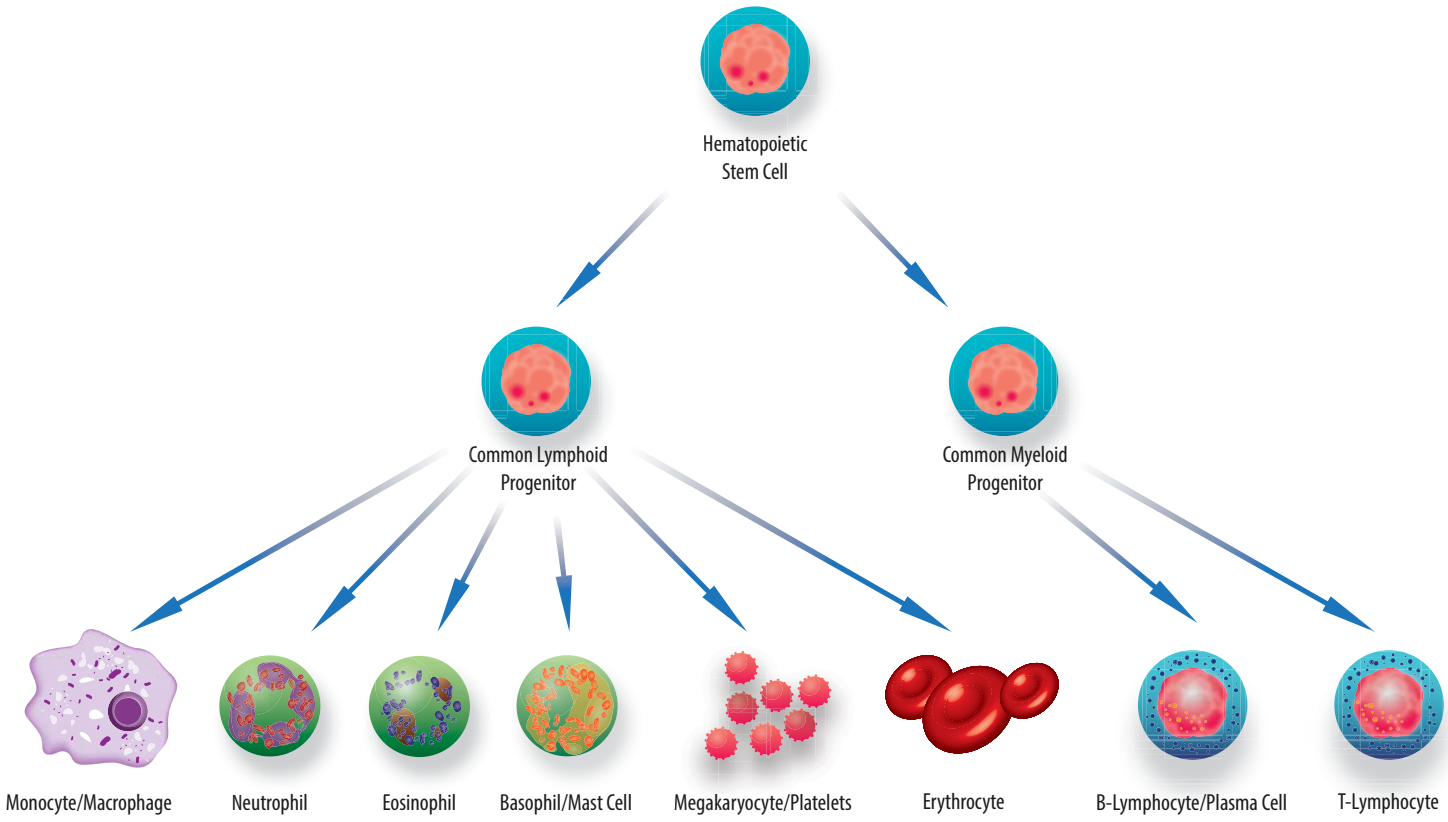
Embryonic Stem Cells (ESCs)

ESCs are pluripotent stem cells derived from the inner mast of blastocyst than can differentiate into three primary germ layers: ectoderm, endoderm and mesoderm.



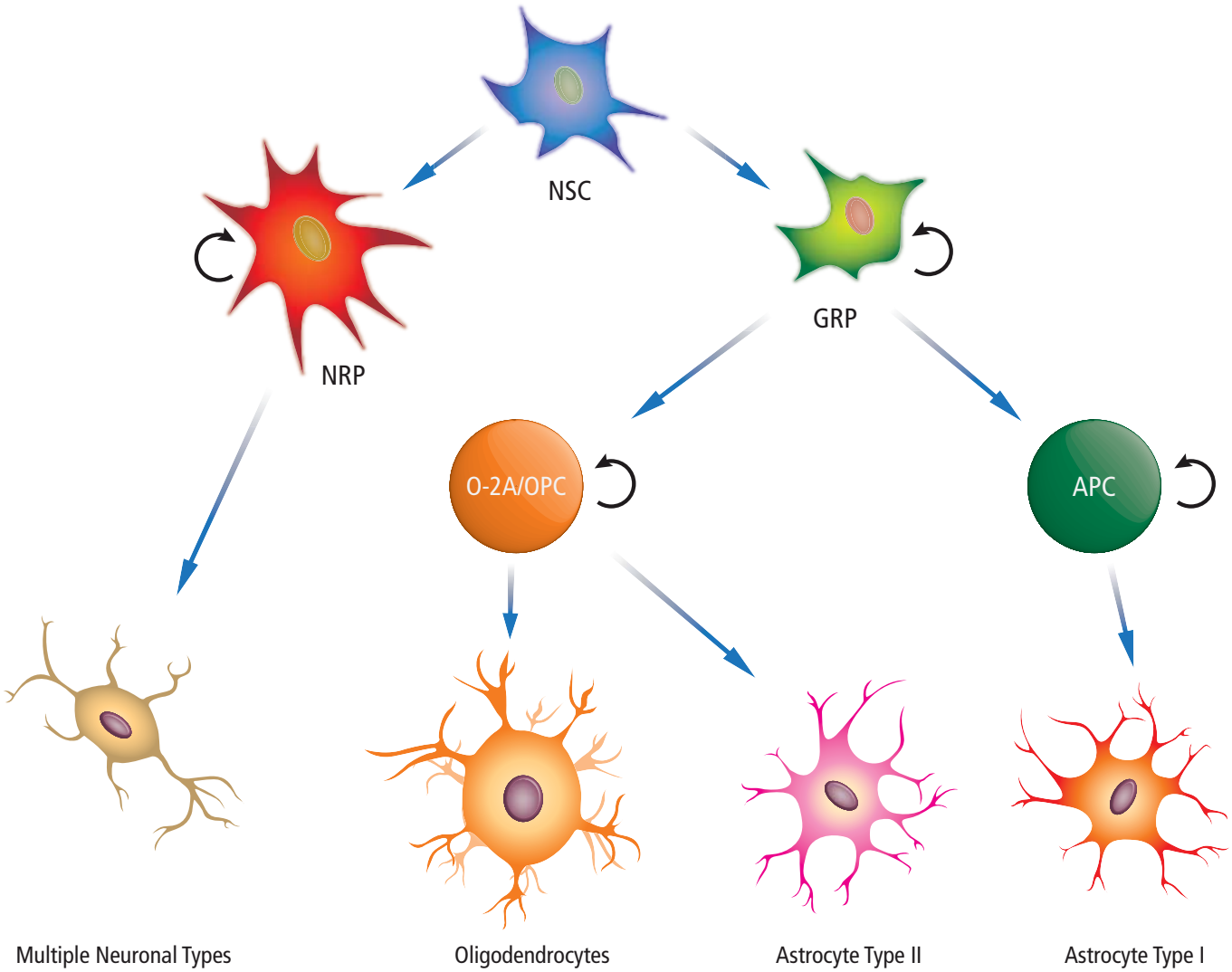
Hematopoietic Stem Cells (HSCs)

HSCs are multipotent stem cells that give rise to all blood cell types from the myeloid and lymphoid linages



Neural Stem Cells (NSCs)

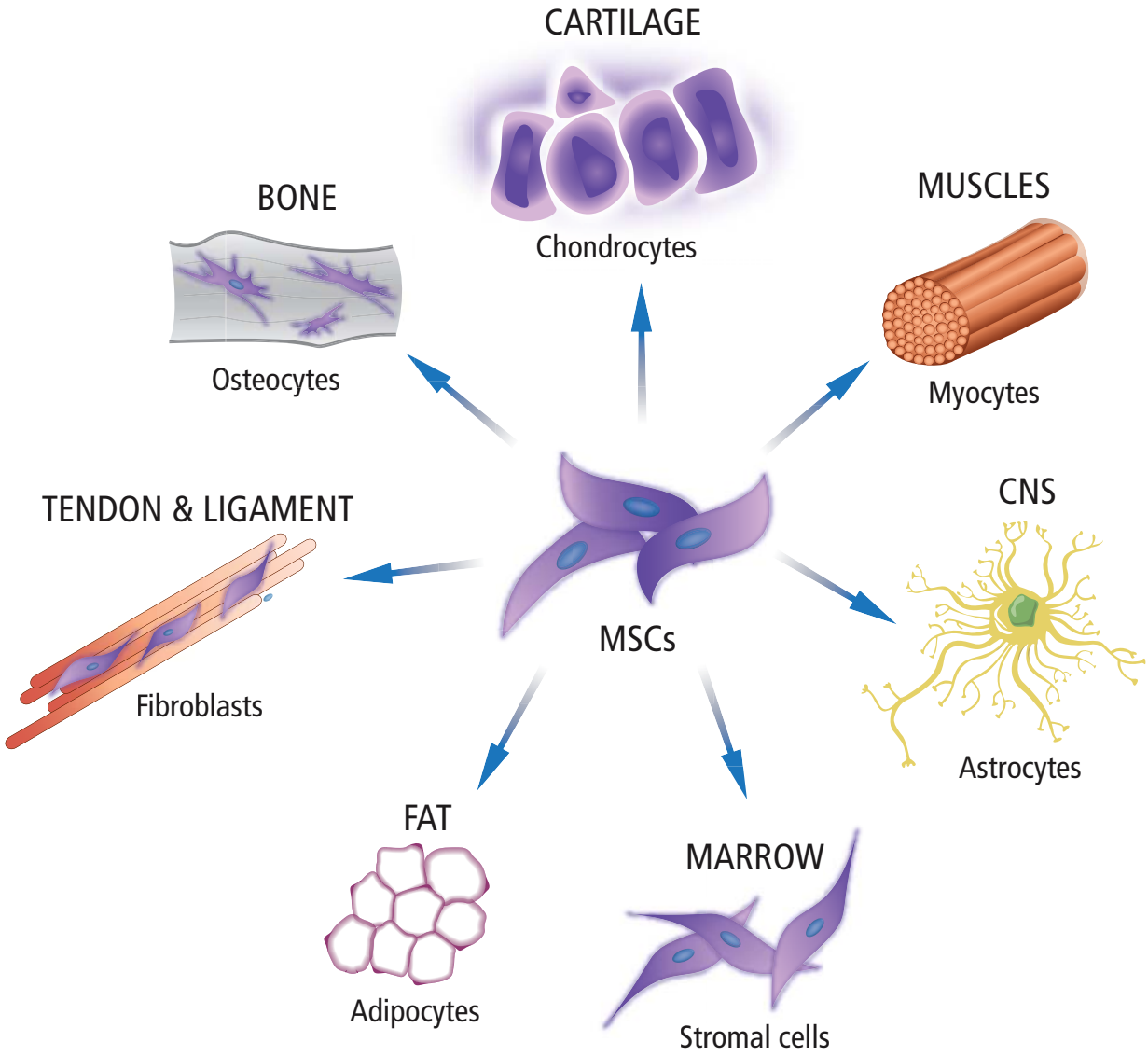
NSCs are multipotent stem cells that produce the main phenotypes of the nervous system.



NSC	Neural Stem Cell	O-2A/OPC	Oligodendrocyte-type 2 Astrocyte Precursor cell
NRP	Neuronal-Restricted Precursor cells	APC	Astrocyte Precursor cell
GRP	Glial-Restricted Precursor cells	↻	Self Renewal

Mesenchymal Stem Cells (MSCs)

MSCs are multipotent stem cells, that given exposure to different inductive agents can differentiate into adipocytes, fibroblasts, stromal cells, astrocytes, myocytes, chondrocytes, and osteocytes.



Activin A

Activin A is a TGF-β family member that exhibits a wide range of biological activities including regulation of cellular proliferation and differentiation, and promotion of neuronal survival. Elevated levels of Activin A in human colorectal tumors and in postmenopausal women have been implicated in colorectal and breast cancers, respectively. The biological activities of Activin A can be neutralized by inhibins and by the diffusible TGF-β antagonist, follistatin. Human, murine, and rat Activin A share 100% amino acid sequence identity.

Woodruff, T.K. et al. (Apr. 1998) Biochem. Pharmacol. 7, 953-963.

Activin B

Activin B is a TGF-β family member that exhibits a wide range of biological activities including regulation of embryogenesis, osteogenesis, hematopoiesis, reproductive physiology and hormone secretion from the hypothalamic, pituitary and gonadal glands. Activin B, like certain other members of the TGF-β family, signals through the ActRII receptor (Activin Receptor type II).

Woodruff, T.K. et al. (Apr. 1998) Biochem. Pharmacol. 7, 953-963.

Amphiregulin

Amphiregulin is an EGF related growth factor that signals through the EGF/TGF-α receptor, and stimulates growth of keratinocytes, epithelial cells and some fibroblasts. Amphiregulin also inhibits the growth of certain carcinoma cell lines. Synthesized as a transmembrane protein, Amphiregulin's extracellular domain is proteolytically processed to release the mature protein. There are 6 conserved cysteine residues, which form 3 intramolecular disulfide bonds essential for biological activity.

Artemin

Artemin is a disulfide-linked homodimeric neurotrophic factor structurally related to GDNF, Neurturin and Persephin. These proteins belong to the cysteine-knot superfamily of growth factors that assume stable dimeric protein structures. Artemin, GDNF, Persephin and Neurturin all signal though a multicomponent receptor system, composed of RET (receptor tyrosine kinase) and one of the four GFRα (α1-α4) receptors. Artemin prefers the receptor GFRα3-RET, but will use other receptors as an alternative. Artemin supports the survival of all peripheral ganglia such as sympathetic, neural crest and placodally derived sensory neurons, and dompaminergic midbrains neurons. The functional human Artemin ligand is a disulfide-linked homodimer, of two 12.0 kDa polypeptide monomers. Each monomer contains seven conserved cysteine residues, one of which is used for inter-chain disulfide bridging and the others are involved in intramolecular ring formation known as the cysteine knot configuration.

Baloh, R.H. et al. (Dec. 1998) Neuron 6, 1291-1302.

BAFF

BAFF, a member of the TNF superfamily of ligands, is expressed in T cells, macrophages, monocytes and dendritic cells. BAFF is involved in stimulation of B and T cell function, and is an important survival and maturation factor for peripheral B cells. BAFF signals through three different TNF receptors TACI, BCMA and BAFF-R. The human BAFF gene codes for a 285 amino acid type II transmembrane protein containing a 46 amino acid cytoplasmic domain, a 21 amino acid transmembrane domain, and a 218 amino acid extracellular domain.

MacKay, F. and Ambrose, C. (June-Aug. 2003) Cytokine Growth Factor Rev. 3-4, 311-324.
Kalled, S.L. et al. (Feb 7, 2003) Expert Opin. Ther. Targets 1, 115-123.

BD-1, -2, -3, -4

Defensins (α and β) are cationic peptides with a broad spectrum of antimicrobial activity that comprise an important arm of the innate immune system. The α-defensins are distinguished from the β-defensins by the pairing of their three disulfide bonds. To date, six human β-defensins have been identified; BD-1, BD-2, BD-3, BD-4, BD-5 and BD-6. β-defensins are expressed on some leukocytes and at epithelial surfaces. In addition to their direct antimicrobial activities, they can act as chemoattractants towards immature dendritic cells and memory T cells. The β-defensin proteins are expressed as the C-terminal portion of precursors and are released by proteolytic cleavage of a signal sequence and

BD-1, -2, -3, -4 cont'd

in some cases, a propeptide sequence. β-defensins contain a six-cysteine motif that forms three intramolecular disulfide bonds.

Lehrer, R.I. and Ganz, T. (2002) Curr. Opin. Immunol. 14, 96-102.
Tang, Y.Q. et al. (1999) Science 286, 498-502.

BDNF

BDNF is a member of the NGF family of neurotrophic growth factors. Like other members of this family, BDNF supports neuron proliferation and survival. BDNF can bind to a low affinity cell surface receptor called LNGFR, which also binds other neurotrophins such as NGF, NT-3 and NT-4. However, BDNF mediates its neurotrophic properties by signaling through a high affinity cell surface receptor called gp145/trkB. BDNF is expressed as the C-terminal portion of a 247 amino acid polypeptide precursor, which also contains a signal sequence of 18 amino acid residues and a propeptide of 110 amino acid residues. Human and mouse BDNF sequences are identical.

Friedman, W.J. and Greene, L.A. (1999) Exp. Cell. Res. 253, 131-142.

Betacellulin

Betacellulin is an EGF-related polypeptide growth factor that signals through the EGF receptor. It is produced in several tissues, including the pancreas, small intestine, and in certain tumor cells. Betacellulin is a potent mitogen for retinal pigment epithelial cells and vascular smooth muscle cells. Human Betacellulin is initially synthesized as a glycosylated 32.0 kDa transmembrane precursor protein, which is processed by proteolytic cleavage to produce the mature sequence.

Seno, M. et al. (1996) Growth Factors. 13, 181-191.

BMP-2

BMP-2 is a potent osteoinductive cytokine, capable of inducing bone and cartilage formation in association with an osteoconductive carrier such as collagen and synthetic hydroxyapatite. In addition to its osteogenic activity, BMP-2 appears to play an important role in cardiac morphogenesis, and is expressed in a variety of other tissues including lung, liver, spleen, prostate, ovary, and small intestine. The functional form of BMP-2 is a 26 kDa protein composed of two identical 114 amino-acid polypeptide chains (monomers) linked by a single disulfide bond. Each BMP-2 monomer is expressed as the C-terminal part of a precursor polypeptide, which also contains a 23 amino-acid signal sequence for secretion, and a 259 amino-acid propeptide. After dimerization of this precursor, the covalent bonds between the propeptide (which is also a disulfide-linked homodimer) and the mature BMP-2 ligand are cleaved by a furin-type protease.

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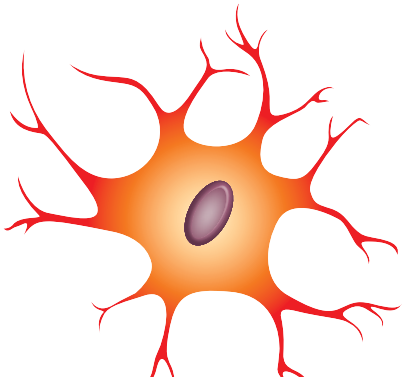
Urist, M.R. (1965) Science 150, 893-899.

BMP-3

TGF-β family members are key modulators of cell proliferation, differentiation, matrix synthesis, and apoptosis. As implied by their name, BMPs initiate, promote, and regulate the development, growth and remodeling of bone and cartilage. In addition to this role, BMPs are also involved in prenatal development and postnatal growth, remodeling and maintenance of a variety of other tissues and organs. BMP-3 is abundantly found in adult bone, and to a lesser extent fetal cartilage. BMP-3 inhibits osteogenesis and bone formation by activating a signaling cascade that antagonizes the signaling of pro-osteogenic BMPs.

Chang, C.H. et al. (1994) J. Bio. Chem. 45, 28227-28234.
Chang, D et al. (2004) Growth Factors. 22, 233-241.
Lens, S.M. et al. (1998) Semin. Immunol. 10, 491-499.
Scheufer, C. et al. (1999) J. Mol. Biol. 287, 103-115.
Urist, M.R. (1965) Science 150, 893-899.



BMP-4

Bone morphogenetic proteins (BMPs) constitute a subfamily within the TGF-β superfamily of structurally related signaling proteins. Members of this superfamily are widely distributed throughout the body and are involved in diverse physiological processes during both pre- and postnatal life. Like BMP-7, BMP-4 is involved in the development and maintenance of bone and cartilage. Reduced expression of BMP-4 is associated with a number of bone diseases, including the heritable disorder Fibrodysplasia Ossificans Progressiva.

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Chang, D et al. (2004) Growth Factors. 22, 233-241.
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Scheufer, C. et al. (1999) J. Mol. Biol. 287, 103-115.
Urist, M.R. (1965) Science 150, 893-899.

BMP-5

TGF-β family members are key modulators of cell proliferation, differentiation, matrix synthesis, and apoptosis. As implied by their name, BMPs initiate, promote, and regulate the development, growth and remodeling of bone and cartilage. In addition to this role, BMPs are also involved in prenatal development and postnatal growth, remodeling and maintenance of a variety of other tissues and organs. BMP-5 is expressed in the nervous system, lung and liver. It is a known regulator for dendritic growth in sympathetic neurons.

Chang, C.H. et al. (1994) J. Bio. Chem. 45, 28227-28234.
Chang, D et al. (2004) Growth Factors. 22, 233-241.
Lens, S.M. et al. (1998) Semin. Immunol. 10, 491-499.
Scheufer, C. et al. (1999) J. Mol. Biol. 287, 103-115.
Urist, M.R. (1965) Science 150, 893-899.

BMP-6

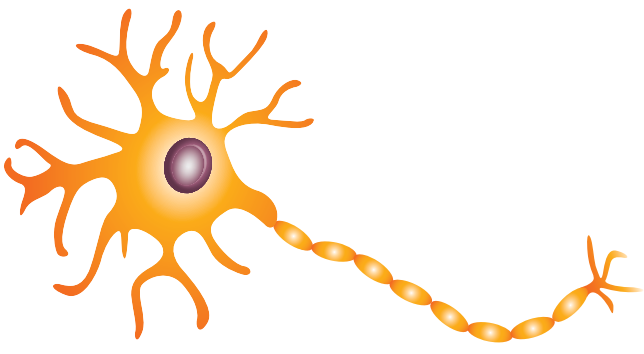
TGF-β family members are key modulators of cell proliferation, differentiation, matrix synthesis, and apoptosis. As implied by their name, BMPs initiate, promote, and regulate the development, growth and remodeling of bone and cartilage. In addition to this role, BMPs are also involved in prenatal development and postnatal growth, remodeling and maintenance of a variety of other tissues and organs. Increasing evidence indicates that BMP-Smad signaling has a tumor suppressing activity and that BMPs can inhibit tumor growth. BMP-6 is abnormally expressed in breast cancer cell lines; however, its function in promoting breast cancer development is unknown.

Chang, C.H. et al. (1994) J. Bio. Chem. 45, 28227-28234.
Chang, D et al. (2004) Growth Factors. 22, 233-241.
Lens, S.M. et al. (1998) Semin. Immunol. 10, 491-499.
Scheufer, C. et al. (1999) J. Mol. Biol. 287, 103-115.
Urist, M.R. (1965) Science 150, 893-899.

BMP-7

TGF-β family members are key modulators of cell proliferation, differentiation, matrix synthesis, and apoptosis. As implied by their name, BMPs initiate, promote, and regulate the development, growth and remodeling of bone and cartilage. In addition to this role, BMPs are also involved in prenatal development and postnatal growth, remodeling and maintenance of a variety of other tissues and organs. BMP-7, also known as osteogenic protein-1 or OP-1, is a potent bone inducing agent, which in the presence of appropriate osteoconductive carrier (e.g. collagen sponge or synthetic hydroxyapatite) can be used in the treatment of bone defects. A bone-graft substitute, called OP-1™ implant, made of recombinant human BMP-7 associated with bovine bone-derived collagen, has recently been approved by the FDA as a device for treating critical-size bone fractures. The potential use of BMP-7 in dental reconstructive surgeries is currently under investigation.

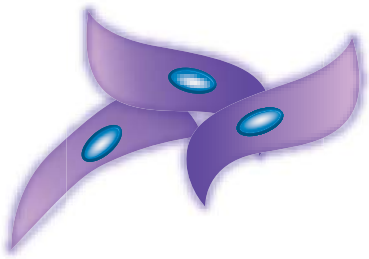
Chang, C.H. et al. (1994) J. Bio. Chem. 45, 28227-28234.
Chang, D et al. (2004) Growth Factors. 22, 233-241.
Lens, S.M. et al. (1998) Semin. Immunol. 10, 491-499.
Scheufer, C. et al. (1999) J. Mol. Biol. 287, 103-115.
Urist, M.R. (1965) Science 150, 893-899.



BMP-13/CDMP-2

BMP-13 is expressed in hypertrophic chondrocytes during embryonic development of long bones. Continued postnatal expression of BMP-13 in articular cartilage suggests that it plays a regulatory role in the growth and maintenance of articular cartilage. Adenovirus-mediated BMP-13 gene transfer to rabbit bone marrow stem cells have been reported to augment periosteal repair of osteochondral defects. The functional form of BMP-13/CDMP-2 is a disulfide-linked homodimer of two 120 amino-acid polypeptide chains. This 27.5 kDa protein is obtained by proteolytic processing of a biologically inactive precursor protein of 97.7 kDa.

Chang, C.H. et al. (1994) J. Bio. Chem. 45, 28227-28234.
Chang, D et al. (2004) Growth Factors. 22, 233-241.
Lens, S.M. et al. (1998) Semin. Immunol. 10, 491-499.
Scheufer, C. et al. (1999) J. Mol. Biol. 287, 103-115.
Urist, M.R. (1965) Science 150, 893-899.



BMP-14/CDMP-1 see GDF-5

Cardiotrophin-1 (CT-1)

CT-1 is a member of the IL-6 family of cytokines which also includes LIF, CNTF, OSM (Oncostatin M), IL-11, IL-6 and possibly NT-1/ BSF-3. CT-1 is a pleiotropic cytokine which is expressed in various tissues including the adult heart, skeletal muscle, ovary, colon, prostate and fetal lung and signals through the LIF receptor and the gp130 receptor subunit. CT-1 has the ability to induce cardiac myocyte hypertrophy, and enhances the survival of cardiomyocyte and different neuronal populations.

Pennica, D. et al. (1995) J. Biol. Chem. 270, 10915-10922.

CDNF

CDNF is a secreted neurotrophic factor that is expressed in brain, neuronal and certain non-neuronal tissues. It has been shown to promote survival, growth and function of dopamine specific neurons. CDNF and its structural homolog MANF, each contain an N-terminal saposin-like lipid binding domain, and a carboxyl-terminal domain, which is not homologous to previously characterized protein structures. CDNF and MANF can prevent 6-OHDA induced degeneration of dopaminergic neurons by triggering survival pathways in a rat experimental model of Parkinson disease.

Lindholm, P. et al. (2007) Nature 448, 73-77.
Lindholm, P. and Saarma, M. (2010) Dev. Neurobiol. 70, 360-371.

CNTF

CNTF is a potent neural factor that was originally characterized as a vital factor for the survival of chick ciliary neurons in vitro. CNTF is also important for the survival of other neural cell types including primary sensory neurons, motor neurons, basal forebrain neurons and type 2 astrocytes. CNTF is highly conserved across species and exhibits cross-species bioactivity.

Lam, A. et al. (1991) Gene. 102, 271-276.
Bazan, J.F. (1991) Neuron 7, 197-208.

CTGF

CTGF is a member of the CCN family of secreted cysteine rich regulatory proteins and is the major mitogenic and chemoattractant protein produced by umbilical vein and vascular endothelial cells. CTGF stimulates the proliferation and differentiation of chondrocytes, induces angiogenesis, promotes cell adhesion of fibroblasts, endothelial, and epithelial cells, and binds to IGF, TGF-β1, and BMP-4. Cell migration and adhesion are signaled through binding to specific cell surface integrins and to heparin sulfate proteoglycans. CTGF (98 a.a.), a lower molecular weight isoform containing the C-terminal portion of the full length CTGF protein, exerts full heparin binding, cell adhesion, and mitogenic CTGF activity.

Ball, D.K. et al. (Feb. 2003) Reproduction 125, 271-284.
Kumar, S. et al. (1999) J. Biol. Chem. 24, 17123-17131.

CYR61

CYR61 is a member of the CCN family of secreted cysteine rich regulatory proteins. CYR61 induces angiogenesis by stimulating the proliferation, migration, and adhesion of endothelial cells. Cell migration and adhesion are mediated through binding to specific cell surface integrins and to heparin sulfate proteoglycans. Increased expression of CYR61 is associated with several types of cancer, and correlates with the progression and estrogen independence of human breast cancers. Recombinant human CYR61 is a 39.5 kDa protein containing 357 amino acid residues. It is composed of four distinct structural domains (modules); the IGF binding protein (IGFBP) domain, the von Willebrand Factor C (VWFC) domain, the Thrombospondin type-I (TSP type-1) domain, and a C-terminal cysteine knot-like (CTCK) domain.

Brigstock, D.R. et al. (2002) Angiogenesis 5 (3), 153-165.
Menendez, J.A. et al. (June 2003) Endocr. Relat. Cancer 10 (2), 141-152.

DKK-1

DKK-1 is a member of the DKK protein family which also includes DKK-2, DKK-3 and DKK-4. DKK-1 was originally identified as a Xenopus head forming molecule that behaves as an antagonist for Wnt signaling. Subsequent studies have shown that DKK-1 and DKK-4 play an important regulatory role in the Wnt/β-catenin signaling pathway by forming inhibitory complexes with LDL receptor-related proteins 5 and 6 (LRP5 and LRP6), which are essential components of the Wnt/β-catenin signaling system. LPR5 and LPR6 are single-pass transmembrane proteins that appear to act as co-receptors for Wnt ligands involved in the Wnt/β-catenin signaling cascade. It has been suggested that by inhibiting Wnt/β-catenin signaling, which is essential for posterior patterning in vertebrates, DKK-1 permits anterior development. This notion is supported by the finding that mice deficient of DKK-1 expression lack head formation and die during embryogenesis.

Niehrs, C. (2006) Oncogene, 25:7469-7481.
Krupnik, VE. et al. (1999) Gene 238:301-313.

DLL-1

Human sDLL-1 comprises the extracellular signaling domain of DLL-1, a member of a structurally-related family of single-pass type I trans-membrane proteins that serve as ligands for Notch receptors. It is expressed in the heart and pancreas, and to a lesser extent in various other tissues. DLL-1 functions to specifically activate the Notch-1 and Notch-2 receptors. The Notch signaling pathway regulates endothelial cell differentiation, proliferation and apoptosis, and is essential for the development, maintenance and remodeling of the vascular system. DLL-1 suppresses differentiation of hematopoietic progenitor cells into the B-cell lineage while promoting differentiation to T-cell and NK cell precursors.

Leong, K.G. and Gao, W.Q. (2008) Differentiation 76 (6), 699-716.
dePooler, R.F. and Zunigna-Pflucker, J.C. (2007) Methods Mol. Biol. 380, 73-81.

DLL-4

Human sDLL-4 comprises the extracellular signaling domain of DLL-4, a member of a structurally-related family of single-pass type I trans-membrane proteins that serve as ligands for Notch receptors. DLL-4 functions to specifically activate the Notch-1 and Notch-4 receptors. The Notch signaling pathway regulates endothelial-cell differentiation, proliferation and apoptosis, and is essential for the development, maintenance and remodeling of the vascular system. Targeted deletion of the DLL-4 gene in mice resulted in severe vascular defects and death before birth. Up-regulation of DLL-4 expression has been implicated in the vascular development of certain tumors.

Thurston, G. and Kitajewski, J. (2008) Br. J. Cancer 99, 1204-1209.
Yan, M. and Plowman, G.D. (2007) Clin. Cancer Res. 13, 7243-7246

EGF

EGF is a potent growth factor that stimulates the proliferation of various epidermal and epithelial cells. Additionally, EGF has been shown to inhibit gastric secretion, and to be involved in wound healing. EGF signals through a receptor known as ErbB-1, which is a class I tyrosine kinase receptor. This receptor also binds with TGF-α and VGF (vaccinia virus growth factor).

Carpenter, G. and Cohen, S. (1990) J. Biol. Chem. 265, 7709-7712.
Burgess, A.W. (1989) In Br. Med. Bull. 45, Growth Factors, Waterfield, M.D. ed., Chruchill Livingstone, Edinburgh, 401-424.

EGF Receptor

EGF Receptor (EGFR, ErbB1) is a transmembrane protein that exerts tyrosine kinase activity upon ligand induced activation. EGFR can be activated by binding EGF or at least six other structurally related protein ligands, including TGF-α, HB-EGF, Betacellulin (BTC), Amphiregulin, Epiregulin, and Epigen. Upon activation, EGFR initiates a signaling cascade which includes dimerization and internalization, tyrosine phosphorylation, DNA synthesis of target genes, and, ultimately, cell proliferation. EGFR signaling plays a role in the growth and differentiation of normal cells, but elevated EGFR activity is correlated with the development and pathogenesis of certain cancers.

EG-VEGF

EG-VEGF is a secreted angiogenetic mitogen growth factor expressed in the steroidogenic glands, ovary, testis, adrenal and placenta. EG-VEGF induces proliferation and migration fenestration (formation of membrane discontinuities) in capillary endothelial cells derived from endocrine glands. The human EG-VEGF gene codes for a 105 amino acid polypeptide containing an N-terminal signal sequence of 19 amino acids.

Carmeliet, P. (2001) Nature, 412 (6850), 868-869.
Li, M. et al. (2001) Mol. Pharmacol. 4, 692-698.

Epigen

Epigen is an EGF-related polypeptide growth factor tha signals through the ErbB receptor-1 (ErbB-1). It is produced in several tissues, including the testis, liver, heart and in certain tumor cells. Epigen is mitogenic for fibroblasts and epithelial cells Human Epigen is initially synthesized as a glycosylated 14.7 kDa transmembrane precursor protein, which is processed by proteolytic cleavage to produce a mature soluble sequence.

Strachan, L. et al. (2001) J Bio. Chem. 276, 18265-18271.

Epiregulin

Epiregulin is an EGF related growth factor that binds specifically to EGFR (ErbB1) and ErbB4, but not ErbB2 or ErbB3. It is expressed mainly in the placenta and peripheral blood leukocytes and in certain carcinomas of the bladder, lung, kidney and colon. Epiregulin stimulates the proliferation of keratinocytes, hepatocytes, fibroblasts and vascular smooth muscle cells. It also inhibits the growth of several tumor-derived epithelial cell lines. Human Epiregulin is initially synthesized as a glycosylated 19.0 kDa transmembrane precursor protein, which is processed by proteolytic cleavage to produce a 6.0 kDa mature secreted sequence.

Shirakata, Y. et al. (2000) J. Bio. Chem. 275, 5748-5753.

FGF Family

Proteins of the FGF family of growth factors manifest only a modest degree of primary sequence homology, yet share the ability to signal through one or more of four tyrosine kinase receptors called FGFR1 through FGFR4. The FGFs play a central role during prenatal development and postnatal growth and regeneration of a wide variety of tissues, by promoting cellular proliferation and differentiation. All members of the FGF family bind, with varying degrees of affinity, to heparin sulfate proteoglycans, which serve as an extracellular storage site and in some cases appear to be involved in the activation of the FGF receptors.

Ornitz, D.M. and Itoh, N. (2001) Genome Biology 2 (3): reviews 3005.1-3005.12.

FGF-1/FGF-acidic

FGF-acidic is one of 23 known members of the FGF family. FGF-acidic is a non-glycosylated heparin binding growth factor that is expressed in the brain, kidney, retina, smooth muscle cells, bone matrix, osteoblasts, astrocytes and endothelial cells. FGF-acidic has the ability to signal through all the FGF receptors.

FGF-2/FGF-basic

FGF-basic is one of 23 known members of the FGF family. FGF-basic is a non-glycosylated heparin binding growth factor that is expressed in the brain, pituitary, kidney, retina, bone, testis, adrenal gland liver, monocytes, epithelial cells and endothelial cells. FGF-basic signals through FGFR 1b, 1c, 2c, 3c and 4.

FGF-4

FGF-4 is a heparin binding growth factor that is a member of the FGF family. FGF-4 signals through the FGFR 1c, 2c, 3c, and 4. FGF-4 plays a role in angiogenesis, vertebrate limb development and development of stomach cancer.

FGF-5

FGF-5 is a secreted heparin binding growth factor that belongs to the FGF family. FGF-5 binds to FGFR 1c and 2c, and plays a regulatory role in the hair growth cycle.

FGF-6

FGF-6 is a secreted heparin binding growth factor that is a member of the FGF family. FGF-6 is expressed in leukemia cell lines with platelet megakaryocytic differentiation potential. It signals through FGFR 1c, 2c, and 4. It is also found to play a role in skeletal muscle development.

FGF-7/KGF

KGF/FGF-7 is one of 23 known members of the FGF family. KGF/FGF-7 is a mitogen factor specific for epithelial cells and keratinocytes. KGF/FGF-7 signals through FGFR 2b. KGF/FGF-7 plays a role in kidney and lung development, angiogenesis, and wound healing.

FGF-8

FGF-8 (FGF-8b) is a heparin binding growth factor belonging to the FGF family. There are 4 known alternate spliced forms of FGF8; FGF-8A, FGF-8B, FGF-8E and FGF-8F. The human and murine FGF8-A and -B are identical unlike human and mouse FGF8E and F are 98% identical. FGF-8 targets mammary carcinoma cells and other cells expressing the FGF receptors.

FGF-16

FGF-16 is a heparin binding growth factor that is a member of the FGF family. FGF-16 signals through FGFR 2c and 3c. FGF-16 plays a role in the development of the central nervous system.

FGF-17

FGF-17 is a heparin binding growth factor that is a member of the FGF family. FGF-17 signals through the FGFR 1c, 2c, 3c, and 4. FGF-17 signals induction and patterning of embryonic brain.

FGF-19

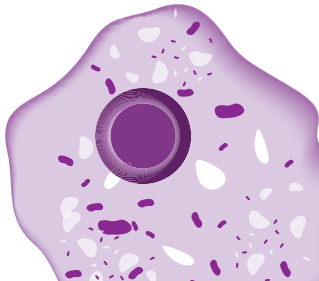
The FGF family plays central roles during prenatal development and postnatal growth and regeneration of a variety of tissues, by promoting cellular proliferation and differentiation. FGF-19, a member of the FGF family, is a high affinity heparin dependent ligand for FGFR4. FGF-19 is expressed during brain development and embryogenesis. FGF-19, FGF-21 and FGF-23 constitute an atypical FGF subfamily whose ligands act as circulating hormones and require the participation of a Klotho protein as a co-receptor for their signaling.

FGF-20

FGF-20 is secreted heparin binding growth factor that is a member of the FGF family. FGF-20 signals through the FGFR 2c and 3c and is expressed during limb and brain development.

FGF-21

FGF-21 is a secreted growth factor that is a member of the FGF family. FGF-21, in the presence of β-Klotho as a protein cofactor, signals through the FGFR 1c and 4 receptors and stimulates insulin independent glucose uptake by adipocytes.



FGF-23

The FGF family plays a central role during prenatal development and postnatal growth and regeneration of a variety of tissues, by promoting cellular proliferation and differentiation. FGF-23, FGF-21 and FGF-19 constitute an atypical FGF subfamily whose ligands act as circulating hormones and require the participation of a Klotho protein as a co-receptor for their signaling. FGF-23 is a bone-derived hormone that acts in the kidney to regulate phosphate homeostasis and vitamin D metabolism. The signaling receptor for FGF-23, a Klotho-FGFR1 (IIIc) complex, is an essential regulator of the renal sodium phosphate co-transporter and key vitamin D-metabolizing enzymes CYP27B1 and CYP24A1.

Flt3-Ligand

Flt3-Ligand is a growth factor that regulates proliferation of early hematopoietic cells. Flt3-Ligand binds to cells expressing the tyrosine kinase receptor Flt3. Flt3-Ligand, by itself does not stimulate proliferation of early hematopoietic cells, but synergizes with other CSFs and interleukins to induce growth and differentiation. Unlike SCF, Flt3-Ligand exerts no activity on mast cells. Multiple isoforms of Flt3-Ligand have been identified. The predominant biologically active form is anchored to the cell surface as the extracellular domain of a transmembrane protein (209 a.a.). The membrane-bound isoform can be proteolytically cleaved to generate a biologically active soluble isoform.

Hannum, C. et al. (1994) Nature 368, 643-648.
Mackarehtschian, K. et al. (1995) Immunity 3, 147-161.

Follistatin

Follistatin is a secreted protein that binds to ligands of the TGF-β family and regulates their activity by inhibiting their access to signaling receptors. It was originally discovered as an activin antagonists whose activity suppresses expression and secretion of the pituitary hormone FSH (follicle stimulating hormone). In addition to being a natural antagonist, follistatin can inhibit the activity of other TGF-β ligands including BMP-2,-4,-6,-7, Myostatin, GDF-11, and TGF-β1. Follistatin is expressed in the pituitary, ovaries, decidual cells of the endometrium, and in some other tissues. Recombinant human Follistatin is a 31.5 kDa protein containing 288 amino acids. Its primary structure contains three cysteine-rich domains (called FS domains), each followed by a protease-inhibitory kazal domain.

Inouye, S. et al. (1991) Endocrinology 2, 815-822.
DePaolo, L.V. (1997) Proc. Soc. Exp. Biol. Med. 4, 328-39.

FRP-1

Secreted Frizzled Related Proteins (sFRPs) modulate Wnt signaling by binding directly to Wnt proteins in a manner that affects their receptor binding and signaling capabilities. sFRP-1 is a widely distributed protein that can bind directly to Wnt-1, Wnt-2, and possibly other Wnt proteins, and generally exerts anti-proliferative effects consistent with activity as a Wnt antagonist. It also inhibits apoptosis and has been found to be down-regulated in many solid tumors, but up-regulated in uterine leiomyomas.

Goodwin, A.M. and D'Amore P.A. (2002) Angiogenesis 5 (1-2), 1-9.

Galectin-1

Lectins, of either plant or animal origin, are carbohydrate binding proteins that interact with glycoprotein and glycolipids on the surface of animal cells. The Galectins are lectins that recognize and interact with beta-galactoside moieties. Galectin-1 is an animal lectin that has been shown to interact with CD3, CD4, and CD45. It induces apoptosis of activated T-cells and T-leukemia cell lines and inhibits the protein phosphatase activity of CD45.

Yang, R.Y. et al. (2003) Cell Mol. Life Sci. 2, 267-276.

G-CSF

G-CSF is a hematopoietic growth factor that stimulates the development of committed progenitor cells to neutrophils and enhances the functional activities of the mature end-cell. It is produced in response to specific stimulation by a variety of cells including macrophages, fibroblasts, endothelial cells and bone marrow stroma. G-CSF is being used clinically to facilitate hematopoietic recovery after bone marrow transplantation. Human and murine G-CSF are cross-species reactive.

Lu, H.S. et al. (1989) Arch. Biochem. Biophys. 268, 81-92.

GDF-2

GDF-2 belongs to the TGF-β cytokine family whose members play an important role during prenatal development and postnatal growth, remodeling and maintenance of a variety of tissues and organs. GDF-2 is expressed mainly in nonparenchymal cells of the liver, but is also found in other various cells and tissues. GDF-2 can signal through the ALK1 receptor and has been implicated in a number of physiologic events. These include regulation of the hepatic reticuloendothelial system, glucose homeostasis, and iron homeostasis, as well as the inhibition of angiogenesis.

Brown, M. et al. (2005) J. Bio. Chem. 280, 25111-25118.

GDF-3

GDF-3 is a member of the TGF-β superfamily of growth and differentiation factors, and is highly homologous to GDF-9. Unlike most TGF-β family members, GDF-3 and GDF-9 are not disulfide-linked dimers. GDF-3 is expressed in adult bone marrow, spleen, thymus, and adipose tissue. The expression of GDF-3 is upregulated in high-fat-fed wild-type FABP4/aP2 null mice and was associated with obesity, but not with the related hyperglycemia/hyperinsulinemia which characterizes Type 2 diabetes.

Witthuhn, B.A. and Bernlohr, D.A. (May 2001) Cytokine 3, 129-135.

GDF-5/CDMP-1/BMP-14

GDF-5 is expressed in long bones during embryonic development and postnatally in articular cartilage. Mutations in the GDF-5 gene have been implicated in Grebe Syndrome, which is characterized by short stature, extra digits, short and deformed extremities, and in Hunter-Thompson type dwarfism. The mature and functional form of GDF-5 is a homodimer of two 120 amino-acid polypeptide chain (monomers) linked by a single disulfide bond. Each GDF-5 monomer is expressed as the C-terminal part of a precursor polypeptide, which also contains a 27 amino-acid signal peptide and a 354 amino-acid propeptide. This precursor undergoes intracellular dimerization, and upon secretion it is processed by a furin-type protease.

Chang, C.H. et al. (1994) J. Bio. Chem. 45, 28227-28234.
Chang, D. et al. (2004) Growth Factors. 22, 233-241.
Lens, S.M. et al. (1998) Semin. Immunol. 10, 491-499.
Scheufer, C. et al. (1999) J. Mol. Biol. 287, 103-115.
Urist, M.R. (1965) Science 150, 893-899.

GDF-11

GDF-11 is a myostatin-homologous protein that acts as an inhibitor of nerve tissue growth. GDF-11 has been shown to suppress neurogenesis through a myostatin-like pathway, which involves arrest of progenitor cell-cycle in the G1 phase. Similarities between myostatin and GDF-11, which are 90% identical in their amino acid sequence, suggests that the regulatory mechanisms responsible for maintaining proper tissue size during neural and muscular development might be the same. GDF-11 is highly homologous to Myostatin/GDF-8 sharing 90% amino-acid sequence identity.

Langley, B. et al. (2002) J. Biol. Chem. 277, 49831-49840.
Wu, H.H. et al. (2003) Neuron 37, 197-207.

GDF-15/MIC-1

GDF-15 belongs to the TGF-β cytokine family whose members play an important role during prenatal development and postnatal growth, remodeling, and m aintenance of a variety of tissues and organs. GDF-15 is expressed predominantly in placenta and to a much lesser extent in various other tissues. The presence of GDF-15 in amniotic fluid and its elevated levels in the sera of pregnant women suggest a role for GDF-15 in gestation and embryonic development. GDF-15 generally exerts tumor suppressive activities and is one of the predominant factors produced and secreted in response to activation of the p53 pathway. Interestingly, the serum level of GDF-15 is positively correlated with neoplastic progression of several tumor types, including certain colorectal, pancreatic, and prostate cancers.

Moore, A.G. et al. (2000) J. Clin. Endocrinol. Metab. 85, 4781-4788.
Strelau, J. et al. (2000) J. Neural Transm. Suppl. 60, 273-276.
Yang, H. et al. (2003) Mol. Cancer Ther. 10, 1023-1029.

GDNF

GDNF is a disulfide-linked homodimeric neurotrophic factor structurally related to Artemin, Neurturin and Persephin. These proteins belong to the cysteine-knot superfamily of growth factors that assume stable dimeric protein structures. GDNF signals through a multicomponent receptor system, composed of a RET and one of the four GFRα (α1-α4) receptors. GDNF specifically promotes dopamine uptake and survival and morphological differentiation of midbrain neurons. Using Parkinson's disease mouse model, GDNF has been shown to improve conditions such as bradykinesia, rigidity, and postural instability. The functional human GDNF ligand is a disulfide-linked homodimer, of two 15 kDa polypeptide chains called monomers. Each monomer contains seven conserved cysteine residues, one of which (Cys 101) is used for inter-chain disulfide bridging and the others are involved in intramolecular ring formation known as the cysteine knot configuration.

Lin, L.F. et al. (1993) Science 260, 1130-1132.
Lindahl, M. et al. (2000) Mol. Cell Neurosci. 15, 522-533.
Trupp, M. et al. (1996) Nature 381, 785-789.

GM-CSF

GM-CSF is a hematopoietic growth factor that stimulates the development of neutrophils and macrophages and promotes the proliferation and development of early erythroid megakaryocytic and eosinophilic progenitor cells. It is produced in endothelial cells, monocytes, fibroblasts and T-lymphocytes. GM-CSF inhibits neutrophil migration and enhances the functional activity of the mature end-cells. The human and murine molecules are species-specific and exhibit no cross-species reactivity. Recombinant human GM-CSF is a 14.6 kDa globular protein consisting of 128 amino acids containing two intramolecular disulfide bonds and two potential N-linked glycosylation sites.

Nemunaitis, J. et al. (1991a) Blood 77, 2065-2071.
Wong, G.C. et al. (1985) Science 228, 810-815.

HB-EGF

HB-EGF is an EGF related growth factor that signals through the EGF receptor, and stimulates the proliferation of smooth muscle cells (SMC), fibroblasts, epithelial cells, and keratinocytes. HB-EGF is expressed in numerous cell types and tissues, including vascular endothelial cells and SMC, macrophages, skeletal muscle, keratinocytes, and certain tumor cells. The ability of HB-EGF to specifically bind heparin and heparin sulfate proteoglycans is distinct from other EGF-like molecules, and may be related to the enhanced mitogenic activity, relative to EGF, that HB-EGF exerts on smooth muscle cells. The human HB-EGF gene encodes a 208 amino acid transmembrane protein, which can be proteolytically cleaved to produce soluble HB-EGF.

Iwamoto, R and Mekada E. (Dec. 2000) Cytokine and Growth Factor Reviews. 11, 335-344.

Heregulin-β1

Heregulin/Neuregulin is a family of structurally related polypeptide growth factors derived from alternatively spliced genes (NRG1, NRG2, NRG3 and NRG4). To date, there are over 14 soluble and transmembrane proteins derived from the NRG1 gene. Proteolytic processing of the extracellular domain of the transmembrane NRG1 isoforms release soluble growth factors. HRG-β1contains an Ig domain and an EGF-like domain that is necessary for direct binding to receptor tyrosine kinases erb3 and erb4. This binding induces erb3 and erb4 heterodimerization with erb2, stimulating intrinsic kinase activity, which leads to tyrosine phosphorylation. Although HRG-β1 biological effects is still unclear, it has been found to promote motility and invasiveness of breast cancer cells which may also involve up-regulation of expression and function of the autocrine motility-promoting factor (AMF).

Xiong, S. et al. (Feb. 2001) Cancer Research 61, 1727-1732.

HGF

HGF is a mesenchymally derived potent mitogen for mature parenchymal hepatocyte cells and acts as a growth factor for a broad spectrum of tissues and cell types. HGF signals through a transmembrane tyrosine kinase receptor known as MET. Activities of HGF include induction of cell proliferation, motility, morphogenesis, inhibition of cell growth, and enhancement of neuron survival. HGF is a crucial mitogen for liver regeneration processes, especially after partial hepatectomy and other liver injuries. Human and murine HGF are cross-reactive. Human HGF is expressed as a linear 697 amino

HGF cont'd

acid polypeptide precursor glycoprotein. Proteolytic processing of this precursor generates the biologically active form of HGF, which consists of two polypeptide chains (α-chain and β-chain) held by a single disulfide bond resulting in formation of a biologically active heterodimer. The α-chain consists of 463 amino acid residues and four kringle domains. The β-chain consists of 234 amino acid residues.

Naldini, L. et al. (1991) EMBO J. 10, 2867-2878.
Zarnegar, R. and Michalopoulos, G.K. (1995) J. Cell. Biol. 129, 1177-1180.

IFN-γ

IFN-γ is an acid-labile interferon produced by CD4 and CD8 T lymphocytes as well as activated NK cells. IFN-γ receptors are present in most immune cells, which respond to IFN-γ signaling by increasing the surface expression of class I MHC proteins. This promotes the presentation of antigen to T-helper (CD4+) cells. IFN-γ signaling in antigen-presenting cells and antigen-recognizing B and T lymphocytes regulate the antigen-specific phases of the immune response. Additionally, IFN-γ stimulates a number of lymphoid cell functions including the anti-microbial and anti-tumor responses of macrophages, NK cells, and neutrophils. Human IFN-γ is species-specific and is biologically active only in human and primate cells.

Billiau, A. and Vandenbroeck, K. (2001) Cytokine Reference. Oppenheim, J.J. and Feldman, M. ed., Academic Press, London, 642-675.
Gray, P.W. et al. (1982) Nature 295, 503-508.

IGF-I, -II

The IGFs are mitogenic polypeptide growth factors that stimulate the proliferation and survival of various cell types including muscle, bone, and cartilage tissue in vitro. IGFs are predominantly produced by the liver, although a variety of tissues produce the IGFs at distinctive times. The IGFs belong to the Insulin gene family, which also contains insulin and relaxin. The IGFs are similar by structure and function to insulin, but have a much higher growth-promoting activity than insulin. IGF-II expression is influenced by placenta lactogen, while IGF-I expression is regulated by growth hormone. Both IGF-I and IGF-II signal through the tyrosine kinase type I receptor (IGF-IR), but, IGF-II can also signal through the IGF-II/Mannose-6-phosphate receptor. Mature IGFs are generated by proteolytic processing of inactive precursor proteins, which contain N-terminal and C-terminal propeptide regions. Recombinant human IGF-I and IGF-II are globular proteins containing 70 and 67 amino acids, respectively, and 3 intra-molecular disulfide bonds.

D'Ercole, A.J. (1996) Endocrin. Metab. Clin. North Am. 25, 573-590.

IL-1α

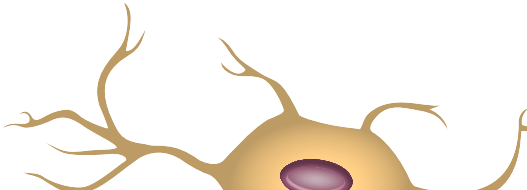
IL-1α is a non-secreted proinflammatory cytokine produced in a variety of cells including monocytes, tissue macrophages, keratinocytes and other epithelial cells. Both IL-1α and IL-1β binds to the same receptor and has similar if not identical biological properties. These cytokines have a broad range of activities including, stimulation of thymocyte proliferation, by inducing IL-2 release, B-cell maturation and proliferation, mitogenic FGF-like activity and the ability to stimulate the release of prostaglandin and collagenase from synovial cells. However, whereas IL-1β is a secreted cytokine, IL-1α is predominantly a cell-associated cytokine.

Dinarello, C.A. (1989) Adv. Immunol. 44, 153-205.
Gubler, U. et al. (1986) J. Immunol. 36 (7), 2492-2497.

IL-1β

IL-1β is a proinflammatory cytokine produced in a variety of cells including monocytes, tissue macrophages, keratinocytes and other epithelial cells. Both IL-1α and IL-1β binds to the same receptor and has similar if not identical biological properties. These cytokines have a broad range of activities including stimulation of thymocyte proliferation, by inducing IL-2 release, B-cell maturation and proliferation, mitogenic FGF-like activity and the ability to stimulate the release of prostaglandin and collagenase from synovial cells. However, whereas IL-1β is a secreted cytokine, IL-1α is predominantly a cell-associated cytokine.

Dinarello, C.A. (1989) Adv. Immunol. 44, 153-205.
Gubler, U. et al. (1986) J. Immunol. 36 (7), 2492-2497.



IL-2

IL-2 is a powerful immunoregulatory lymphokine produced by T-cells in response to antigenic or mitogenic stimulation. IL-2/IL-2R signaling is required for T-cell proliferation and other fundamental functions which are essential for the immune response. IL-2 stimulates growth and differentiation of B-cells, NK cells, lymphokine activated killer cells, monocytes, macrophages and oligodendrocytes. Recombinant human IL-2 is a 15.5 kDa protein, containing 134 amino acid residues including one intrachain disulfide bond.

Smith K.A. (2001) Cytokine Reference. Oppenheim, J.J. and Feldman, M. ed., Academic Press, London, 113-125.
Smith, K.A. (1998) Science 240, 1169-1176.

IL-3

IL-3 is a hematopoietic growth factor that promotes the survival, differentiation and proliferation of committed progenitor cells of the megakaryocyte, granulocyte-macrophage, erythroid, eosinophil, basophil and mast cell lineages. Produced by T cells, mast cells and eosinophils, IL-3 enhances thrombopoiesis, phagocytosis, and antibody-mediated cellular cytotoxicity. Its ability to activate monocytes suggests that IL-3 may have additional immunoregulatory roles. Many of the IL-3 activities depend upon co-stimulation with other cytokines. IL-3 is a species-specific, variably glycosylated cytokine.

Ihle, J.N. (1991) In Peptide Growth Factors and their Receptors I, Sporn, M.B. and Roberts, A.B. eds., Springer-Verglag, New York, 541-575.
Ihle, J.N. (1992) Chem. Immunol. 51, 65-106.

IL-4

IL-4 is a pleiotropic cytokine that regulates diverse T and B cell responses including cell proliferation, survival and gene expression. Produced by mast cells, T cells and bone marrow stromal cells, IL-4 regulates the differentiation of naive CD4+ T cells into helper Th2 cells, characterized by their cytokine-secretion profile that includes secretion of IL-4, IL-5, IL-6, IL-10, and IL-13, which favor a humoral immune response. Another dominant function of IL-4 is the regulation of immunoglobulin class switching to the IgG1 and IgE isotypes. Excessive IL-4 production by Th2 cells has been associated with elevated IgE production and allergy.

Keegan, A.D. (2001) Cytokine Reference. Oppenheim, J.J. and Feldman, M. ed., Academic Press, London, 127-135.
Paul, W.E. (1991) Blood 77, 1859-1870.

IL-5

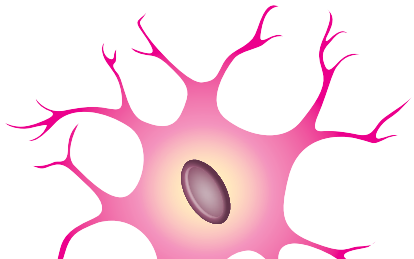
IL-5 is a hematopoietic growth factor that stimulates the proliferation and activation of eosinophils. Produced by mast cells, T cells, and eosinophils, IL-5 plays an important role in inducing cell-mediated immunity against parasitic infections and certain tumors. Elevated levels of IL-5 lead to Eosinophilia, which may result in the induction of asthma and other allergic diseases. Human and murine IL-5 are cross-species reactive.

Sanderson, C.J. (1992) Blood 79, 3101.
Campbell, H.D. et al. (1987) PNAS USA 84, 6629.

IL-6

IL-6 is a pleiotropic cytokine that plays an important role in host defense by regulating immune and inflammatory responses. Produced by T cells, monocytes, fibroblasts, endothelial cells and keratinocytes, IL-6 has diverse biological functions. It stimulates B-cell differentiation and antibody production, synergizes with IL-3 in megakaryocyte development and platelet production, induces expression of hepatic acute-phase proteins, and regulates bone metabolism. IL-6 signals through the IL-6 receptor system that consists of two chains, IL-6Rα and gp130. Murine IL-6 is inactive on human cells, while both human and murine are equally active on murine cells.

Matsuda, T. and Hirano, T. (2001) Cytokine Reference. Oppenheim, J.J. and Feldman, M. ed., Academic Press, London, 537-563.
Kishimoto, T. (1992) Science 258, 593-597.



IL-6 Receptor α

IL-6 mediates its biological effects through the type I IL-6 receptor system that consists of two chains, IL-6Rα and gp130. The IL-6Rα chain is the binding component specific to IL-6; while the gp130 only transmits signals of IL-6 when bound to IL-6Rα. The gp130 also can transmit signals from LIF, OSM, CNTF, IL-11 and CT-1 in conjunction with other receptor subunits. The low-affinity binding site for IL-6 is composed of IL-6Rα alone. IL-6Rα is expressed in a wide range of cells including T cells, fibroblasts and macrophages. Soluble IL-6Rα which consists of only the extracellular domain of the IL-6Rα chain, acts as an agonist of IL-6 activity at low concentrations.

Hibi, M and Hirano, T. (2001) Cytokine Reference. Oppenheim, J.J. and Feldman, M. ed., Academic Press, London, 1761-1773.
Sugita, T. et al. (1990) J. Exp. Med. 171, 2001-2009.

IL-7

IL-7 is a hematopoietic growth factor, which affects primarily early B and T cells. Produced by thymic stromal cells, spleen cells and keratinocytes, IL-7 can also co-stimulate the proliferation of mature T cells in combination with other factors such as ConA and IL-2. Human and murine IL-7 is cross-species reactive.

Namen, A.E. et al. (1988b) J. Esp. Med. 167, 988-1002.
Spits, H. (2001) Cytokine Reference. Oppenheim, J.J. and Feldman, M. ed., Academic Press, London, 137-153.

IL-10

IL-10 is an immunosuppressive cytokine produced by a variety of mammalian cell types including macrophages, monocytes, T cells, B cells and keratinocytes. IL-10 inhibits the expression of proinflammatory cytokines such as IL-1 and TNF-α. Like IL-4, IL-10 enhances humoral immune responses and attenuates cell-mediated immune reactions. Human IL-10 is active on murine cells, but murine IL-10 is inactive on human cells.

Malefyt, R. (2001) Cytokine Reference. Oppenheim, J.J. and Feldman, M. ed., Academic Press, London, 1061-1067.
Mehrotra, M. et al. (1998) J. Immunol. 160, 2637-2644.

IL-11

IL-11 is a multifunctional cytokine produced by stromal cells such as fibroblasts, epithelial cells and osteoclasts. It is expressed in a wide variety of tissues including thymus, lung, bone, connective tissue and central nervous system. IL-11 plays an important regulatory role in hematopoiesis by stimulating growth of myeloid, erythroid and megakaryocyte progenitor cells. It also regulates bone metabolism, inhibits production of proinflammatory cytokines and protects against gastromucosal injury.

Fitzgerald, K.A. et al. (2001)The Cytokine Factsbook, Academic Press, London, 95-98.
Quesniaux, V.F. et al. (1993) Int. Rev. Exp. Pathol. 34, 205-214.

IL-12

IL-12 is a potent regulator of cell mediated immune responses and it induces IFN-γ production by NK and T cells. It is produced by activated monocytes/macrophage cells, B lymphocytes and connective tissue type mast cells. Among its biological activities IL-12 promotes the growth and activity of activated NK, CD4+ and CD8+ cells and induces the development of IFN-γ producing Th1 cells. Recombinant Human IL-12 is a 75.0 kDa heterodimeric glycoprotein consisting of disulfide-linked 35 kDa (p35) and 40 kDa (p40) subunits (503 total amino acid residues).

Atkins, M.B. et al. (1997) Clin. Cancer Res. 3, 409-417.
Gately, M.K. et al. (1991) J. Immunol. 147, 874-882.

IL-13

IL-13 is an immunoregulatory cytokine produced primarily by activated Th2 cells, and also by mast cells and NK cells. Targeted deletion of IL-13 in mice resulted in impaired Th2 cell development and indicated an important role for IL-13 in the expulsion of gastrointestinal parasites. IL-13 exerts anti-inflammatory effects on monocytes and macrophages and it inhibits the expression of inflammatory cytokines such as IL-1β, TNF-α, IL-6 and IL-8. IL-13 has also been shown to enhance B cell proliferation

IL-13 cont'd

and to induce isotype switching resulting in increased production of IgE. Blocking of IL-13 activity inhibits the pathophysiology of asthma. Human and murine IL-13 are cross-species reactive.

McKenzie, A.N.J. and Matthews, D.J. (2001) Cytokine Reference. Oppenheim, J.J. and Feldman, M. ed., Academic Press, London, 203-211.

Wills-Karp, M. et al. (1998) Science 282, 2258-2261.

IL-15

IL-15 is an immunomodulating cytokine that stimulates the proliferation of T lymphocytes and shares many biological properties with IL-2. IL-15 exerts its biological activities primarily on T cells. It is also essential in the development, survival and activation of NK cells. Increased expression of IL-15 has been implicated with rheumatoid arthritis, inflammatory bowel disease and diseases affiliated with retroviruses HIV and HTLV-I. Human IL-15 is biologically active on mouse cells as measured by the dose-dependent stimulation of the proliferation of mouse CTLL cells.

Grabstein, K.H. et al. (1994) Science 264, 965-968.

Waldmann, T.A. and Tagaya, Y. (2001) Cytokine Reference. Oppenheim, J.J. and Feldman, M. ed., Academic Press, London, 214-223.

IL-31

Human IL-31 is a T-cell derived cytokine that shares several structural and functional characteristics with IL-6, Oncostatin M, LIF, and Cardiotrophin-1. It signals through a receptor complex comprised of GPL (GP130-like, IL-31RA) and OSMR (Oncostatin M receptor). GPL/OSMR signaling is a strong activator of STAT3 and STAT5, and can also activate STAT1, Jak1, and Jak2 signaling pathways. IL-31 regulated immune responses have been implicated in skin physiology and inflammatory skin diseases.

Dillion, S. R. et al. (Jul. 2004) Nature Immunol. 5 (7), 752-760.

KLF4, KLF4-TAT

KLF4 is a member of the Kruppel-like factor (KLF) family of zinc finger transcription factors. Members of this family have in common 3 contiguous C2H2-type zinc fingers at the carboxyl terminus that comprise the DNA-binding domain. KLF4 is highly expressed in skin and gut epithelial tissues, but is also found in various other cells and tissues, including vascular endothelial cells, lymphocytes, lung, and testis. It is an important regulator of the cell cycle, transcription, and cell differentiation. Together with Sox2, Oct4, and cMyc, KLF4 can induce the reprogramming of primary human fibroblasts to a pluripotent state. KLF4 and other transcription factors can be introduced into cells by DNA transfection, viral infection, or microinjection. Protein transduction using TAT fusion proteins represents an alternative methodology for introducing transcription factors into primary as well as transformed cells.

Evans, P.M. and Liu, C. (2008) Acta. Biochem. Biophys. Sin. 40, 554-5564.

Ghaleb, A.M. et al. (2005) Cell Res. 15, 92-96.

Klotho

Klotho is a glycosylated protein that plays an important role in the regulation of phosphate and calcium homeostasis. Human Klotho exists in both membrane bound and secreted forms, and is predominantly expressed in the kidney convoluted tubules, and to a lesser extent, in the brain, reproductive organs, endocrine glands, urinary bladder, skeletal muscle, placenta, and colon. The full length transmembrane form has a large extracellular domain composed of two homologous subunits termed KL1 and KL2, which contain 516 and 439 amino acid residues, respectively. The predominant circulating form, which is derived from alternative RNA splicing, contains the KL1 subunit and constitutes the N-terminal sequence of transmembrane Klotho. A third Klotho protein of about 128 kDa has been identified in the blood and cerebrospinal fluid. This circulating protein arises from the action of an as yet unidentified protease which cleaves transmembrane Klotho just above and/or within the plasma membrane. Klotho has been shown to play a key role in the signaling cascade of fibroblast growth factor-23 (FGF-23), a bone derived hormone that acts in the kidney to inhibit phosphate reabsorption and vitamin D biosynthesis. Klotho promotes FGF-23 signaling through binding to FGFRI (IIIc) which converts this canonical FGF receptor into a specific receptor for FGF-23. In the absence of Klotho the function of FGF-23 is literally abolished.

Kuro-o, M. (2008) Trends Endocrinol. Metab. 9, 239-245.

Kurosult, H. and Kuro, O.M. (2008) Curr. Opin. Nephrol. Hypertens. 17, 368-372.

LIF

LIF is a pleiotrophic factor produced by multiple cell types including T cells, myelomoncytic lineages, fibroblasts, liver, heart and melanoma. LIF promotes long-term maintenance of embryonic stem cells by suppressing spontaneous differentiation. Other activities include the stimulation of acute phase protein synthesis by hepatocytes, stimulation of differentiation of cholinergic nerves, and suppression of adipogenesis by inhibiting the lipoprotein lipase in adipocytes. Human and murine LIF show cross reactivity.

Lin28, Lin28-TAT

Lin28 is a RNA-binding protein that belongs to a diverse family of structurally-related transcription factors. Lin28 is found abundantly in embryonic stem cells (ESCs), and to a lesser extent in placenta and testis. Lin28 has been shown to block let-7 microRNA processing and maturation, a necessary step in the differentiation of stem cells and certain cancer cell lines. Together with Sox2, Oct4, and Nanog, Lin28 can induce the reprogramming of primary human fibroblasts to a pluripotent state. Lin28 and other regulatory proteins can be introduced into cells by DNA transfection, viral infection, or microinjection. Protein transduction using TAT fusion proteins represents an alternative methodology for introducing proteins into primary as well as transformed cells. Recombinant human Lin28-TAT is a 24.4 kDa protein containing 222 amino acid residues, including a 13-residue C-terminal TAT peptide.

Xu, B. et al. (2009) RNA, 15, 357-361.

MANF

MANF is a secreted neurotrophic factor that is expressed in the brain, neuronal and certain non-neuronal tissues. It has been shown to promote survival, growth and function of dopamine specific neurons. MANF and its structural homolog CDNF, each contain an N-terminal saposin-like lipid binding domain, and a carboxyl-terminal domain, which is not homologous to previously characterized protein structures. MANF and CDNF can prevent 6-OHDA induced degeneration of dopaminergic neurons by triggering survival pathways in a rat experimental model of Parkinson disease. Recombinant human MANF is an 18.1 kDa protein consisting of 158 amino acids including 8 cysteine residues.

Lindholm, P. et al. (2007) Nature 448, 73-77.
Lindholm, P. and Saarma, M. (2010) Dev. Neurobiol. 70, 360-371.

M-CSF

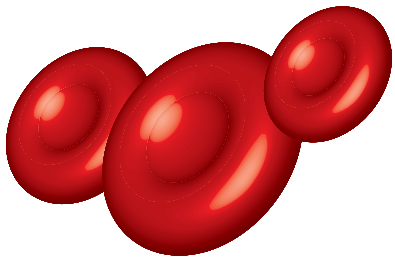
M-CSF is a potent hematopoietic factor produced by a variety of cells including lymphocytes, monocytes, fibroblasts, endothelial cells, myoblasts and osteoblasts. It is a key regulator of cellular proliferation, differentiation, and survival of blood monocytes, tissue macrophages and their progenitor cells. M-CSF has been shown to play important roles in modulating dermal thickness, and male and female fertility. M-CSF is clinically used in the treatment of infection, malignancies and atherosclerosis. It facilitates hematopoietic recovery after bone marrow transplantation. Human M-CSF is reactive in murine systems, but the murine molecule exhibits no activity on human cells.

Stanley, E.R. (1994) The Cytokine Handbook (ed. A.W. Thomson) Academic Press, San Diego, 387-418.
Stanley, E.R. (1985) Methods Enzymol. 116, 564-587.

Midkine

Midkine and its functionally-related protein Pleiotrophin are heparin-binding neurotrophic factors that signal through the same receptor, known as anaplastic lymphoma kinase (ALK). MK plays an important regulatory role in epithelial-mesenchymal interactions during fetal development and in postnatal lung development. MK chemoattracts embryonic neurons, neutrophils and macrophages, and by signaling through the ALK receptor, it exerts angiogenic, growth and survival activities during tumorgenesis. Recombinant human Midkine is a 13.4 kDa protein containing 123 amino acid residues including five intra-molecular disulfide bonds.

Iwasaki, W. et al. (1997) EMBO J. 16, 6936-6946.
Muramatsu, T. (2002) J. Biochem. 3, 359-371.



Myostatin

Myostatin/GDF-8 is a TGF-β family member that acts as an inhibitor of skeletal muscle growth. This muscle-specific cytokine interacts with Activin type I and type II receptors, and suppresses myoblast proliferation by arresting cell-cycle in the G1 phase. Suppression of myostatin activity facilitates muscle formation and may be useful in reducing and/or preventing adiposity and type-2 diabetes. Myostatin activity can be blocked by the Activin-binding protein Follistatin, and by the propeptide of Myostatin.

Langley, B. et. al. (2002) J. Biol. Chem. 227, 49831-49840.
McPherron, A.C. et. al. (1997) Nature 387, 83-90.

Nanog, Nanog-TAT

Nanog is a regulatory protein that is associated with undifferentiated pluripotent cells. The expression of Nanog, which is suppressed in all adult tissues, is restricted to embryonic stem cells and to certain pluripotent cancer cells. Decreased expression of Nanog is strongly correlated with cell differentiation. Nanog, most likely, acts as an intracellular regulator, which helps maintain pluripotency and self renewal via a STAT3 independent pathway. The introduction of Nanog, along with Sox2, Oct4, Lin28, into primary human fibroblasts was sufficient to confer a pluripotent state upon the fibroblast genome. The reprogrammed cells thus obtained resemble ESC in morphology and gene expression. Protein transduction using TAT fusion proteins represents an alternative methodology for introducing transcription factors into primary as well as transformed cells. Recombinant human Nanog is a 34.7 kDa protein, which is synthesized as a 304 amino acid polypeptide lacking a signal sequence for secretion. Recombinant human Nanog-TAT is a 36.2 kDa protein, which is synthesized as a 304 amino acid polypeptide plus a 13-residue C-terminal TAT peptide.

Hart, A.H. et al. (2004) Dev. Dyn. 230 (1), 187-198.
Mitsui, K. et al. (2003) Cell 113 (5), 631-642.

Neuropoietin

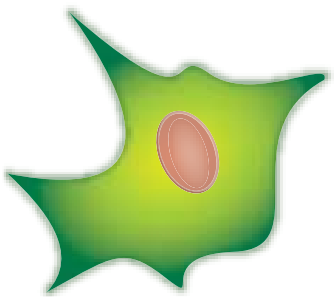
Neuropoietin is a newly identified member of the IL-6 cytokine family. Members of this family, including IL-6, IL-11, Oncostatin M, Leukemia Inhibitory factor (LIF), Cardiotrophin-1 (CT-1), Cardiotrophin-like cytokine, and CNTF, display a four-helix bundle structure, and signal through gp130-containing receptor complexes. Neuropoietin, which is predominantly expressed in neuroepithelia during embryonic life, acts through a receptor complex comprising CNTF receptor-α component, gp 130, and LIF receptor. Like CNTF, it promotes the survival of embryonic motor neurons and could increase the proliferation of neural precursor cells in the presence of EGF and FGF-2. Interestingly, the human Neuropoietin gene has evolved toward a pseudogene, suggesting that the alternative signaling via CNTF is an effective compensatory pathway.

Derouet, D. et al. (2004) PNAS USA 101, 4827-4832.

Neurturin

Neurturin is a disulfide-linked homodimer neurotrophic factor structurally related to GDNF, Artemin, and Persephin. These proteins belong to the cysteine-knot family of growth factors that assume stable dimeric structures. Neurturin signals through a multicomponent receptor system, composed of RET and one of four GFRα (α1-α4) receptors. Neurturin promotes the development and survival of sympathetic and sensory neurons by signaling through a receptor system composed of RET and GFRα2. The functional form of human Neurturin is a disulfide-linked homodimer, of two 11.8 kDa polypeptide monomers (206 total amino acid residues). Each monomer contains seven conserved cysteine residues, one of which (Cys 69) is used for inter-chain disulfide bridging and the others are involved in intramolecular ring formation known as the cysteine knot configuration.

Baloh, R.H. et al. (Dec. 1998) Neuron 6, 1291-1302.
Kotzbauer, P.T. et al. (1996) Nature 384, 467-470.
Saarma, M. (2001) Trends. Neurosci. 24, 427-429.



β-NGF

β-NGF is a neurotrophic factor structurally related to BDNF, NT-3 and NT-4. These proteins belong to the cysteine-knot family of growth factors that assume stable dimeric structures. β-NGF is a potent neurotrophic factor that signals through its receptor β-NGFR, and plays a crucial role in the development and preservation of the sensory and sympathetic nervous systems. β-NGF also acts as a growth and differentiation factor for B lymphocytes and enhances B-cell survival. The functional form of human β-NGF is a noncovalently disulfide-linked homodimer, of two 13.5 kDa polypeptide monomers (240 total amino acid residues). The three disulfide bonds are required for biological activity.

Levi-Montalcini, R. (1987) Science 237, 1154-1162.
Ullrich, A. et al. (1983) Nature 303, 821-825.

Noggin

Noggin belongs to a group of diffusible proteins which bind to ligands of the TGF-β family and regulate their activity by inhibiting their access to signaling receptors. The interplay between TGF-β ligands and their natural antagonists has major biological significance during development processes, in which cellular response can vary considerably depending upon the local concentration of the signaling molecule. Noggin was originally identified as a BMP-4 antagonist whose action is critical for proper formation of the head and other dorsal structures. Consequently, Noggin has been shown to modulate the activities of other BMPs including BMP-2,-7,-13, and -14. Targeted deletion of Noggin in mice results in prenatal death and recessive phenotype displaying a severely malformed skeletal system. Conversely, transgenic mice over-expressing Noggin in mature osteoblasts display impaired osteoblastic differentiation, reduced bone formation, and severe osteoporosis.

Brunet, L.J. et al. (1998) Science 280, 1455-1457.
Minina, E. (2001) Development 128, 4523-4534.

NOV

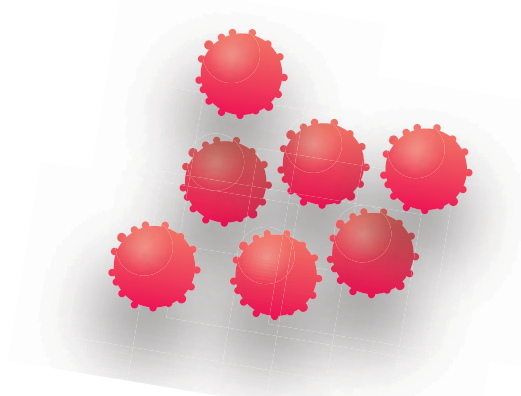
NOV is a member of the CCN family of secreted cysteine rich regulatory proteins. The full length NOV protein contains four structural domains that confer distinct, and sometimes opposing, biological activities. Elevated expression of NOV is associated with certain tumors, including Wilm's tumor and most nephroblastomas. However, in other tumor types and certain cancer cell lines, increased tumorgenicity and proliferation is correlated with decreased NOV expression. Additionally, NOV induces cell adhesion and cell migration by signaling through specific cell surface integrins and by binding to heparin sulfate proteoglycans and to fibulin 1C. NOV has also been reported to exert proangiogenic activities. Recombinant human NOV is a 36.2 kDa protein containing 331 amino acid residues. It is composed of four distinct structural domains (modules); the IGF binding protein (IGFBP) domain, the von Willebrand Factor C (VWFC) domain, the Thrombospondin type-I (TSP type-1) domain, and a C-terminal cysteine knot-like (CTCK) domain.

Perbal, B. et al. (1999) PNAS 96 (3), 869-874.
Perbal, B. et al. (2001) J. Clin. Pathol. Mol. Pathol. 54, 57-59.

NT-3

NT-3 is a neurotrophic factor structurally related to β-NGF, BDNF, and NT-4. These proteins belong to the cysteine-knot family of growth factors that assume stable dimeric structures. NT-3 is expressed by neurons of the central nervous systems and can signal through the trk receptors. NT-3 promotes the growth and survival of nerve and glial cells. The amino acid sequences of human, murine and rat NT-3 are identical. Recombinant human NT-3 is a noncovalently linked homodimer, of two 13.6 kDa polypeptide monomers (240 total amino acid residues).

Maisonpierre, P.C. et al. (1991) Genomics 10, 558-568.
Meakin S.O. and Shooter, E.M. (1992) Trends Neurosci. 15, 323-331.
Yancopoulos, G.D. et al. (1990) Cold Spring Harbor Symp. Quant. Biol. LV, 371-379.



NT-4

NT-4 is a neurotrophic factor structurally related to β-NGF, BDNF, and NT-3. These proteins belong to the cysteine-knot family of growth factors that assume stable dimeric structures. NT-4 is expressed in the prostate, thymus, placenta and skeletal muscle. NT-4 can signal through the LNGFR and trkB receptors and promotes the survival of peripheral sensory sympathetic neurons. Recombinant human NT-4 is a noncovalently linked homodimer, of two 14.0 kDa polypeptide monomers (260 total amino acid residues).

Ip, N.Y. et al. (1992) PNAS USA 89, 3060-3064.
Klein, R. et al. (1992) Neuron 8, 947-956.

Oncostatin M

Oncostatin M (OSM) is a growth and differentiation factor that participates in the regulation of neurogenesis, osteogenesis and hematopoiesis. Produced by activated T cells, monocytes and Kaposi's sarcoma cells, OSM can exert both stimulatory and inhibitory effects on cell proliferation. It stimulates the proliferation of fibroblasts, smooth muscle cells and Kaposi's sarcoma cells, but, inhibits the growth of some normal and tumor cell lines. It also promotes cytokine release (e.g. IL-6, GM-CSF and G-CSF) from endothelial cells, and enhances the expression of low-density lipoprotein receptor in hepatoma cells. OSM shares several structural and functional characteristics with LIF, IL-6, and CNTF. Human OSM is active on murine cells.

Bruce, A.G. and Rose, T.M. (2001) Cytokine Reference. Oppenheim, J.J. and Feldman, M. ed., Academic Press, London, 585-598.
Rose, T.M. and Bruce, A.G. (1991) PNAS USA 88,8641-8645.

p16-INK4a, p16-INK4a-TAT

p16-INK4a is a nuclear protein that regulates the cell cycle by inhibiting cyclin dependent kinase-4 (CDK4) and CDK6. p16-INK4a inhibits CDK activity by binding to the CDK molecules in a manner that interferes with their ability to interact with cyclin D. This activity has the effect of suppressing tumor formation and growth, and of inducing replicative senescence in various normal cells, including stem cells. The expression of p16-INK4a steadily increases with age and tends to accumulate in stem cell compartments. The deletion, rearrangement, or mutation of the p16-INK4a gene is frequently found in melanomas as well as in certain other types of cancer. Protein transduction using TAT fusion proteins represents an alternative methodology for introducing transcription factors and other nuclear proteins into primary as well as transformed cells. Recombinant p16-INK4a is a 16.5 kDa protein containing 156 amino acid residues. Recombinant p16-INK4a-TAT is an 18.1 kDa protein containing 168 amino acid residues including a 13-residue C-terminal TAT peptide (GGYGRKKRRQRRR).

Nishino, J. et al. (2008) Cell 135, 227-239.
Okamoto, A. et al. (1994) PNAS USA 91, 11045-11049.

PDGF-AA, -AB, -BB

PDGFs are disulfide-linked dimers consisting of two 12.0-13.5 kDa polypeptide chains, designated PDGF-A and PDGF-B chains. The three naturally occurring PDGFs; PDGF-AA, PDGF-BB and PDGF-AB, are potent mitogens for a variety of cell types including smooth muscle cells, connective tissue cells, bone and cartilage cells, and some blood cells. The PDGFs are stored in platelet α-granules and are released upon platelet activation. The PDGFs are involved in a number of biological processes, including hyperplasia, chemotaxis, embryonic neuron development, and respiratory tubule epithelial cell development. Two distinct signaling receptors used by PDGFs have been identified and named PDGFR-α and PDGFR-β. PDGFR-α is high-affinity receptor for each of the three PDGF forms. On the other hand, PDGFR-β interacts with only PDGF-BB and PDGF-AB. Recombinant human PDGF-AA is a 28.5 kDa disulfide-linked homodimer of two A chains (250 total amino acids). Recombinant human PDGF-AB is a 25.5 kDa disulfide-linked dimer, consisting of one A chain and one B chain (234 total amino acids). Recombinant human PDGF-BB is a 24.3 kDa disulfide-linked homodimer of two B chains (218 total amino acids).

Collins, T. et al. (1987) Nature 328, 621-624.
Heldin, C.H. (1992) EMBO J. 11, 4251-4259.
Ross, R. et al. (1986) Cell 46, 155-169.

PDGF-CC

The platelet-derived growth factor (PDGF) family of heparin-binding growth factors consists of five known members, denoted PDGF-AA, PDGF-BB, PDGF-AB, PDGF-CC and PDGF-DD. The mature and active form of these proteins, an anti-parallel disulfide-linked dimer of two 12-14 kDa polypeptide chains, is obtained through proteolytic processing of biologically inactive precursor proteins, which contain an N-terminal CUB domain and a PDGF/VEGF homologous domain. The PDGFs interact with two related protein tyrosine kinase receptors, PDGFR-α and PDGFR-β, and are potent mitogens for a variety of cell types, including smooth muscle cells, connective tissue cells, bone and cartilage cells, and certain tumor cells. They play an important role in a number of biological processes, including hyperplasia, chemotaxis, embryonic neuron development, and respiratory tubules epithelial cell development. Mature PDGFs are stored in platelet α-granules and are released upon platelet activation. PDGF-AA, -AB, -BB and –CC signal primarily through the PDGF-Rα receptor, whereas PDGF-DD interacts almost exclusively with the PDGF-Rβ receptor.

Collins, T et al. (1987) Nature 328, 621-624.
Heldin, C.H. (1992) EMBO J. 11, 4251-4259.
Ross, R et al. (1986) Cell 46, 155-169.

Persephin

Persephin is a disulfide-linked homodimer neurotrophic factor structurally related to GDNF, Artemin, and Neurturin. These proteins belong to the cysteine-knot family of growth factors that assume stable dimeric structures. Persephin signals through a multicomponent receptor system, composed of RET and one of four GFRα (α1-α4) receptors. The GFRα4 was first identified in chicken and was later shown to be the preferential binding subunit for Persephin. Persephin promotes the survival of ventral midbrain dompaminergic neurons and motor neurons after sciatic nerve oxotomy, and like GNDF, promotes ureteric bud branching. However, in contrast to GDNF and Neurturin, Persephin does not support survival of peripheral neurons. Recombinant human Persephin is a disulfide-linked homodimer, composed of two 10.3 kDa polypeptide chains (192 total amino acid residues). Each chain contains seven conserved cysteine residues, one of which (Cys 63) is used for inter-chain disulfide bridging and the others are involved in intramolecular ring formation known as the cysteine knot configuration.

Lindahl, M. et al. (2000) Mol. Cell Neurosci. 15, 522-533.
Saarma, M. (2001) Trends Neurosci. 24, 427-429.

Pleiotrophin

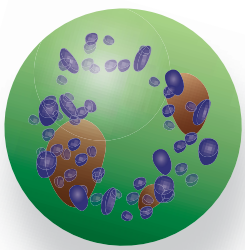
Pleiotrophin and Midkine are structurally related heparin-binding neurotrophic factors, whose expression is developmentally regulated. The expression pattern of these neurotrophic factors suggests function in neurogenesis, cell migration, secondary organogenetic induction, and mesoderm epithelial interaction. The expression of PTN increases during the process of brain embryogenesis and reaches maximum levels at time of birth. The physiological roles of PTN and Midkine are largely unknown, but these neurotrophins have been implicated in the pathogenesis of neuroblastomas. Recombinant human Pleiotrophin is a 15.4 kDa protein containing 136 amino acid residues and five intra-molecular disulfide bonds.

Muramatsu, T. (1993) Int. J. Dev. Biol. 1, 183-188.
Muramatsu, T. (2002) J. Biochem. 3, 359-371.

PlGF-1

PlGF-1 is an angiogenic factor that belongs to the cysteine-knot superfamily of growth factors. PlGF-1 is expressed in placental tissues, colon and mammary carcinomas. It signals through the VEGFR-1/FLT1 receptor and stimulates endothelial cell proliferation and migration. Recombinant human PlGF-1 is a 29.7 kDa disulfide-linked homodimeric protein of two 131 amino acid polypeptide chains.

Park, J.E. et al. (1994) J. Biol. Chem. 269 (41), 25646-25654.



PlGF-2

PlGF-2 is an angiogenic factor that belongs to the cysteine-knot superfamily of growth factors. PlGF-2 is expressed in umbilical vein endothelial cells and placenta. It signals through the VEGFR-1/FLT1 receptor and stimulates endothelial cell proliferation and migration. PlGF-2 also signals through Neuropilin and can bind with high affinity to heparin. Recombinant human PlGF-2 is a 34.0 kDa disulfide-linked homodimeric protein of two 150 amino acid polypeptide chains.

Hauser, S.D. and Weich, H.A. (1993) Growth Factors 9, 259-268.
Mig, M. et al. (1998) J. Biol. Chem. 273, 22272-22278.

PlGF-3

PlGF-3 is an angiogenic factor that belongs to the cysteine-knot superfamily of growth factors. PlGF-3 is expressed exclusively in the placenta. It signals through the VEGFR-1/FLT1 receptor and stimulates endothelial cell proliferation and migration. PlGF-3 lacks heparin binding affinity. Recombinant human PlGF-3 is a 45.7 kDa disulfide-linked homodimeric protein of two 203 amino acid polypeptide chains.

Yang, W. et al. (2003) J. Reprod. Immunol. 60, 53-60.

R-Spondin-1

R-Spondin-1 (Rspo-1) belongs to the (Rspo) family of Wnt modulators. Currently, the family consists of four structurally related secreted ligands (Rspo 1-4), all containing furin-like and thrombospondin structural domains. Rspo-1 is expressed in certain areas of the developing central nervous system, as well as in adrenal glands, ovary, testis, thyroid, and trachea. Rspo-1 can interact with the Frizzled/LRP6 receptor complex in a manner that stimulates the Wnt/beta-catenin signaling pathway.

Binnerts, M.E. et al. (2007) PNAS USA 104, 14700-147005.

SCF

SCF is a hematopoietic growth factor that exerts its activity by signaling through the c-Kit receptor. SCF and c-Kit are essential for the survival, proliferation and differentiation of hematopoietic cells committed to the melanocyte and germ cell lineages. Human SCF manifests low activity on murine cells, while murine and rat SCF are fully active on human cells. Recombinant human SCF is an 18.4 kDa polypeptide containing 165 amino acid residues, which corresponds to the sequence of the secreted soluble form of SCF.

Anderson, D.M. et al. (1990) Cell 63, 235-243.
Ullich, T.R. et al. (1991) Blood 78, 645.

SCGF-α, -β

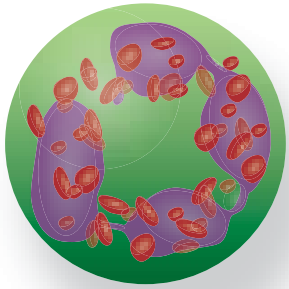
SCGF-α and β are hematopoietic growth factors that exert their activity at early stages of hematopoiesis. The SCGFs are non-glycosylated, species-specific cytokines that can support growth of primitive hematopoietic cells, and in combination with EPO or GM-CSF, promote proliferation or erythroid or myeloid progenitors, respectively. Recombinant human SCGF-α is a 33.9 kDa polypeptide containing 305 amino acid residues. Recombinant human SCGF-β is a 25.0 kDa polypeptide containing 227 amino acid residues.

Hiraoka, A. et al. (1997) PNAS USA 14, 7577-7582.

SDF-1α, -1β/CXCL12

SDF-1α and β are stromal derived CXC chemokines, and signal through the CXCR4 receptor. SDF-1α and β chemoattract B and T cells, and have been shown to induce migration of CD34+ stem cells. Additionally, the SDF-1 proteins exert HIV suppressive activity in cells expressing the CXCR4 receptor. Recombinant human SDF-1α is an 8.0 kDa protein containing 68 amino acid residues. Recombinant human SDF-1β is an 8.5 kDa protein containing 72 amino acid residues.

D'Apuzzo, M. et al. (1997) Eur. J. Immunol. 27, 1788-1793.
Oberlin, E. et al. (1996) Nature 382, 833-835.



Sonic Hedgehog

Members of the Hedgehog (Hh) family are highly conserved proteins which are widely represented throughout the animal kingdom. The three known mammalian Hh proteins, Sonic (Shh), Desert (Dhh) and Indian (Ihh) are structurally related and share a high degree of amino-acid sequence identity (e.g., Shh and Ihh are 93% identical). The biologically active form of Hh molecules is obtained by autocatalytic cleavage of their precursor proteins and corresponds to approximately the N-terminal one half of the precursor molecule. Although Hh proteins have unique expression patterns and distinct biological roles within their respective regions of secretion, they use the same signaling pathway and can substitute for each other in experimental systems. Recombinant human Sonic Hedgehog is a 20.0 kDa protein consisting of 176 amino acid residues, including an N-terminal Ile-Val-Ile sequence substituted for the naturally occurring chemically modified Cys residue.

Kumar, S. et al. (1996) Genetics 3, 965-972.
Lanske, B et al. (1996) Science 273, 663-666.

Sox2, Sox2-TAT

Sox2, also known as sex determining region Y (SRY)-box 2, belongs to a diverse family of structurally-related transcription factors whose primary structure contains a 79-residue DNA-binding domain, called high mobility group (HMG) box. It plays an essential role in maintaining the pluripotency of embryonic stem cells (ESC) and determination of cell fate. Microarray analysis showed that Sox2 regulates the expression of multiple genes involved in embryonic development including FGF-4, YES1 and ZFP206. Sox2 acts as a transcriptional activator after forming a ternary complex with Oct3/4 and a conserved non-coding DNA sequence (CNS1), located approximately 2 kb upstream of the RAX promoter. The introduction of Sox2, Oct4, Myc, and Klf4, into human dermal fibroblasts isolated from a skin biopsy of a healthy research fellow was sufficient to confer a pluripotent state upon the fibroblast genome. The reprogrammed cells thus obtained resemble ESC in morphology, gene expression, and in the capacity to form teratomas in immune-deficient mice. Sox2 and other transcription factors have been introduced into cells by DNA transfection, viral infection, or microinjection. Protein transduction using TAT fusion proteins represents an alternative methodology for introducing transcription factors and other nuclear proteins into primary as well as transformed cells. Recombinant human Sox2 is a 34.3 kDa protein containing 317 amino-acid residues. Recombinant human Sox2-TAT is a 36 kDa protein containing 330 amino-acid residues, including the 317 residues of full-length Sox2 and a 13-residue C-terminal TAT peptide (GGYGRKKRRQRRR).

Guo, J. et al. (2009) Biochem. Biophys. Res. Commun. 390, 1081-1086.

TGF-α

TGF-α is an EGF-related polypeptide growth factor that signals through the EGF receptor, and stimulates the proliferation of a wide range of epidermal and epithelial cells. It is produced by monocytes, keratinocytes, and various tumor cells. TGF-α induces transformation anchorage independence in cultured cells. Human, murine and rat TGF-α are cross-species reactive. Recombinant human TGF-α is a 50 amino acid polypeptide (5.5 kDa) which shares approximately 40% sequence homology with EGF, including 6 conserved cysteine residues, which form 3 intramolecular disulfide bonds.

Bosenberg, M.W. et al. (1992) Cell 71, 1157-1165.
Burgess, A.W. et al. (1989) In Br. Med. Bulletin 45, Growth Factors, Waterfeld, M.D. ed., Churchill Livingstone, London, 401-424.

TGF-β1, -β2, -β3

The three mammalian isoforms of TGF-β, TGF-β1, β2, β3, signal through the same receptor and elicit similar biological responses. They are multifunctional cytokines that regulate cell proliferation, growth, differentiation and motility as well as synthesis and deposition of the extracellular matrix. They are involved in various physiological processes including embryogenesis, tissue remodeling and wound healing. They are secreted predominantly as latent complexes which are stored at the cell surface and in the extracellular matrix. The release of biologically active TGF-β isoform from a latent complex involves proteolytic processing of the complex and /or induction of conformational changes by proteins such as thrombospondin-1.

TGF-β1: TGF-β1 is the most abundant isoform secreted by almost every cell type. It was originally identified for its ability to induce phenotypic transformation of fibroblasts and recently it has been

TGF-β1, -β2, -β3 cont'd

implicated in the formation of skin tumors. Recombinant human TGF-**β1** is a 25.0 kDa protein composed of two identical 112 amino acid polypeptide chains linked by a single disulfide bond.

TGF-β2: TGF-**β2** has been shown to exert suppressive effects on IL-2 dependent T-cell growth, and may also have an autocrine function in enhancing tumor growth by suppressing immuno-surveillance of tumor development. Recombinant human TGF-**β2** is a 25.0 kDa protein composed of two identical 112 amino acid polypeptide chains linked by a single disulfide bond.

TGF-β3: The physiological role of TGF-**β3** is still unknown but its expression pattern suggests a role in the regulation of certain development processes. Recombinant TGF-**β3** is a 25.0 kDa protein composed of two identical 112 amino acid polypeptide chains linked by a single disulfide bond.

Flanders, K.C. and Roberts, A.B. (2001) Cytokine Reference. Oppenheim, J.J. and Feldman, M. ed., Academic Press, London, 719-746.

TIGAR, TIGAR-TAT

TIGAR is a p53-inducible enzyme that catalyzes the hydrolysis of fructose-2-6 bisphosphate (F-2-6-BP) to fructose-6-phosphate and inorganic phosphate. F-2-6-BP is a powerful activator of 6-phosphofructose-1 kinase, the rate limiting enzyme of glycolysis. By lowering the intracellular level of F-2-6-BP, TIGAR expression leads to increased glucose processing via the pentose phosphate pathway, the major cellular source for NADPH. Protein transduction using TAT fusion proteins represents an alternative methodology for introducing transcription factors and other intracellular proteins into primary as well as transformed cells. Recombinant human TIGAR-TAT is a 36 kDa protein containing 284 amino-acid residues, including the 271 residues of full-length TIGAR fused to a 13-residue C-terminal peptide containing the TAT transduction domain (GGYGRKKRRQRRR).

Bensaad, K et al. (2006) Cell 126, 107-120.

TNF-α

TNF-α is a pleiotropic pro-inflammatory cytokine secreted by various cells including adipocytes, activated monocytes, macrophages, B cells, T cells and fibroblasts. It belongs to the TNF family of ligands and signals through two receptors, TNFR1 and TNFR2. TNF-α is cytotoxic to a wide variety of tumor cells and is an essential factor in mediating the immune response against bacterial infections. TNF-α also plays a role in the induction of septic shock, auto immune diseases, rheumatoid arthritis, inflammation, and diabetes. Recombinant human TNF-α is a soluble 157 amino acid protein (17.4 kDa) which corresponds to the C-terminal extracellular domain of the full length transmembrane protein.

Aggarwal, B.B. et al. (2001) Cytokine Reference. Oppenheim, J.J. and Feldman, M. ed., Academic Press, London, 413-434.

TNF Receptor Type I

TNFR1 belongs to the TNFR superfamily of transmembrane proteins, and is expressed in most cell types. Binding of either TNF-α or TNF-β to TNFR1 initiates a signal transduction pathway that results in the activation of the transduction factor NFkB, whose target genes are involved in the regulation of inflammatory responses, and, in certain cells induce apoptosis. Soluble TNF Receptor I (sTNFR1) is capable of inhibiting TNF-α and TNF-β activities by acting as a decoy receptor that serves as a sink for the TNF ligands. Recombinant human sTNFR1 is an 18.3 kDa protein (162 amino acid residues) comprising the cysteine rich ligand binding portion of the extracellular domain of the TNFR1 protein.

Aggarwal, B.B. et al. (2001) Cytokine Reference. Oppenheim, J.J. and Feldman, M. ed., Academic Press, London, 1619-1632.

TNF Receptor Type II

TNFR2 is a member of the TNFR superfamily of transmembrane proteins, and is expressed in immune cells and certain endothelial cells. It is a high affinity receptor for TNF-α but manifests a lower affinity to TNF-β. Signaling through this receptor regulates various biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, coagulation, and neurotransmission. Soluble TNFR2 is capable of inhibiting TNF-α induced activities by acting as a decoy receptor. Recombinant human sTNFR2 is an 18.9 kDa protein (174 amino acid residues) comprising the cysteine rich ligand binding portion of the extracellular domain of the TNFR2 protein.

Aggarwal, B.B. et al. (2001) Cytokine Reference. Oppenheim, J.J. and Feldman, M. ed., Academic Press, London, 1619-1632.

TPO

TPO is a lineage specific growth factor, produced in the liver, kidney and skeletal muscle. It stimulates the proliferation and maturation of megakaryocytes, and promotes increased circulating levels of platelets *in vivo*. TPO signals through the c-mpl receptor and acts as an important regulator of circulating platelets. Human and murine TPO exhibits cross-species reactivity. Recombinant human TPO is a fully biologically active 174 amino acid polypeptide (18.6 kDa), which contains the erythropoietin-like domain of the full length TPO protein.

Kuter, D.J. et al. (2001) Cytokine Reference. Oppenheim, J.J. and Feldman, M. ed., Academic Press, London, 965-982.

VEGF-A

VEGF-A is a potent growth and angiogenic cytokine. It stimulates proliferation and survival of endothelial cells, and promotes angiogenesis and vascular permeability. Expressed in vascularized tissues, VEGF plays a prominent role in normal and pathological angiogenesis. VEGF signals through three receptors; *fms*-like tyrosine kinase (flt-1), KDR gene product (the murine homolog of KDR is the flk-1 gene product) and the flt4 gene product. Due to its increased acidity, $VEGF_{121}$ circulates more freely than other VEGF forms, which bind more tightly with vascular heparin sulfates.

Clauss, M. et al. (1990) J. Exp. Med. 172, 1535-1545.
Leung, D.W. et al. (1989) Science 246, 1306-1309.

VEGF-B

VEGF-B, a member of the VEGF family, is a potent growth and angiogenic cytokine. It promotes DNA synthesis in endothelial cells, helps regulate angiogenesis and vascular permeability, and inhibits apoptosis in certain smooth muscle cells and neurons. VEGF-B is expressed in all tissues except the liver. It forms cell surfaced-associated disulfide linked homodimers and can form heterodimers with VEGF-A. There are two known isoforms, formed by alternative splicing, which have been designated $VEGF\text{-}B_{167}$ and $VEGF\text{-}B_{186}$. Both forms have identical amino-terminal sequences encoding a “cysteine knot” like structural motif, but differ in their carboxyl-terminal domains. Both VEGF-B isoforms signal only through the VEGFR1 receptor.

VEGF-C, -D

VEGF-C and VEGF-D, members of the VEGF/PDGF family of structurally related proteins, are potent angiogenic cytokines. They promote endothelial cell growth, lymphangiogesis, and can also affect vascular permeability. VEGF-C is expressed in various tissues, but is not produced in peripheral blood lymphocytes. VEGF-D is highly expressed in the lung, heart, small intestine and fetal lung, and at lower levels in the skeletal muscle, colon, and pancreas. They form cell surfaced-associated non-covalent disulfide linked homodimers, and can bind and activate both VEGFR-2 (flk1) and VEGFR-3 (flt4) receptors. During embryogenesis, VEGF-C and VEGF-D may play a role in the formation of the venous and lymphatic vascular systems. VEGF-D also participates in the growth and maintenance of differentiated lymphatic endothelium in adults. Both VEGF-C and VEGF-D are over-expressed in certain cancers, and the resulting elevated levels of VEGF-C or VEGF-D tend to correlate with increased lymphatic metastasis.

Breen, E.C. (2007) J. Cell Biochem. 102, 1358-1367.
Roy, H. et al. (2006) FEBS Lett. 580, 2879-2887.

Vitronectin

Vitronectin is a secreted glycoprotein which is synthesized in the liver. It circulates primarily in monomeric form, but can undergo conformational change to a structure that forms disulfide linked multimers. The multimeric Vitronectin can efficiently bind to and incorporate into the extracellular matrix. Within the matrix, Vitronectin can support cell adhesion through binding to various integrins and other proteoglycans. Additionally, recombinant vitronectin can function as a chemically defined matrix component in human embryonic stem cell renewal media.

Braam, S.R. et al. (2008) Stem Cells 26, 2257-2265.
Ruoslahti, E. et al. (1985) Artheriosclerosis 5, 581-594.

WISP-1

WISP-1 is a member of the CCN family of secreted cysteine rich regulatory proteins. It is expressed in the heart, kidney, lung, pancreas, placenta, ovary, small intestine and spleen. WISP-1 is a beta catenin regulated protein that can contribute to tumorigenesis and has also been shown to play a role in bone development and fracture repair. Human WISP-1 is a 38.1 kDa protein containing 345 amino acid residues. It is composed of four distinct structural domains (modules); the IGF binding protein (IGFBP) domain, the von Willebrand Factor C (VWFC) domain, the thrombospondin type-1 repeat (TSP type-1) domain, and a C-terminal cystine knot-like (CTCK) domain.

Desnoyers, L. et al. (2001) J. Biol. Chem. 276, 47599-47607.
Pennica, D. et al. (1998) PNAS USA 95, 14717-14722.
Xu, et al. (2000) Genes Dev. 14, 585-595.

Wnt-1

Wnt-1 is a secreted protein that signals through the Frizzled family of cell surface receptors and is required for normal embryonic development. Wnt-1 activation induces a complex signaling cascade that ultimately leads to the increased expression of over fifty genes. An important component of Wnt-1 signaling is the stabilization, and resulting accumulation, of the intracellular signaling protein, beta-catenine. Wnt signaling induces and maintains the transformed phenotype and, in certain embryonic cell lines, supports self renewal in the absence of significant differentiation. Elevated levels of Wnt proteins are associated with tumorigenesis and are present in numerous human breast cancers.

Brennan, K.R. and Brown, A.M. (2004) J. Mammary Gland Biol. Neoplasia 9, 119-131.
Sakanaka, C. et al. (2000) Recent Prog. Horm. Res. 55, 225-236.

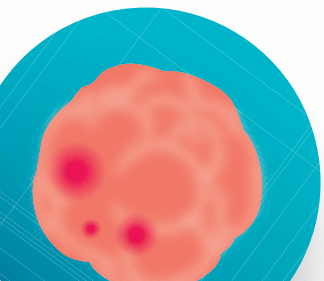
Wnt-3a

Wnt-3a belongs to the Wnt family of signaling proteins that play a key role in maintaining the integrity of embryonic and adult tissues. Expression of Wnt-3a occurs primarily along the dorsal midline across overlapping regions of the Central Nervous System (CNS). Wnt-3a signaling is essential for various morphogenetic events including embryonic patterning, cell determination, cell proliferation, CNS development, and cytoskeletal formation. Like other members of this family, Wnt-3a contains a highly conserved lipid modified cysteine rich domain that is essential for cell signaling. During a biochemical process called the canonical Wnt pathway; Wnt family members bind to and activate seven-pass transmembrane receptors of the Frizzled family ultimately leading to the disruption of β-Catenein degradation. Intracellular accumulation of β-Catenin increases translocation of the protein into the nucleus where it binds to TCF/LEF transcription factors to promote gene expression. Lack of Wnt signaling disrupts transcriptional activation of tumor suppressor genes and has shown to result in neoplastic transformation, oncogenesis, and human degenerative diseases.

Castelo-Branco, G. et al. (2003) PNAS USA 100, 12747-12752.
Fischer, L. et al. (2002) J. Cell Biochem. 84, 816-831.

Wnt-7a

Wnt-7a belongs to the Wnt family of signaling proteins that play a key role in maintaining the integrity of embryonic and adult tissues. It is expressed in placenta, kidney, testis, uterus, fetal lung and fetal and adult brain. Most Wnt proteins can signal though a mechanism called the canonical Wnt pathway, in which Wnt proteins bind to and activate seven-pass transmembrane receptors of the Frizzled family ultimately leading to the disruption of β-Catenein degradation. Intracellular accumulation of β-Catenin increases translocation of the protein into the nucleus where it binds to TCF/LEF transcription factors and induces the expression of numerous genes. Increased Wnt/β-Catenein signaling is associated with tumorigenesis in a diverse set of human cancers. However, Wnt-7a/Frizzled-9 signaling has been shown to act as a tumor suppressor in non-small cell lung cancers.



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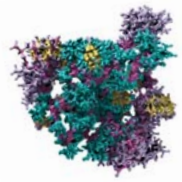

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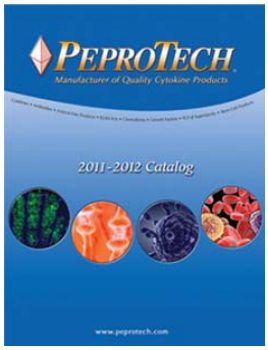
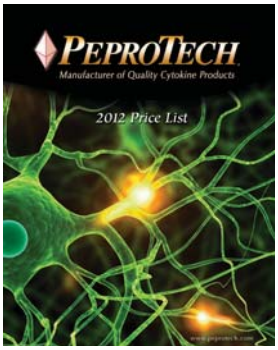
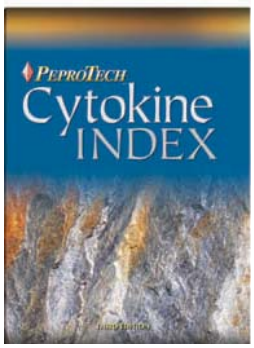
USB Catalog
A USB version of PeproTech's Product Catalog, which includes a comprehensive list of our products, their prices, and other relevant product information.



Catalog
PeproTech's Product Catalog includes a comprehensive list of our products, their prices, and other relevant product information, such as procedure overviews and technical support.



Price List
PeproTech's Price List offers our customers a comprehensive resource for the pricing of our products, as well as some supplemental product information.



Cytokine INDEX
This 312 page INDEX contains encyclopedic coverage of more than 350 cytokines.

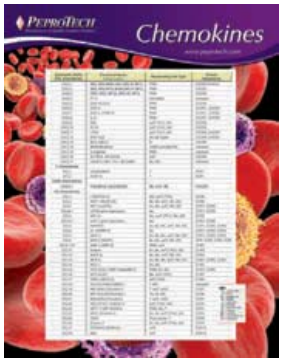
Antibody & ELISA Resource Guide
This publication describes the production, application and availability of PeproTech's Antibodies and ELISA Development Kits.

VEGF/PDGF Family
This 17″ x 24″ color poster includes relavant information about VEGF/PDGF members.

Neurotrophins/ Neuropoietic Poster
This 17″x24″ color poster displays relevant information about Neurotrophins and Neuropoietic Cytokines.

Product Update
PeproTech's Product Update is a quarterly newsletter featuring any new products that we have begun selling since the previous issuance of our Product Catalog.

The Focus
The FOCUS is a collection of articles that have been published in previous UPDATE editions.

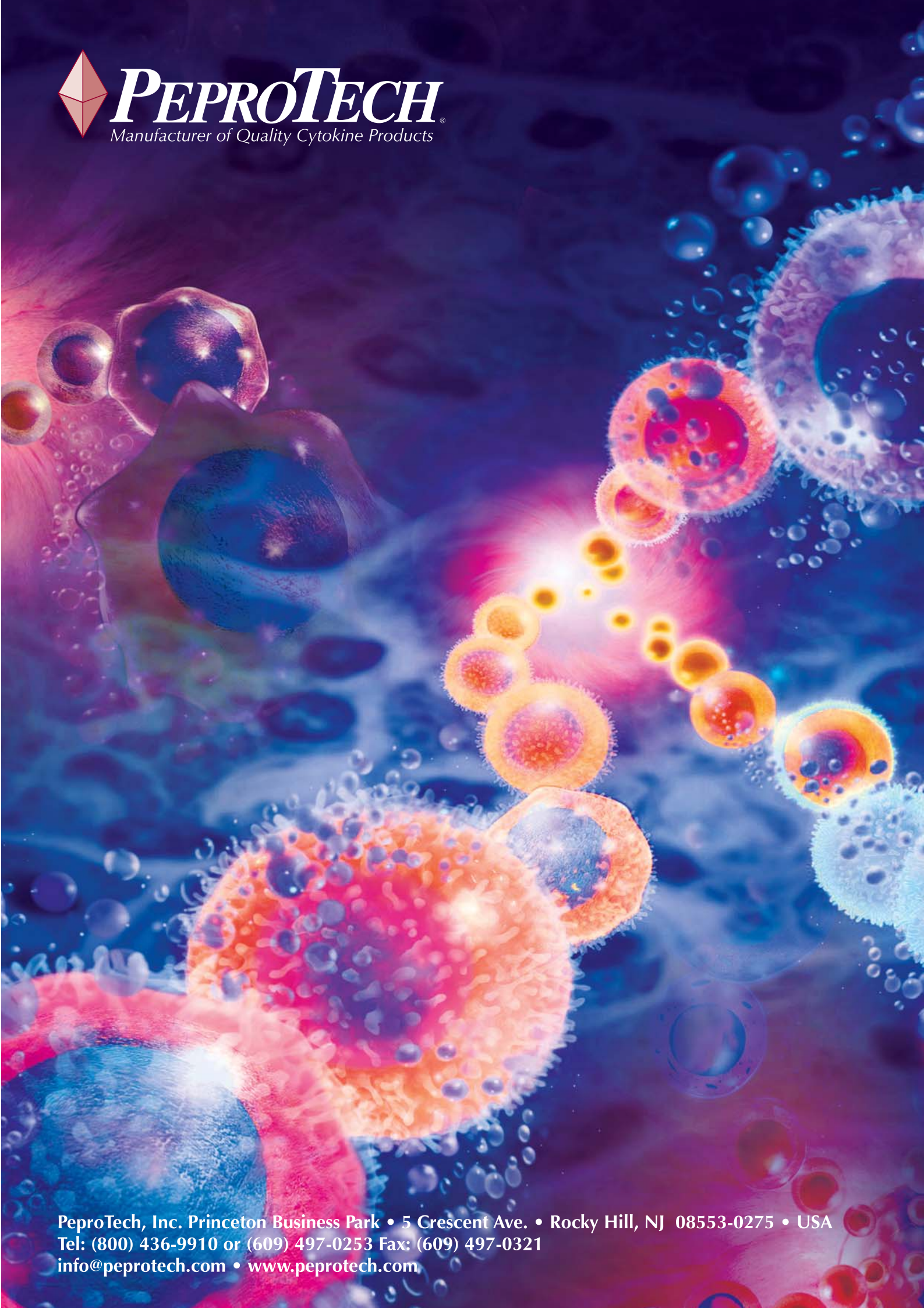


Chemokines
This 17″ x 24″ color poster displays the Systematic Nomenclature for Chemokines.

TNF Superfamily
This 17″ x 24″ color poster displays the TNF Superfamily nomenclature.

FGF Family
This 17″ x 24″ color poster displays the FGF cytokine family and includes target cells, receptors, and more.

TGFβ Superfamily
This 17″ x 24″ color poster includes relevant information about the TGFβ Superfamily.



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