# Functions of PDGF-A and -C: Essential Ligands for the PDGF Alpha Receptor

Hans Boström

Department of Medical Biochemistry
Göteborg University
2003

ISBN 91-628-5574-3

© Hans Boström
Department of Medical Biochemistry
Göteborg University, Sweden

Printed by Intellecta Docusys AB
Göteborg, Sweden 2003

Recorded on AGAIN! - Rob McConnell and the Boss Brass

# TAKE THE "A" CHAIN [1)]

BARITONE SAX

By BILLY STRAYHORN
*Arranged by ROB McCONNELL*

SAMBA IN 2 (𝅗𝅥=144)

1 2 3 4 5

6 7 8-11 4 12

13 14 15 16 17

18 19 20 21 22

23 24-31 8 24 32 33 34

35 36 37 38

39 40 41 42

43 44 44 45 46

1) As previously reported (Betsholtz, 1986).

## Abstract

Altered protein kinase activity is a contributing factor in many diseases including most forms of human malignancies, and there is reason to believe that protein kinases will prove to be major drug targets in the treatment of cancer. Protein kinases mediate most of the signal transduction in eukaryotic cells, regulating cellular events such as transcription, metabolism, proliferation, cytoskeletal rearrangement, migration, differentiation and apoptosis. Protein phosphorylation also plays a critical role in intercellular communication during embryonic development, especially through activation of receptor tyrosine kinases (RTK:s).

This study focuses on the Platelet-derived growth factor (PDGF) family, secreted molecules whose functions are to bind to, and activate, two structurally related RTK:s, PDGF receptor alpha and beta. To gain insight into the developmental role of PDGF-A and -C, three lines of mice were generated in which these genes were modified by gene targeting.

As a common principle, PDGF-A and -C, secreted from epithelial cells, induced proliferation, and possibly migration, of mesenchymal progenitor cells expressing PDGF receptor alpha.

Mice deficient for PDGF-A died perinatally and displayed defective lung development due to lack of alveolar formation. This phenotype was coupled to a loss of alveolar smooth muscle cells and reduced parenchymal elastin, resulting in a picture resembling emphysema.

The sixth exon in PDGF-A is normally alternatively spliced, and, when present, it confers binding to extracellular matrix structures. The second line of mice generated carried a mutation in the sixth exon splice acceptor, so that only the short, freely diffusible form of PDGF-A was being produced. Analysis of these mice suggested overlapping functions for PDGF-A and -C, and also revealed that extracellular retention of PDGF-A is important for normal development of gastrointestinal villi.

Depending on genetic background, PDGF-C negative mice died postnatally due to a cleft palate accompanied by moderate spina bifida. Interestingly, PDGF-A / PDGF-C double deficient mice were phenotypically indistinguishable from mice carrying a null mutation in PDGF receptor alpha. Taken together, the results of this study imply that PDGF-A and -C are the physiologically important ligands for PDGF receptor alpha during development.

*Keywords: platelet-derived growth factor, PDGF-A, PDGF-C, PDGF alpha receptor, extracellular retention, gene targeting, mouse development, epithelial-mesenchymal interaction, lung, alveolar formation, spina bifida, gastrointestinal villus*

ISBN 91-628-5574-3

## List of publications

This thesis is based on the following publications, referred to in the text by their Roman numerals.

I. PDGF-A signaling is a critical event in lung alveolar myofibroblast development and alveogenesis.
Bostrom, H., Willetts, K., Pekny, M., Leveen, P., Lindahl, P., Hedstrand, H., Pekna, M., Hellstrom, M., Gebre-Medhin, S., Schalling, M., Nilsson, M., Kurland, S., Tornell, J., Heath, J. K. and Betsholtz, C.
*Cell 85, 863-873 (1996)*

II. PDGF-A / PDGF alpha-receptor signaling is required for lung growth and the formation of alveoli but not for early lung branching morphogenesis.
Bostrom, H., Gritli-Linde, A., and Betsholtz, C.
*Dev. Dyn. 223(1):155-162 (2002)*

III. PDGF-C is a new protease-activated ligand for the PDGF alpha-receptor.
Li, X., Ponten, A., Aase, K., Karlsson, L., Abramsson, A., Uutela, M., Backstrom, G., Hellstrom, M., Bostrom, H., Li, H., Soriano, P., Betsholtz, C., Heldin, C.H., Alitalo, K., Ostman, A. and Eriksson, U.
*Nature Cell Biol. 2(5):302-9 (2000)*

IV. PDGF-C and -A exert both overlapping and specific developmental functions through the PDGF alpha-receptor.
Ding, H., Wu, X., Bostrom, H., Kim, I., Koh, G.Y., Soriano, P., Betsholtz, C., Tam, P.P.L. and Nagy, A.
*Manuscript (2003)*

V. Mice engineered to produce only the short form of PDGF-A point to the importance of extracellular retention of growth factors.
Bostrom, H., Ding, H., Bianconi, S., Nagy, A. and Betsholtz, C.
*Manuscript (2003)*

Reprints were made with permission from the publishers.

# Table of contents

Abbreviations ........ 8
Introduction ........ 9
Protein kinases - major information bearing molecules ........ 9
Altered protein tyrosine kinase activity and human disease ........ 11
Platelet-derived growth factors - secreted molecules that activate two receptor tyrosine kinases ........ 17
PDGF isoforms ........ 18
Extracellular retention of PDGF isoforms ........ 22
PDGF receptors ........ 23
Expression of PDGF and PDGF receptors ........ 27
Developmental functions of PDGF - lessons from gene targeting in mice ........ 31
Gene targeting - methodological aspects ........ 31
Platelet-derived growth factor mutants ........ 34
Complex phenotypic alterations in PDGF-A knockout mice ........ 34
The importance of extracellular retention of PDGF-A ........ 38
Mice deficient for PDGF-B display cardiac and vascular defects ........ 39
Cell-type-specific ablation of PDGF-B by the creation of chimeric mice and conditional gene targeting ........ 40
PDGF-C negative mice show cleft palate and spina bifida ........ 41
PDGF receptor mutants ........ 42
Patterning of the somites and development of neural-crest-derived mesenchyme is dependent on PDGFRα ........ 42
PDGFR-β negative mice phenocopy PDGF-B knockouts ........ 43
Cell autonomous requirement for PDGF receptors revealed by chimeric analyses ........ 43
Selective mutations in PDGF receptors suggest both overlapping and specific functions for RTK-activated signaling pathways ........ 44
The generation of PDGF-A / PDGF-C double deficient mice provides an important piece in the PDGF puzzle ........ 47
Critical PDGF ligand/receptor specificities in mouse development ........ 48
Analogous functions for PDGFs and their target cells ........ 49
Summary and clinical perspective ........ 51
Acknowledgments ........ 55
References ........ 57

## Abbreviations

| | |
|---|---|
| ABL | Abelson tyrosine kinase |
| ApoE | apolipoprotein E |
| ASMA | alpha smooth muscle actin |
| ATP | adenosine triphosphate |
| CSF1R | colony stimulating factor 1 receptor (also called FMS) |
| CUB | domain found in complement, urchin-like EGF protein and bone morphogenetic protein-1 |
| E | embryonic day |
| EGF | epidermal growth factor |
| FGF | fibroblast growth factor |
| GDNF | glial derived neurotrophic factor |
| GIST | gastrointestinal stromal tumor |
| Grb2 | growth factor receptor bound protein 2 |
| FLT3 | FMS-related tyrosine kinase 3 |
| KIT | stem cell factor receptor |
| MAPK | mitogen-activated protein kinase |
| MEN | multiple endocrine neoplasia |
| Neo | neomycin (resistance gene) |
| NGF | nerve growth factor |
| P | postnatal day |
| PDGF | platelet-derived growth factor |
| PDGFR | PDGF receptor |
| PI3K | phosphatidyl inositol 3' kinase |
| PLCγ | phospholipase C-gamma |
| PlGF | placenta growth factor |
| Ras-GAP | GTPase-activating protein for Ras |
| RET | receptor for GDNF |
| RTK | receptor tyrosine kinase |
| SH2 | src homology 2 domain |
| SHP2 | SH2 tyrosine phosphatase 2 |
| Sos1 | son of sevenless homologue 1 |
| SPARC | secreted protein, acidic, and rich in cysteine |
| STAT | signal transducers and activators of transcription |
| TGFβ | transforming growth factor beta |
| VEGF | vascular endothelial growth factor |

# Introduction

*"There is no rest for the messenger til the message is delivered"*

Joseph Conrad, The Rescue (1920)

## *Protein kinases - major information bearing molecules*

In this thesis, the developmental role of two members of the Platelet-Derived Growth Factor (PDGF) family, PDGF-A and -C, has been studied by the creation of loss-of-function mutations through gene targeting in mice.

The action of PDGF is mediated via two receptor tyrosine kinases, members of the larger gene superfamily of protein kinases. In an attempt to place PDGF within its evolutionary context, one may ask: what is the function of protein kinases in general?

Protein phosphorylation (and dephosphorylation) is the most prevalent reversible covalent modification of proteins in the human cell, and this mechanism provides a cellular infrastructure for the sensing of extracellular signals and the coordination of intracellular events such as transcription, metabolism, proliferation, cytoskeletal rearrangement, migration, differentiation and apoptosis.

While dephosphorylation is performed by phosphatases, protein kinases phosphorylate proteins by transferring phosphoryl groups from ATP to specific amino acid residues.

For a long time, protein kinases where presumed to exist exclusively in eukaryotic cells, but recent biomedical research including the near-complete sequencing of several genomes has shown that the picture is more complex and

that protein phosphorylation is a major mechanism for transducing information in all living organisms (Kennelly and Potts, 1996). However, clear trends in the distribution of kinase subgroups can be seen, which imply divergent functions.

In bacteria, the most common protein kinases are protein-histidine kinases, while the Hanks-type (formerly called "eukaryotic") protein kinases can be subdivided according to their ability to phosphorylate either serine/threonine or tyrosine residues. Hanks-type protein kinases are uncommon in prokaryotes, but represent one of the largest protein superfamilies in mammals, constituting about 1.7% of all human genes. In total, 518 protein kinase genes have been identified in the human genome (Manning et al., 2002b).

| Group | Yeast kinases | Fly kinases | Worm kinases | Human kinases |
|---|---|---|---|---|
| Serine/Threonine kinase | 115 | 168 | 302 | 340 |
| Tyrosine kinase | 0 | 32 | 90 | 90 |
| Tyrosine kinase-like | 0 | 17 | 15 | 43 |
| RGC & Atypical | 15 | 23 | 47 | 45 |
| **Total** | **130** | **240** | **454** | **518** |

**Table 1.** Protein kinase distribution by major groups. Modified from (Manning et al., 2002b).

Interestingly, when comparing the distribution of kinase subgroups in different eukaryotes, it is evident that although serine/threonine protein kinases are abundant already in primitive organisms such as brewer's yeast (*Saccharomyces cerevisiae*), tyrosine kinases seem to be largely restricted to multicellular organisms (Table 1).

Tyrosine kinases, in turn, form two major groups due to their subcellular location, cytoplasmic and receptor tyrosine kinases (RTKs) (Fig. 1A). Given the assumption that tyrosine kinase signaling is important for intercellular communication in the developing multicellular organism, RTKs are especially interesting since they possess an extracellular domain that can respond to external stimuli, in addition to an intracellular tyrosine kinase domain (Fig. 1B).

Indeed, until recently RTKs were presumed to exist only in metazoans, but now the first receptor tyrosine kinase in a choanoflagellate, a unicellular organism suspected to be closely related to animals, has been identified (King and Carroll, 2001). This suggests that the evolution of receptor tyrosine kinases may have been a prerequisite for the emergence of the animal kingdom, with the increased coordination of cellular events that is necessary when multiple cells have to harmonize their collective destinies in the building of tissues and organs.

There is now ample evidence that cell signaling by RTKs plays an important role in the control of most fundamental cellular processes in the metazoans (reviewed in Hunter, 2000; Schlessinger, 2000).

## Altered protein tyrosine kinase activity and human disease

Tyrosine kinases are interesting not only from the viewpoint of developmental biology, but also highly relevant from a medical perspective. If tyrosine kinases are fundamental for normal cell signaling, what happens when those signals are disturbed?

In principle, mutations in TKs can lead to either diminished or increased activation of downstream signaling pathways.

Inactivation of TKs has been demonstrated to be a causative factor in several developmental disorders, for instance severe combined immunodeficiency disease

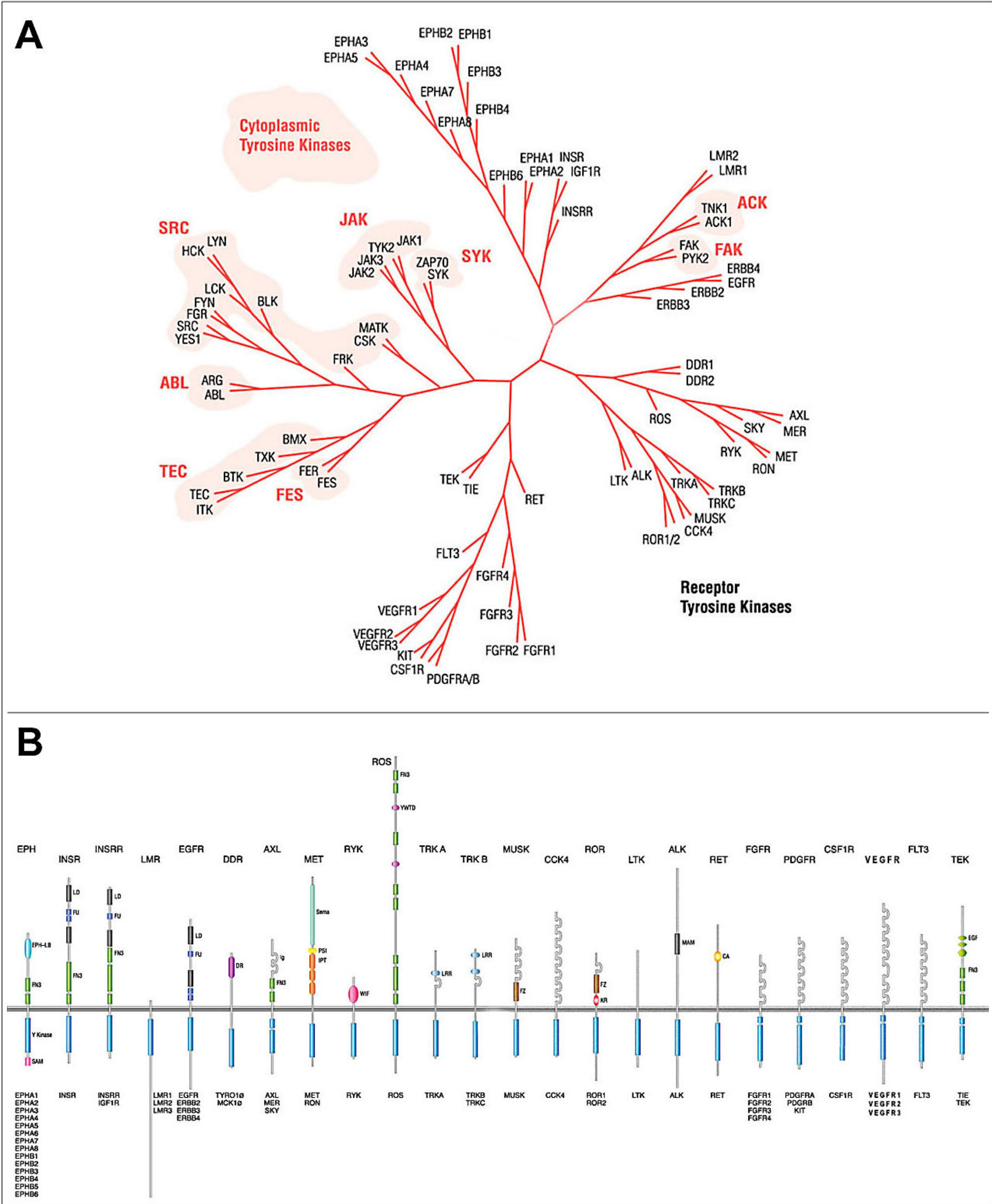


**Figure 1.** The human tyrosine kinases. **A**) Phylogenetic tree of cytoplasmic and receptor tyrosine kinases. **B**) Structure of receptor tyrosine kinases. Modified with permission from www.cellsignal.com

(mutations in JAK3 and ZAP70), retinal dystrophy (MER), hereditary lymphedema (VEGFR3), hereditary agammaglobulinemia (BTK) and Hirschsprung's disease (RET) (reviewed in Hunter, 2002). New drugs targeted to potentiate TK signaling may provide relief for these and other disorders.

Tissue regeneration is another area in which activation of primarily RTKs could be of major importance, by inducing resting progenitor cells to proliferate. Clinical trials with glial derived neurotrophic factor (GDNF) treatment for Parkinson's and nerve growth factor (NGF) for Alzheimer's diseases are under way (Maimone et al., 2001). Therapeutic angiogenesis by vascular endothelial growth factor (VEGF) gene transfer shows promising results on peripheral arterial disease and ischemic heart disease (Morishita, 2002), even if combination therapy with other factors, such as PDGF-BB, are likely to be necessary to ensure long-term functionality of the newly formed blood vessels (Blau and Banfi, 2001).

The GDNF receptor tyrosine kinase RET is a good example of the duality of TK dysregulation. As mentioned above, inactivation of RET causes Hirschsprung's disease, a congenital absence of enteric neurons in the hindgut. Germline mutations that lead to constitutive activation of RET, on the other hand, is the cause for multiple endocrine neoplasia type 2 (MEN 2), an inherited cancer syndrome (Jhiang, 2000).

Deregulated (autonomous) cell growth is a hallmark of all benign and malignant neoplasms. In 1980, it was demonstrated that v-src, the protein responsible for malignant transformation in Rous sarcoma virus, was a tyrosine kinase (Collett et al., 1980; Hunter and Sefton, 1980), and in 1983 another viral oncogene, v-sis, was shown to be highly homologous to a known mitogen, the PDGF receptor tyrosine kinase ligand PDGF-B (Devare et al., 1983;

Doolittle et al., 1983; Waterfield et al., 1983). This historical finding gave rise to the idea that so called "oncogenes" in truth were mutated versions of normal growth-promoting genes. Further support for this hypothesis came when it was discovered that a constitutively active truncated EGF-receptor gene, v-erbB, was responsible for the transforming ability of the avian erythroblastosis virus (Downward et al., 1984; Ullrich et al., 1984).

Today, it is a well-established dogma that cancer occurs when mutations in the genome of a cell lead to a selective growth advantage, and more than 30 recessive tumor suppressor genes and 100 dominant oncogenes have been identified (Futreal et al., 2001). Tyrosine kinases form the largest group of dominant oncogenes with structural homology, and at least 45 group members have been implied in human cancers (reviewed in Blume-Jensen and Hunter, 2001).

From this perspective, it is not surprising that academic scientists and pharmaceutical companies have identified protein kinases as major drug targets. A landmark event in this work occurred in 2001, when the first specific protein-kinase inhibitor, imatinib mesylate (Glivec®) was approved for clinical use. Imatinib was developed to target the mutated Abelson tyrosine kinase (BCR-ABL) found in Philadelphia-chromosome positive chronic myelogenous leukemia (CML), and clinical trials demonstrated surprisingly positive results, especially in late chronic phase CML (Kantarjian et al., 2002).

Imatinib is an ATP competitive inhibitor, and in addition to the effect on ABL, it also inhibits c-KIT and the PDGF receptors. c-KIT (and PDGF receptor alpha) mutations are causative factors in gastrointestinal stromal tumors (GIST), and rearrangement of the PDGF beta receptor by fusion with the TEL gene is responsible for a subgroup of myeloproliferative diseases. Preliminary studies

indicate that imatinib is efficient also in treating these disorders (see Capdeville et al., 2002, for a review on the development and clinical trials of imatinib mesylate).

Imatinib was the first specific protein-kinase inhibitor to be developed, but it certainly will not be the last. In fact, protein kinases have now become the second most important group of drug targets after G-protein-coupled receptors. While most drugs are aimed at various forms of cancer, other interesting therapeutic areas exist and protein-kinase inhibitors may prove to be useful immunosuppressants, as well as anti-fibrotic and anti-inflammatory drugs (reviewed in Cohen, 2002).

To summarize, modulation of protein kinase activity will likely emerge as an important clinical paradigm in the following decades, with a range of drugs available for the treatment of cancers and other diseases. In order to rationally develop efficient compounds, and use them with good clinical results, we need to increase our understanding of the fundamental biology of protein kinase signaling. The focus of this thesis - studies on the developmental role of platelet-derived growth factors - is one such example.

## Platelet-derived growth factors - secreted molecules that activate two receptor tyrosine kinases

> *"I hold it probable that in the germ cells there exist fine internal differences which predetermine the subsequent transformation to a determinant substance; not differences which are mere potencies present in the germ cells, but actual material differences so fine that we have not as yet been able to demonstrate them."*
>
> Rudolf Virchow, Die Cellularpathologie (1858)

The history of platelet-derived growth factor (PDGF) dates back about thirty years, when Balk observed that serum was more efficient than plasma in promoting proliferation of fibroblasts *in vitro* (Balk, 1971). Subsequently, this growth promoting activity was located to platelets (Kohler and Lipton, 1974; Ross et al., 1974), and around 1980, PDGF was purified from human thrombocytes (Antoniades et al., 1979; Heldin et al., 1979; Raines and Ross, 1982). Since this discovery, several reports have shown that PDGF by no means is restricted to the platelets, but rather is produced in a large number of different cell types.

The structural and functional properties of PDGF and PDGF receptors in health and disease have been extensively studied over the last two decades (for a detailed review, see Heldin and Westermark, 1999). It is not my intention to give a comprehensive summary here, but rather focus on aspects of PDGF biology that are important as a background to the present study.

## PDGF isoforms

Platelet-derived growth factor is a family of five identified disulfide-linked homo- and heterodimers (PDGF-AA, -BB, -AB, -CC and -DD) that are encoded by four genes (*PDGF A-D*). PDGF-C and -D were discovered only recently (Paper III; Bergsten et al., 2001; Gilbertson et al., 2001; LaRochelle et al., 2001), so most studies have been performed on PDGF-A and -B.

PDGF-A and -B are synthesized as precursor molecules that undergo proteolytic processing. In the mature parts of approximately 100 amino acid residues, eight cysteines are perfectly conserved (Betsholtz et al., 1986). The C- and -D chains of PDGF show the same eight cysteine motif, but three additional amino acids are inserted in the most conserved region (Paper III; Bergsten et al., 2001; Gilbertson et al., 2001; Heldin et al., 2002; LaRochelle et al., 2001).

A similar spacing between cysteine residues is seen also in the vascular endothelial growth factor (VEGF) family (reviewed in (Veikkola and Alitalo, 1999), and this suggests that the PDGFs and VEGFs have evolved by evolutionary divergence from a common ancestral gene (Fig. 2; Bergsten et al., 2001).

The crystal structure of PDGF-BB has been determined (Oefner et al., 1992), and this has revealed the involvement of the conserved cysteines in inter- and intrachain disulfide bonds. The second and fourth cysteines are responsible for interchain dimerization, which arranges the dimer in an antiparallell manner.
The remaining six residues are engaged in a tight cystine knot structure in the PDGF monomer. This simple yet robust structure places PDGF/VEGF in a larger Cystine Knot superfamily, since a similar knot exists also in the nerve growth factor (NGF), transforming growth factor beta (TGFβ) and glycoprotein

hormone (GPH) families. Apart from the cystine knot, these protein families lack sequence similarity, and it is possible that this represents an example of evolutionary convergence (rather than divergence), since the formation of a cystine knot is an efficient mechanism to create loops in a polypeptide chain (Hearn and Gomme, 2000).

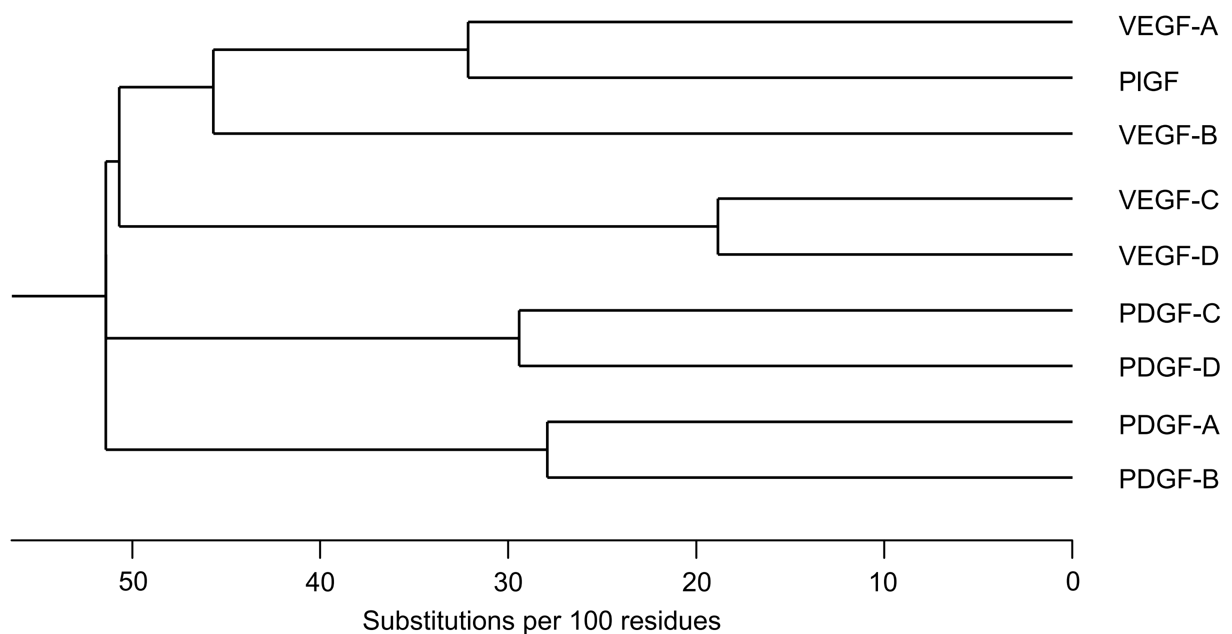


**Figure 2.** Evolutionary relationships in the PDGF / VEGF family.

The three loops in PDGF-B connect two pairs of β-strands. In PDGF-BB, loop one and three from one monomer, and loop two from the other are exposed on each side of the dimer (Oefner et al., 1992). These loops in PDGF-A and -B are the receptor binding epitopes (Andersson et al., 1995; Clements et al., 1991; Fenstermaker et al., 1993; LaRochelle et al., 1992; Ostman et al., 1993). While

it is likely that the corresponding sequences in PDGF-C and -D have the same function, this remains to be shown.

The genomic organization of the PDGF family genes is depicted in Fig. 3 (modified from Uutela et al., 2001).

The genes for PDGF-A and -B are located on human chromosomes 7 and 22 (Betsholtz et al., 1986; Dalla-Favera et al., 1982). PDGF-A and -B are closely structurally related, showing approximately 60% amino acid identity in the mature proteins, encoded mainly by exons 4 and 5 (Betsholtz et al., 1986). Exon 1 encodes a hydrophobic signal sequence that together with exons 2 and 3 is removed during intracellular processing. Exon 7 is largely noncoding, and exon 6 in both PDGF-A and -B encodes a basic carboxyterminal sequence. In PDGF-B, the exon6-encoded sequence may be proteolytically removed, whereas PDGF-A exists in two splice variants, with or without this basic sequence (further discussed under "Extracellular retention").

The genes for PDGF-C and -D, located on human chromosomes 4 and 11 (Uutela et al., 2001), show only about 20% sequence identity with PDGF-A and -B in the PDGF core domain, but 50% amino acid identity with each other (Bergsten et al., 2001). The PDGF core domain is encoded by the fifth and sixth exons in PDGF-C, and the sixth and seventh exons in PDGF-D. As in the "old" PDGFs, the first exon of PDGF-C and -D appears to represent a signal sequence. The second and third exons of PDGF-C and -D contain a sequence that is absent in all other PDGFs and VEGFs, but show similarity with the CUB-domain found in complement subcomponents C1r/C1s, urchin EGF-like protein and bone morphogenetic protein-1 (Bork, 1991). The function of this motif is unknown. It may have a regulatory function since it needs to be cleaved

off after secretion to render PDGF-CC and -DD active (Paper III; Bergsten et al., 2001; Gilbertson et al., 2001; LaRochelle et al., 2001). Putative proteolytic cleavage sites are marked with arrows in Fig. 3.

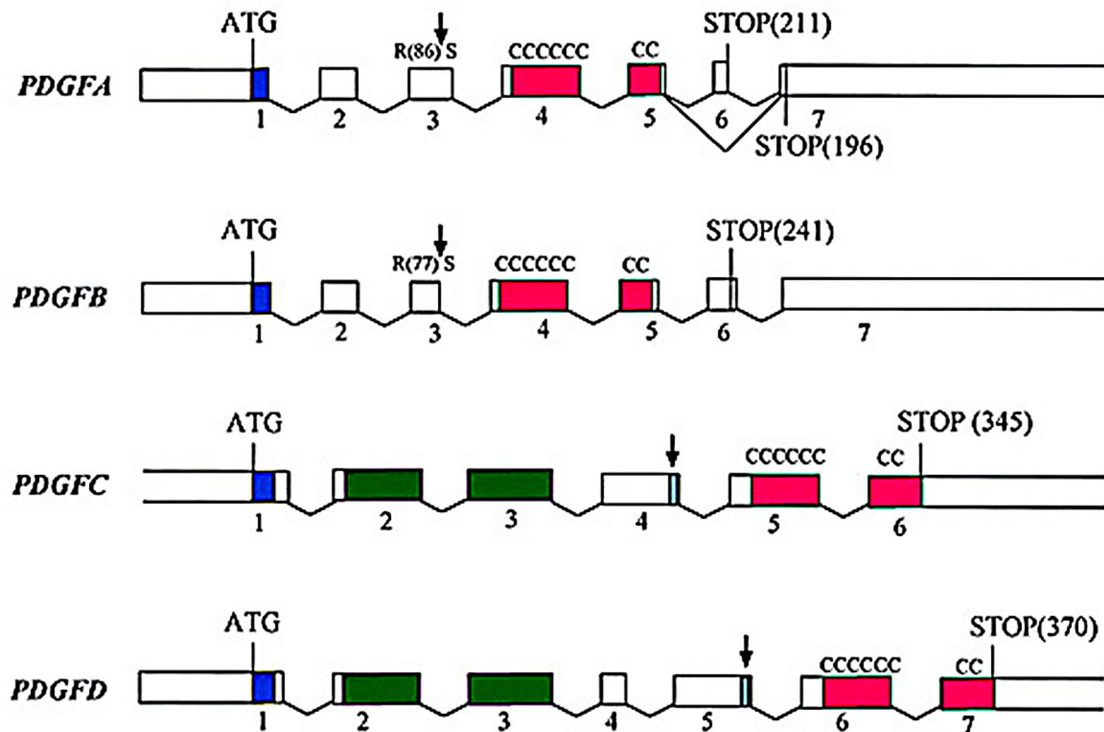


**Figure 3.** Genomic organization of PDGF isoforms. Modified from (Uutela et al., 2001).

Platelets and cells that express both PDGF-A and -B contain PDGF-AA, -BB and -AB dimers, with the major part being the PDGF-AB heterodimer (Hammacher et al., 1988a; Hammacher et al., 1988b; Hart et al., 1990). This indicates that the assembly of these PDGF dimers is a stochastic process. PDGF-C and -D, on the other hand, have not been reported to form heterodimers.

## Extracellular retention of PDGF isoforms

The sixth exon in both PDGF-A and -B encodes a highly basic stretch of amino acids that was originally identified as a nuclear targeting signal (Lee et al., 1987; Maher et al., 1989), but later studies have shown that the probable biological function of this motif is to confer binding to extracellular matrix structures (Kelly et al., 1993; LaRochelle et al., 1991; Pollock and Richardson, 1992; Raines and Ross, 1992). While PDGF has been reported to bind to several extracellular matrix molecules such as SPARC (Raines et al., 1992), collagens (Somasundaram and Schuppan, 1996) and thrombospondin (Hogg et al., 1997), it is likely that the most important binding is that to negatively charged groups in heparan sulfate proteoglycans (Andersson et al., 1994; Feyzi et al., 1997; Lustig et al., 1996).

PDGF-A exists in two isoforms due to alternative splicing of the sixth exon, thus producing one short, freely diffusible form of PDGF-A, and one long, matrix associated form (Betsholtz et al., 1986; Bonthron et al., 1988; Rorsman et al., 1988). The short form of PDGF-A almost always predominates, but the long form has been found in most cell lines and tissues expressing PDGF (Matoskova et al., 1989). The significance of this alternate transcript is illustrated by the fact that the basic stretch of amino acids in PDGF-A is highly evolutionary conserved between *Xenopus Laevi* (Mercola et al., 1988), mouse and man (Rorsman and Betsholtz, 1992), and that it is highly similar not only to the corresponding sequence in PDGF-B, but also to a differentially spliced exon in Vascular Endothelial Growth Factor (VEGF) (Betsholtz et al., 1990) and Placenta Growth Factor (PlGF) (Maglione et al., 1993).

PDGF-C and -D do not contain the C-terminal retention motif found in PDGF-A and -B. However, CUB-domains can be involved in protein-protein and protein-carbohydrate interactions (Kristiansen et al., 1999; Nakamura et al., 1998), and we have hypothesized that this motif may be important for binding to the pericellular matrix (Paper III).

## *PDGF receptors*

PDGFs exert their effects by binding to two structurally related receptor tyrosine kinases, PDGFR-α and PDGFR-β, which are transmembrane proteins with molecular sizes of 170 and 180 kDa after glycosylation (Claesson-Welsh et al., 1989; Matsui et al., 1989a; Yarden et al., 1986).

The extracellular part of each receptor contains five immunoglobulin-like (Ig) domains. An intracellular tyrosine kinase domain with a characteristic insertion puts the PDGF receptors in a larger family of "split-kinase RTKs" (Manning et al., 2002a), together with KIT, CSF1R/FMS and FLT3 that all have five Ig-domains. The closely related VEGFR1-3 contain seven Ig-domains. The human genome sequence reveals that all these eight genes map to three of the four paraHOX clusters, and probably derive from local cisduplications and transduplications of the single ancestral paraHOX locus (Manning et al., 2002b). Finally, FGFR1-4 are more distantly related split-kinase RTKs with three extracellular Ig-domains.

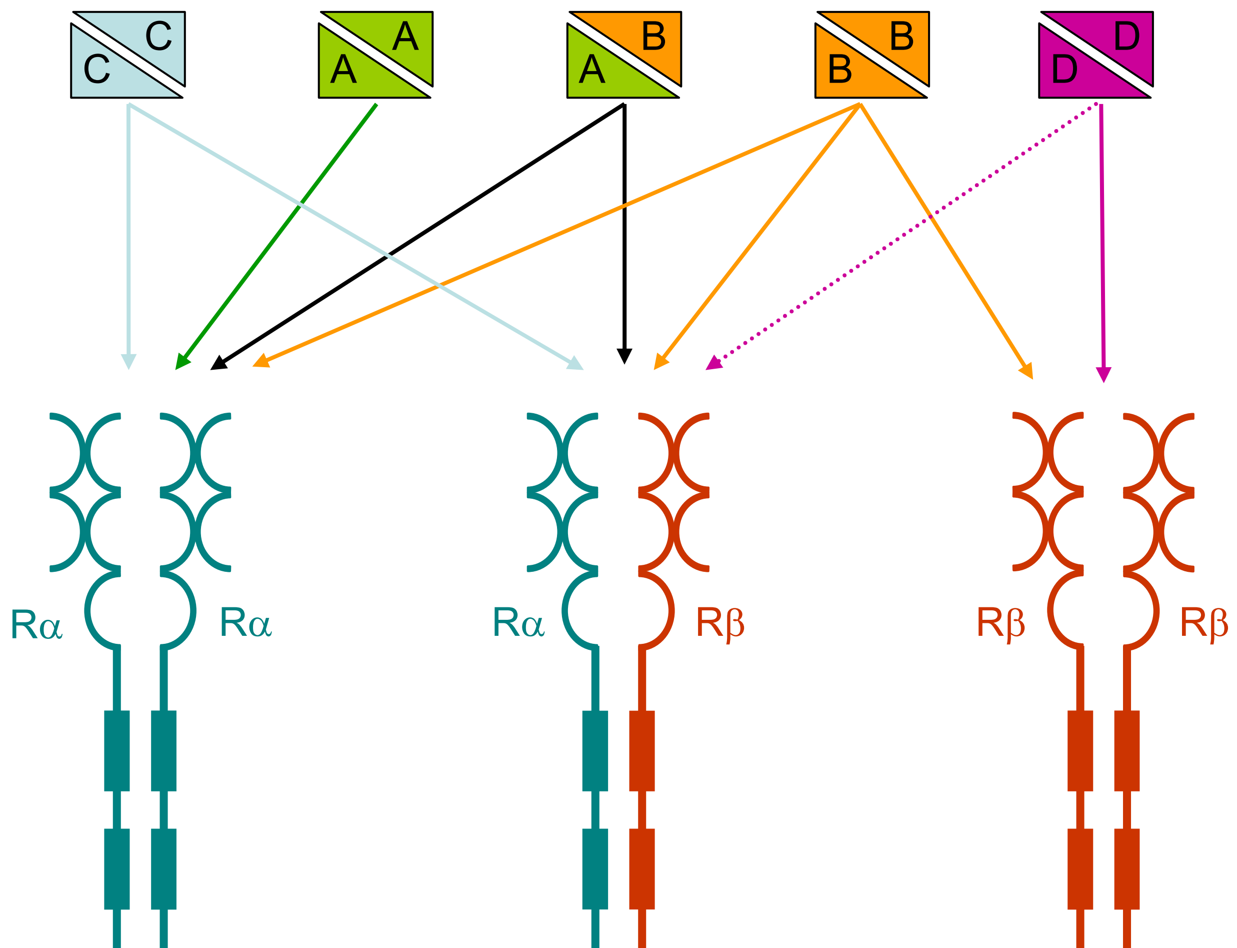


**Figure 4.** PDGF ligand / receptor specificities *in vitro*.

The PDGF receptors, as all other split-kinase RTKs, are monomers in the cell membrane. Because of the dimeric nature of the PDGF ligands, they bind two receptors simultaneously, which leads to receptor dimerization. PDGF-A, -B and -C are high affinity ligands for PDGFRα, while PDGF-B and -D are PDGFRβ agonists *in vitro* (Paper III; Bergsten et al., 2001; Matsui et al., 1989a). Somewhat surprisingly, both PDGF-CC and PDGF-DD seem to be able to induce the PDGFRαβ heterodimer (Gilbertson et al., 2001; LaRochelle et al., 2001), even though they only bind to one of the monomers with high affinity. This leads to the ligand/receptor specificities portrayed in Fig.4.

Activation of PDGF receptors induces a variety of cellular responses, such as proliferation, chemotaxis and antiapoptotic signals (reviewed in Heldin et al., 1998; Heldin and Westermark, 1999). The different receptor dimers transmit partially overlapping, but also partly specific signals. Whereas both receptors direct cytoskeletal rearrangements, only PDGFRβ mediates formation of circular actin ruffles at the dorsal surface of porcine aortic endothelial cells (Eriksson et al., 1992). Additionally, PDGFRβ can promote chemotaxis in certain cell types, such as vascular smooth muscle cells. PDGFRα inhibits migration in these cell types, but can stimulate chemotaxis in other cells (Hosang et al., 1989; Koyama et al., 1992; Matsui et al., 1989b; Osornio-Vargas et al., 1996; Shure et al., 1992; Siegbahn et al., 1990). PDGFRβ also promotes release of intracellular calcium more efficiently than PDGFRα (Diliberto et al., 1992).

From these *in vitro* studies, it would thus seem that PDGFRβ is able to direct certain cellular functions that the PDGFα receptor cannot. Furthermore, PDGF-AB gives a stronger proliferative and migratory effect on cells that express both PDGF receptors, indicating that the PDGFRαβ heterodimer may possess unique signaling properties (Heidaran et al., 1991; Rupp et al., 1994).

Ligand-induced dimerization of the PDGF receptors allows for autophosphorylation of tyrosine residues in *trans* between the two receptor monomers. The three outermost Ig loops of the receptors are responsible for ligand binding, with loop two being the most important. The dimeric complex is further stabilized by direct interaction between the receptors (reviewed in Heldin et al., 1998).

Following autophosphorylation, downstream signaling molecules will attach to phosphotyrosines in a sequence-specific manner. In the PDGF receptors this is accomplished by Src homology 2 (SH2) domain-containing proteins, including phosphatidylinositol 3' kinase (PI3K), phospholipase C-γ (PLC-γ), src family tyrosine kinases, signal transducers and activators of transcription (Stats), SHP2 tyrosine phosphatase, and adaptor molecules, for instance Grb2/Sos1 and Nck.

Most such molecules can bind to both PDGF receptors, but there are exceptions. The GTPase-activating protein for Ras (Ras-GAP) only binds to PDGFRβ, and the adaptor molecule Crk is specific for PDGFRα (Heldin et al., 1998).

The binding of more than 10 SH2 domain-containing signal transduction molecules to the receptor leads to the activation of a complex network of interacting intracellular signaling pathways. A great effort has been put down in the last decades to map the individual signal transduction conduits in detail, with the assumption being that intrinsic differences in the signaling cascades would lead to specific cellular responses (reviewed in Hunter, 2000; Schlessinger, 2000).

Later studies have challenged this hypothesis, and indicated that activation of RTKs rather provides a general "go" signal that is interpreted differently in diverse cellular contexts. For instance, mutations of distinct tyrosine residues in PDGFRβ demonstrated that various pathways have broadly overlapping effects on the induction of immediate early genes in fibroblasts, and even that FGF and PDGF activation of the same celltype leads to highly similar responses (Fambrough et al., 1999). Furthermore, three studies on cell-fate decision in *Drosophila* showed that marker gene expression was dependent not on specific

intracellular signals, but instead by integration of a generic RTK signal with cell-specific transcription factors (Simon, 2000).

The specificity of PDGF receptor signaling pathways was recently examined by gene targeting in mice (Heuchel et al., 1999; Klinghoffer et al., 2002; Klinghoffer et al., 2001; Tallquist et al., 2000a). These studies, which will be discussed further in the next chapter, suggest both overlapping and specific functions for RTK-activated signaling pathways.

## *Expression of PDGF and PDGF receptors*

Expression of platelet-derived growth factor and PDGF receptors has been detected in a wide variety of cell types (reviewed in Heldin and Westermark, 1999). Many of those studies should be interpreted with caution, however, because the fact that a cell type is able to produce a factor when in culture not necessarily implies an important physiological function.

In adult humans, PDGF is expressed at very low levels in most tissues (Raines and Ross, 1993), and it is possible that the role of PDGF in most aspects of normal adult physiology is negligible. An exception to this may be the central nervous system. Here, a neuroregulatory function for PDGF has been suggested, because high levels of PDGF and PDGF receptor expression has been detected in the normal adult mammalian brain (Sasahara et al., 1991; Yeh et al., 1991). The highest levels of PDGF-B and PDGFR$\alpha$ were found in the olfactory bulb, an interesting observation since replacement of olfactory neurons is known to take place throughout adult life (Lee et al., 1990; Sasahara et al., 1992).

PDGF might also have a role in the regulation of interstitial fluid pressure (Rodt et al., 1996), possibly through PI3K mediated activation of the collagen-binding integrin $\alpha 2\beta 1$ (Heuchel et al., 1999).
Imatinib is a potent inhibitor of PDGFR signaling that has recently been used in clinical trials as a treatment for chronic myelogenous leukemia. The relatively mild adverse effects that have been reported when using this drug support the notion that PDGF signaling is of less importance in adult physiology. Interestingly, one of the more common side effects is oedema, which could be due to loss of PDGF-dependent regulation of interstitial fluid pressure (Esmaeli et al., 2002).

Whereas the importance of PDGF in adult physiology is debatable, a clearer function is seen in pathophysiological situations. As a common theme, PDGF upregulation is coupled to fibroproliferative responses. Examples of this include wound healing, atherosclerosis, glomerulonephritis, pulmonary fibrosis, rheumatoid arthritis and malignant tumors. In most cases, PDGF from platelets, macrophages, epithelial and endothelial cells acts as a paracrine growth factor for PDGF receptor positive fibroblasts and smooth muscle cells. In certain tumors, however, autocrine PDGF receptor activation is probably a rate-limiting event (reviewed in Heldin and Westermark, 1999).

PDGF is also essential for embryonic development. In the early preimplantation embryo, PDGF-A and PDGFR$\alpha$ are expressed in the same cells, suggesting autocrine signaling. Expression studies in later developmental stages have demonstrated that high levels of the PDGF receptors mainly are found in mesenchymal and certain neuroectodermal cells, while the ligands are present in adjacent epithelial and endothelial cells. Notable exceptions to these patterns are

the presence of PDGF-A and -C in the myotome and developing muscle, and of both PDGF-A, -B and possibly -C in neurons of the central nervous system (Aase et al., 2002; Ataliotis and Mercola, 1997; Ding et al., 2000).

The functional significance of PDGF expression patterns in mouse development will be discussed further in the next chapter.

# Developmental functions of PDGF - lessons from gene targeting in mice

> *"The interpretation, and indeed power, of a screen is observer-dependent. There are many possible thematic interpretations of a screen, all woven together as in an Escher print or Bach canon, and none is most 'correct', although some can provide more experimentally useful predictions than others. Even the defined phenotype for any given mutation reflects but one part of the function of the gene, the one that happens to be most prominent or fall in the field of focus of that particular scientist. Rashomon-like, what is deemed distinctive and important by one observer may go unnoticed by another."*
>
> Mark C. Fishman, The Genomic Cosmos,
> Nature 410, 1033 (2001)

## *Gene targeting - methodological aspects*

Our knowledge of the identity, structure and function of proteins in physiological and pathophysiological processes is being driven in an unprecedented rate by the application of modern molecular and cell biology techniques. While a given gene's function can be predicted, to some extent, from sequence homology analysis, and descriptive studies may show clear chronological and spatial evidence of the association of a protein with a particular process, it is important to consider that this remains simply an association until the target protein is manipulated and the associated process critically altered.

In their ground-breaking paper from 1944, in which DNA for the first time was shown to cause genetic transformation, Avery and co-authors state: "Biologists have long attempted by chemical means to induce in higher organisms predictable and specific changes which thereafter could be transmitted in series as hereditary characters." (Avery et al., 1944). This dream came true 43 years later, when work from the groups of Smithies and Capecchi for the first time resulted in targeted mutation of a mouse gene at its chromosomal location (Doetschman et al., 1987; Thomas and Capecchi, 1987).

Gene targeting, or homologous recombination in embryonic stem cells (ES cells), is now a routine technique that is used to modify the mouse genome at any given locus. In addition to the first gene "knock-out" studies, in which a gene was completely silenced, it is now possible to create inducible and tissue-specific alterations, with mutations ranging from a single base pair to chromosomal rearrangements (for reviews on gene targeting and inducible systems, see Muller, 1999; Nagy, 2000).

So far, more than 10% of the ~30000 mouse genes have been knocked out. This has revolutionized the field of murine genetics and allowed the analysis of diverse aspects of gene function in the context of the whole animal.

From a medical point of view, knock-out mice are interesting as models for human disease. A recent retrospective study highlights that mouse loss-of-function phenotypes indeed can correlate well with drug efficacy in humans:

The 100 best-selling drugs modulate 43 known human drug targets. The corresponding mouse genes have been knocked out in 34 instances. Out of these, 29 show a direct correlation between the resulting phenotype and the therapeutic effect of the drug (Zambrowicz and Sands, 2003). It seems obvious that, with

well-defined phenotypic screens, prospective gene targeting studies are likely to provide an important source of targets for future drug development.

Some potential problems in the generation and analysis of knock-out mice should be considered.

Is the knock-out really a knock-out? If coding segments of the gene still exist on the chromosome, truncated versions of the protein could be produced. Even if smaller deletions target structurally important parts of the gene, and/or induce frame shifts, a mutant polypeptide could still affect other physiological processes than the intended. This problem can be avoided by removal of all coding exons, but large deletions may also lead to unwanted loss of regulatory regions for other genes, or even deletion of entire unidentified genes.

An important consideration is that the introduction of a selection marker, usually a neomycin resistance gene (Neo) under the phosphoglycerate kinase (PGK) promoter, could affect gene expression. Several reports have shown that the strong promoter in the selection cassette interferes with the neighboring gene, creating a hypomorphic allele (Muller, 1999). There are exceptions, however, and each case should be judged individually. In one report, introduction of a Neo gene in the mouse Jλ1 region lead to a drastic increase in Jλ1 transcription. When the Neo cassette was enzymatically removed with Cre recombinase, the remaining short loxP sequence was shown to act as a strong repressor of transcription, rendering a hypomorphic Jλ1 allele (Sun and Storb, 2001).

The interpretation of a phenotype is often complicated by functional redundancies at several levels. Knock-out mice may display diverse phenotypes

when studied on different genetic backgrounds. The results are often clearer when mutations are maintained in congenic strains rather than outbred mice, since the effect of multiple modifier loci can be controlled. On the other hand, important phenotypes may be absent in certain strains, and the best strategy is often to place a mutation in the context of several genetic backgrounds with controlled back-crossing.

Compensation within a gene family is also a common phenomenon. For example, PDGF-A, -B, -C (and to some extent -D) can all activate PDGFRα. On sites where the ligands are co-expressed, it is conceivable that necessary levels of receptor activation can be maintained even after inactivation of one PDGF gene.

## *Platelet-derived growth factor mutants*

Mice harboring targeted deletions of all PDGF ligands and receptors except PDGF-D have been generated (Paper I; Paper IV; Leveen et al., 1994; Soriano, 1994; Soriano, 1997), and all heterozygous mutants are healthy and fertile. In the following, unless explicitly stated otherwise, only the phenotypes of homozygous mutant animals will be described.

### Complex phenotypic alterations in PDGF-A knockout mice

PDGF-A is essential for postnatal survival. In a mixed genetic background, PDGF-A knockout (PDGF-$A^{-/-}$) mice either die before E10 or survive until birth (Paper I).

The early lethal phenotype appears to be more common on a C57Bl/B6 background, and consists of growth retardation with increased apoptosis in mesenchymal structures, branchial arch hypotrophy, mild myotome patterning defects and an undulated neural tube (M. Hellström, unpublished observations).

Postnatally surviving PDGF-A$^{-/-}$ mice die within the first few weeks and display several developmental defects. The probable cause of death is a complete lack of alveolar septation in the lungs with concomitant pneumothorax and atelektasis. The alveogenesis defect is coupled to reduced elastin deposition and a loss of α-smooth muscle actin (ASMA) positive interstitial cells, normally located at the tip of the alveolar septa (Paper I). These cells have been described as both "myofibroblasts" (Oldmixon et al., 2001) and "smooth muscle cells" (Kapanci et al., 1974), but there is a clear consensus about the fact that they are mesenchymal cells with a hefty contractile machinery.

At embryonic day 14.5 (E14.5), PDGF-A is expressed by the pulmonary epithelium, while PDGFRα expression is restricted to the adjacent mesenchyme. At E18.5, PDGF-A expression could no longer be detected, and PDGFRα positive cells were scattered in the lung mesenchyme. This scattered subpopulation is missing in PDGF-A deficient mice, and we propose that these cells are the precursors of alveolar myofibroblasts. Furthermore, it appears that the alveolar myofibroblast has a crucial role in alveolar formation and elastin deposition in the lung (Paper I).

In a follow-up study, Lindahl and co-workers attempted to find overlapping markers to prove that the scattered PDGFRα positive cells indeed are progenitors to the alveolar myofibroblasts. This failed, but they showed that these cells probably represent the same cell lineage as tropoelastin positive cells seen at postnatal day 4 (P4), after PDFGRα has been downregulated but before ASMA expression can be detected, and that the third phase of elastin deposition in the lungs was absent in PDGF-A$^{-/-}$ mice. Finally, the absence of the scattered PDGFRα positive population was attributed to lack of multiplication and distal spreading of PDGFRα positive cells between E16.5 and E17.5, probably because

of reduced proliferation and possibly migration. No increase in apoptosis was seen in PDGF-A negative lungs (Lindahl et al., 1997b).

PDGF-AA, expressed by neurons and glial cells, is a potent mitogen for PDGFRα positive O2A oligodendrocyte progenitors *in vitro*. In PDGF-A deficient spinal cords, the proliferation of O2A progenitors was severely reduced (Calver et al., 1998), and mutant mice displayed defective oligodendrogenesis with a dysmyelinating phenotype (Fruttiger et al., 1999).

By examining O2A progenitor cell number in wildtype mice, in transgenic animals overexpressing PDGF-A at various levels, and in heterozygote and homozygote PDGF-A knockout mutants, William Richardson and colleagues have provided some of the most compelling and beautiful arguments regarding the *in vivo* role of PDGF (van Heyningen et al., 2001). When cultured in saturating concentrations of PDGF-A, O2A progenitors in dissociated spinal cords show an acceleration of the cell cycle independent of their previous rate of division, suggesting that cell division normally slows down because of declining PDGF concentrations, and not by cell intrinsic mechanisms.

This hypothesis was further strengthened by the demonstration that O2A progenitor cell number is controlled by the supply of PDGF-A in a linear, unsaturable fashion, ranging from undetectable levels in PDGF-A knockout mice to 10-fold upregulation in quadruple transgenic animals. In the latter case, cell number was still increasing but the mice were not viable.

The authors argue that proliferating O2A cells limit their own proliferation, the mechanism being that the cells continue to multiply until their consumption of PDGF-A matches the paracrine supply of the growth factor (van Heyningen et al., 2001).

Even though the proliferation of O2A progenitors differ in the various mutants, the quantity of postmitotic oligodendrocytes is not affected in heterozygous PDGF-A knockouts or overexpressing transgenic mice. This indicates that while the number of O2A progenitors is controlled by PDGF-A, the number of mature oligodendrocytes is controlled by other mechanisms, probably axon-derived survival factors, since increased apoptosis of oligodendrocytes was observed in PDGF-A transgenic mice (Calver et al., 1998).

Three other described phenotypes in PDGF-A knockout mice draw attention to the theme of paracrine epithelial-mesenchymal signaling by PDGF-A over PDGFRα in mouse development.

Male PDGF-A deficient mice display spermatogenic arrest, likely secondary to a diminished number of Leydig cells and associated reduction of testosterone levels. In the developing testis, tubular epithelial cells express PDGF-A and interstitial mesenchymal cells, which are putative Leydig cell progenitors, express PDGFRα (Gnessi et al., 2000).

In the skin, PDGF-A is expressed in the developing hair follicle epithelium and epidermis, and PDGFRα expression is detected in the underlying mesenchyme. Consequently, PDGF-A deficient mice show mesenchymal defects in the dermis and abnormal hair development with misshapen hair follicles (Karlsson et al., 1999).

A similar expression pattern is seen in the developing gut, and PDGF-A knockouts display abnormal gastrointestinal development with dysmorphogenesis of villi that is most pronounced in the upper small intestine. PDGF-A signaling appears to drive the proliferation of PDGFRα positive progenitor cells that are important for the formation of a tight cluster of mesenchymal cells adjacent to the growing villus (Karlsson et al., 2000).

In both the skin and the gut, proliferation defects were demonstrated by reduced BrdU labeling of mesenchymal cells in PDGF-A negative mice. Signs of premature differentiation were also seen in the gut, in which ASMA expression was detected at earlier timepoints in the mutants than in wildtype littermates.

### The importance of extracellular retention of PDGF-A

As described in the previous chapter, PDGF-A exists in two isoforms due to alternative splicing of the sixth exon. When present, the exon-six-encoded sequence mediates binding to the pericellular matrix. We generated mice with a targeted deletion of the sixth exon splice acceptor in PDGF-A, so that only the short, freely diffusible form of PDGF-A was produced from the recombinant allele (Paper V).

PDGF-A retention deficient mice (PDGF-A*ret*) appear to be healthy and normal. Since we had reason to suspect that PDGF-C might compensate for loss of PDGF-A under certain conditions (see below), we bred PDGF-A*ret* mice against PDGF-C knockouts. Double mutants recapitulated the gastrointestinal phenotype found in PDGF-A negative mice. This was in agreement with the finding of relatively high expression levels of the long form of PDGF-A in the developing gut. Furthermore, the spina bifida sometimes seen in PDGF-C deficient mice (Paper IV) was severely aggravated by the addition of the PDGF-A*ret* allele, indicating that extracellular-matrix bound PDGF-A may contribute to neural arch formation.

Theoretically, retention of PDGF-A could be important for guided cell migration, for eliciting isoform-specific signals or for guaranteeing adequate local concentration of the growth factor. We hypothesize that the latter scenario is the most probable, and the observed phenotypes are the result of a functionally hypomorph situation (discussed in detail in Paper V).

### Mice deficient for PDGF-B display cardiac and vascular defects

PDGF-B deficient mice die between E16 and E18 due to vascular defects (Leveen et al., 1994). During mouse development, PDGF-B is expressed mainly by megakaryocytes and vascular endothelium (Lindahl et al., 1997a). All observed phenotypes in PDGF-B knockouts seem to be caused by defective recruitment of PDGFRβ positive vascular smooth muscle and pericyte progenitors.

Reduced proliferation and possibly migration of these cell populations in PDGF-B deficient mice leads to macroscopically apparent bleedings and edema, coupled to arterial SMC hypotrophy and reduced number of pericytes in smaller vessels. Pericytes are probably important for the mechanical stability of the microvessel wall, since ruptured capillary microaneurysms was a common phenomenon in the PDGF-B knockouts (Lindahl et al., 1997a).

In the kidney and the placenta, specialized pericytes fail to develop. PDGFRβ positive kidney mesangial cells are responsible for establishing the structural integrity of the glomerular tuft, and in PDGF-B knockouts these cells are not properly recruited, leading to the formation of a primitive balloon-like structure (Leveen et al., 1994; Lindahl et al., 1998). An analogous picture is seen in the developing placenta (Ohlsson et al., 1999).

Pericyte deficiency was most pronounced in brown adipose tissue, brain and heart, where cardiac muscle hypotrophy was evident. In other tissues, such as the skin and gastrointestinal tract, the number of pericytes was normal. These sites are obviously less dependent on PDGF-B mediated expansion of pericyte progenitors, either because other growth factors can substitute for PDGF-B, or possibly because intrinsic differences exist in a tissue's ability to form

new vascular SMC/pericyte progenitors from competent mesenchyme (Hellstrom et al., 1999).

### Cell-type-specific ablation of PDGF-B by the creation of chimeric mice and conditional gene targeting

To elucidate the physiological role of PDGF-B from different cellular sources, mice with cell-specific targeted deletions of PDGF-B have been generated by two different approaches.

First, using the Cre/loxP system, PDGF-B was inactivated in Tie1-expressing endothelium and Cam kinase-expressing neurons.

Depending on the efficiency of Cre-mediated recombination, mice with endothelium specific ablation of PDGF-B displayed all phenotypes observed in the PDGF-B knockout. This suggests that the endothelium is the physiologically relevant source of PDGF-B in mouse development. Substantial rescue of several of the phenotypes was seen postnatally. A possible explanation for this finding is that PDGF-B may be rate-limiting only during a certain developmental period, and that other factors then can compensate for the loss of PDGF-B (M. Bjarnegård, unpublished results). Since these mice are viable, it was possible to assess retinal angiogenesis. Here, the failure to recruit pericytes lead to proliferative retinopathy, an interesting observation since analogies can be drawn to ophtalmological complications in human diabetes (Enge et al., 2002).

Studies on mice with neuron-specific deletion of PDGF-B failed to demonstrate any abnormalities in CNS development or astroglial response to injury, questioning the importance of PDGF-B expression in this cell population (Enge et al., 2003).

Using another approach, hematopoietic PDGF-B deletion was accomplished by lethally irradiating mice, followed by transplantation with PDGF-B deficient fetal liver cells that successfully repopulated the bone marrow. Chimeric mice lack PDGF-B in all hematopoietic lineages, including platelets and macrophages, but have a normal vascular and hematologic phenotype (Kaminski et al., 2001). Two follow-up studies on these hematopoietic chimeras challenge old beliefs regarding PDGF's role in pathophysiological situations:

In wound healing, PDGF-B from hematopoietic sources was not required for granulation tissue formation, and surprisingly, vascularization was enhanced in the chimeric situation (Buetow et al., 2001).

In a model of atherosclerosis, hematopoietic chimeras were bred against ApoE deficient mice. This study showed that smooth muscle cell accumulation and fibrous cap formation in atherosclerotic lesions was delayed, but not prevented, in the absence of hematopoietic expression of PDGF-B (Kozaki et al., 2002).

### PDGF-C negative mice show cleft palate and spina bifida

We recently generated mice deficient for PDGF-C (Paper IV). In an outbred genetic background, PDGF-C knockouts are fertile and appear healthy. In 129S1/Sv mice, however, the absence of PDGF-C leads to early postnatal lethality. This is probably due to a complete cleft secondary palate, and an associated inability to suckle. PDGF-C is expressed by the medial edge epithelium in the developing palate, whilst PDGFRα expression can be detected in the underlying mesenchyme. It is likely that PDGF-C drives proliferation of this mesenchymal population, but that remains to be proven.

A proportion of PDGF-C negative mice also display mild spina bifida. In general, spina bifida is associated with improper proliferation and differentiation of the mesodermal cartilage precursor cells that form the neural arches, but this phenotype is poorly characterized in the PDGF-C knockouts.

## PDGF receptor mutants

### Patterning of the somites and development of neural-crest-derived mesenchyme is dependent on PDGFRα

Mice carrying a targeted deletion of PDGFRα die between E8 and E16. Early death seems to be loosely associated to C57Bl/B6 background, and the phenotype at this stage resembles the early picture seen in PDGF-A deficient mice (M. Hellström, personal communication and Soriano, 1997).

Older PDGFRα knockouts display incomplete cephalic closure and cardiovascular defects (Tallquist and Soriano, 2003), suggesting a role for PDGFRα in the development of neural-crest-derived mesenchyme. Skeletal abnormalities including fused ribs and vertebrae, bifurcation in ribs, incomplete development of the sternum, severe spina bifida and poor development of the acromion were frequently seen. This points to a failure of somite patterning. Interestingly, myotomal rather than sclerotomal markers were affected in the PDGFRα deficient mice (Soriano, 1997), indicating that a primary defect in the sclerotome feeds back into the myotome (Tallquist et al., 2000b).

The phenotypes seen in PDGRα mutants are highly similar to those of a naturally occurring mouse mutant, *Patch*, which encompasses the PDGFRα locus. Additional features seen in *Patch* mice, such as a pigmentation defect and the presence of an extra rib, indicates that this mutation is multigenic, and it has been suggested that the “patchy” pigmentation may be related to misexpression of c-KIT (Duttlinger et al., 1995).

Even though the phenotype of PDGFRα deficient mice is more severe than those observed in any of the PDGF ligand knockouts, some similarities have

been described. Thus, in the developing skin and gut, a virtually identical picture to that present in PDGF-A negative mice is seen (Karlsson et al., 1999; Karlsson et al., 2000). In PDGFRα negative lungs, no early lung branching defect was observed, and this is in agreement with the absence of an early pulmonary phenotype in PDGF-A knockouts (Paper II). Since PDGFRα deficient mice die before E16, it was not possible to examine any potential effect on alveolar formation.

### PDGFR-β negative mice phenocopy PDGF-B knockouts

Mice lacking PDGFRβ (Soriano, 1994) have not revealed any phenotypical differences from PDGF-B deficient mice (Hellstrom et al., 1999). This emphasizes the importance of PDGF-B/PDGFRβ signaling in mouse development, but also questions the relevance of other ligand/receptor interactions. If any functions exist for PDGF-DD in signaling over PDGFRββ, or for PDGF-AB and PDGF-CC in signaling over PDGFRαβ, this remains to be shown. PDGF-B may not be a physiologically important ligand for PDGFRα simply because the expression patterns of the two genes are physically separate during murine embryogenesis.

### Cell autonomous requirement for PDGF receptors revealed by chimeric analyses

Mouse chimeras have been used to let cells harboring a targeted gene deletion compete with wildtype cells for representation in different cell lineages. If a competitive disadvantage is observed for the knockout cells, this indicates an intrinsic, cell autonomous, role for the targeted gene in that particular tissue.

Using this methodology, chimeric analysis showed a selection against PDGFRα deficient cells in all tissues affected in the knockout. Additionally,

strong selection for PDGFRα bearing cells was seen in the limb bud, even though no limb development defects have been described in PDGFRα negative mice (Tallquist and Soriano, 2003). This suggests a redundant function for PDGFRα in the limb bud progress zone.

A similar competition assay with wildtype and PDGFRβ negative cells revealed a role for PDGFRβ not only in vascular SMC lineages, but also in gastrointestinal smooth muscle layers as well as cardiac and skeletal muscle (Crosby et al., 1998). In a later study, the selection against PDGFRβ deficient cells in striated muscle was not evident in high degree chimeras, indicating that this competitive disadvantage is rather weak (Tallquist et al., 2000a).

When the degree of chimerism is not too high, chimeric mice are viable, and PDGFRβ chimeras have been used to identify cell autonomous requirements for PDGFRβ in wound healing (Crosby et al., 1999). Here, it was demonstrated that fibroblasts and endothelial cells (or endothelial cell progenitors) require PDGFRβ to participate in reactive connective tissue formation.

### Selective mutations in PDGF receptors suggest both overlapping and specific functions for RTK-activated signaling pathways

As discussed in the previous chapter, activation of the PDGF receptors by ligand binding induces complex intracellular signaling networks. To try to elucidate if the observed functional specificities of the two PDGF receptors *in vivo* depend mainly on differences in spatio-temporal expression patterns or divergent mechanisms of signal transduction, Philippe Soriano and co-workers have generated several lines of gene targeted mice with specific mutations in the PDGFR signaling domains.

Two lines of knockin mice where the intracellular signaling domains of one PDGF receptor was replaced by those from the other PDGFR demonstrates that

the two receptors mediate largely overlapping functions. Mice homozygous for each replacement mutation were completely viable and fertile. However, when stressing the system further, a requirement for PDGFRβ-type signaling is revealed: Heterozygous mice, carrying one PDGFRβ knockout allele and one allele where the intracellular PDGFRβ-domain was replaced with that from PDGFRα, display retinopathy and kidney defects comparable to those seen in PDGF-B deficient mice (Klinghoffer et al., 2001). The authors argue that this may be due to a decreased capacity to sustain MAP kinase activation, but it is also possible that the observed phenotype simply is an effect of a hypomorphic PDGFRβ allele.

Redundancy has also been demonstrated on the level of individual signaling pathways. Several cellular effects, including chemotaxis and actin cytoskeleton rearrangements, have been implicated to be dependent on activation of PI3 kinase signaling (reviewed in Heldin et al., 1998). However, mice with a PDGFRβ mutant for PI3K binding develop normally, indicating functional compensation *in vivo* (Heuchel et al., 1999). A possible requirement for PDGFRβ signaling via PI3K was demonstrated in fluid pressure homeostasis: After treatment with a mast cell degranulating agent, mutant mice were unable to restore interstitial pressure in response to an injection of PDGF-BB.

Phospholipase C γ activation has been suggested to be redundant to PI3K signaling, and for this reason mice were generated that lacked the ability to activate either of these pathways following PDGFRβ stimulation. Even more surprisingly than in the previous study, PLCγ/PI3K mutant mice have no distinguishable phenotype, even though migration and proliferation of mutant cells were substantially affected *in vitro* (Tallquist et al., 2000a). Loss of mutant mesangial cells in experimentally induced glomerulonephritis also indicated

important functions for PDGFRβ mediated activation of PLCγ/PI3K in pathophysiological situations.

In the analysis of an allelic series at the PDGFRα locus, it was demonstrated that while mutations in the binding sites for either Src family kinases or PI3K lead to defective oligodendrocyte development, only PI3K site mutations affected skeletal development (Klinghoffer et al., 2002). Notably, PDGFRα/PI3K signaling mutants also recapitulated the alveogenesis defect seen in PDGF-A deficient mice (Paper I), and the cleft palate seen in PDGF-C knockouts (Paper IV). The observed phenotype in the PI3K mutant was not aggravated with the addition of additional tyrosine-to-phenylalanine mutations, suggesting that PI3K is the major effector of PDGFRα signaling in mouse development.

If this is true, why is there a phenotypic difference between PDGFRα deficient mice and PDGFRα/PI3K signaling mutants? One explanation could be that a mutant PDGFRα would still be able to activate PI3K by means of a heterodimeric receptor complex. Consistent with this idea, embryos with mutations in the PI3K binding sites of both PDGF receptors recapitulate the PDGFRα knockout phenotype (Klinghoffer et al., 2002).

The abovementioned studies demonstrate both significant functional redundancy between different PDGF receptor mediated signaling pathways, and that distinguishable physiological effects can be attributed to specific members of this network. It is still unclear, however, if these results mainly reflect a quantitative difference in intracellular signal strength and duration, or if a true qualitative diversity in the ability to elicit specific cellular responses exists.

### *The generation of PDGF-A / PDGF-C double deficient mice provides an important piece in the PDGF puzzle*

Even though signaling via the two PDGF receptors elicits largely redundant biochemical responses, targeted deletion of PDGFRα and PDGFRβ has demonstrated highly distinct functions in mouse development. It is tempting to suggest that this functional diversity mainly is caused by different gene expression patterns and ligand binding specificities, rather than differences in intracellular signaling. Consistent with this idea, PDGF-B knockouts and PDGFRβ deficient mice are phenotypically indistinguishable (Leveen et al., 1994; Soriano, 1994).

PDGFRα negative mice display a phenotype that is more severe than any single PDGF ligand knockout, and not even PDGF-A/B double deficient mice recapitulated the PDGFRα phenotype (M. Hellström, unpublished observations). Based on ligand binding studies and expression patterns (Paper III; Aase et al., 2002; Ding et al., 2000), we hypothesized that the PDGFRα ligands PDGF-A and -C may have overlapping functions in mouse development.

To test this hypothesis, we generated mice that lacked the ability to produce both PDGF-A and PDGF-C. These double deficient mice reproduced all described PDGFRα phenotypes, including cleft face, cardiac and skeletal abnormalities and loss of interstitial mesenchyme in the kidney cortex (Paper IV).

### *Critical PDGF ligand/receptor specificities in mouse development*

Taken together, the combined results from gene targeting studies in mice suggest that PDGF-AA and PDGF-CC are the essential ligands for PDGFRαα in mouse development, whereas PDGF-BB is the principal dimeric ligand for PDGFRββ (Fig. 5). The possible functions of other ligand/receptor combinations in mouse embryogenesis appear to be redundant, but that does not exclude important roles in adult physiology and pathophysiology.

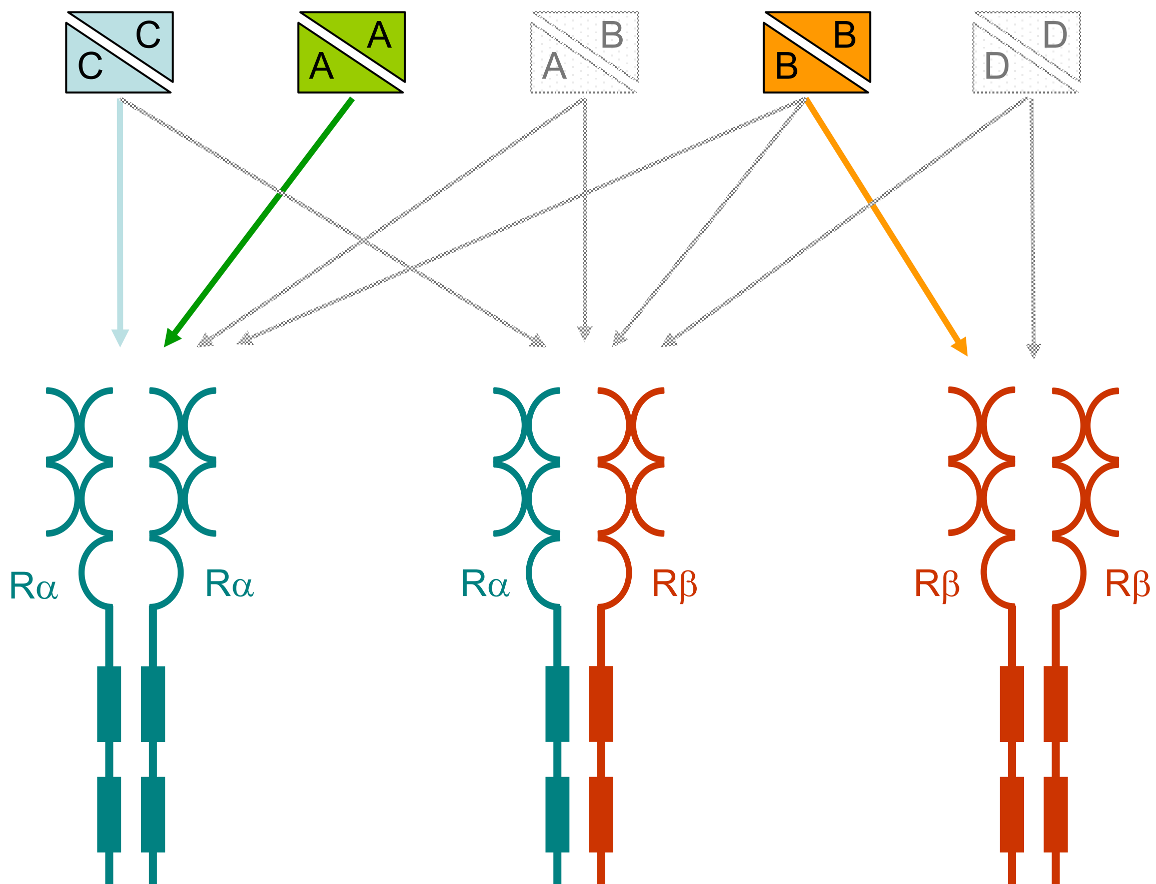


**Figure 5.** Critical PDGF ligand / receptor specificities in mouse development.

## Analogous functions for PDGFs and their target cells

It is evident from the cited reports that platelet-derived growth factors are important for the development of specific mesenchymal and neuroectodermal progenitor populations in mouse embryogenesis. In the construction of a multicellular organism, coordination of multiple cellular events is required, such as proliferation, migration, cytoskeletal reorganization, regulated cell shape, extracellular matrix remodeling, cellular adhesion, differentiation and cell survival. *In vitro* studies have suggested that PDGF probably mediates several of these functions (Heldin and Westermark, 1999).

Reduced proliferation is the only clearly demonstrated cellular effect in mice with targeted PDGF family alleles, as demonstrated by BrdU labeling experiments in PDGF-A knockouts (Karlsson et al., 1999; Karlsson et al., 2000; van Heyningen et al., 2001), as well as PDGF-B and PDGFRβ deficient mutants (Hellstrom et al., 1999).

Increased apoptosis in the cranial neural crest was reported in PDGFRα deficient mice (Soriano, 1997), but a later study by the same author, in which PDGFRα had been specifically disrupted in neural crest cells, failed to show any effect on either proliferation, migration or apoptosis in this cell population. In this study, it was argued that PDGFRα signaling might be important for directing matrix deposition, but an alternative explanation is that a proliferation defect in only a subset of cells would go undetected (Tallquist and Soriano, 2003).

Applying William of Ockham's philosophical razor ("Plurality should not be posited without necessity"), a role for PDGF as a mitogen is sufficient to explain the phenotypes seen in the mouse mutants. Perceived migratory defects could be

secondary to failed expansion of progenitor cell populations. This reasoning leads to the model depicted in Fig. 6.

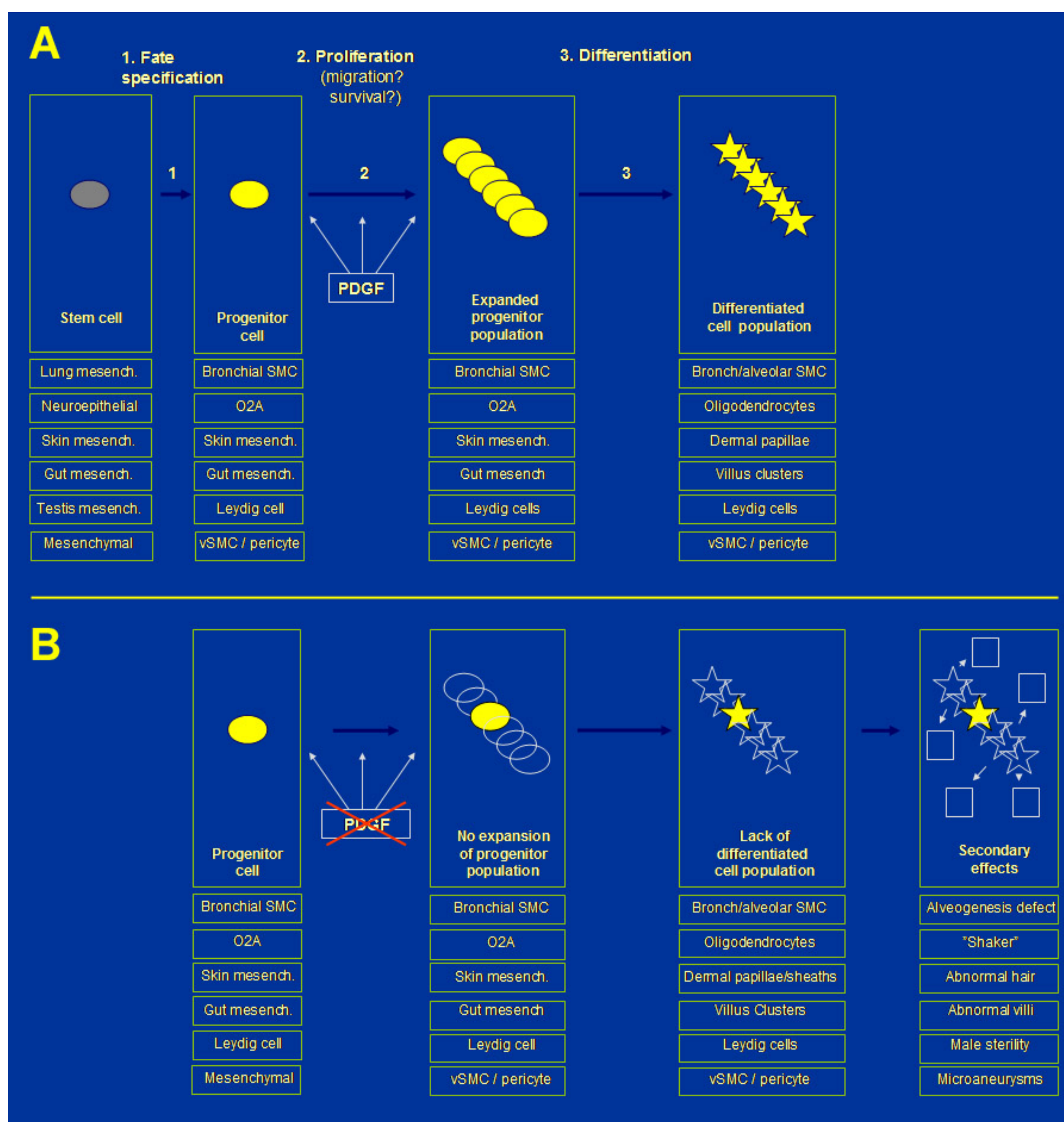


**Figure 6.** Analogous functions for PDGF isoforms. **A)** PDGF regulates the proliferation of PDGFR positive progenitor cells. **B)** In the absence of PDGF, a failed expansion of progenitor cell populations leads to the observed phenotypes. Modified from (Betsholtz et al., 2001).

## Summary and clinical perspective

*"Hence, we cannot categorically deny that perhaps we may be able to grind genes in a mortar and cook them in a beaker after all."*

Herbert J. Muller, Variation due to change in the individual gene,
The American Naturalist 56, 32-50 (1922)

In the present study, a novel high affinity ligand for PDGFRα, PDGF-C, was described (Paper III). Three new tools for genetic studies were designed: mice deficient for PDGF-A (Paper I), PDGF-C (Paper IV), and the retention motif in PDGF-A (Paper V).

I have used these tools to analyze certain functions of PDGF-A and -C in mouse embryogenesis. PDGF-A is required for alveolar myofibroblast development and alveogenesis (Paper I, II), PDGF-C is essential for closure of the neural arches and palate formation (Paper IV), and extracellular retention of PDGF-A is important for normal gastrointestinal development (Paper V).

Interestingly, PDGF-A / PDGF-C double deficient mice recapitulate the phenotype of PDGFRα negative mice, indicating that PDGF-AA and -CC are the essential ligands for PDGFRαα in mouse development (Paper IV).

As I have described in this thesis, PDGF-A deficient mice have already been used by others to demonstrate important functions for PDGF-A in the development of the CNS, skin, gut and testis. The three lines of mice will hopefully be useful tools also in future research. Since PDGF-A retention deficient mice are viable, they can be studied in pathophysiological contexts to elucidate the importance of extracellular retention of PDGF-A in wound healing and other processes.

The phenotypes reported in this study suggest that signaling over the receptor tyrosine kinase PDGFRα regulates mesenchymal progenitor cell populations that are important for normal development of the lung, the intestine, the palate and in closure of the neural tube. It is tempting to speculate that modulation of PDGF activity may be a causative factor in certain diseases affecting these organs. Furthermore, stimulation or inhibition of PDGF signaling could prove to be successful therapeutic approaches in the treatment of such conditions.

To my knowledge, no direct evidence has been presented that implicates PDGF in human cleft palate syndromes, but it is likely that future studies will link a subgroup of orofacial clefts to disturbed PDGF signaling.

In agreement with our mouse knockout data, specific combinations of human PDGFRα promoter haplotypes have been shown to correlate to neural tube defects including spina bifida (Joosten et al., 2001).

A role for PDGFRα in the development of gastrointestinal stromal tumors (GISTs) was recently shown. A subgroup of c-KIT negative GISTs were found to contain activating mutations in PDGFRα. There is reason to believe that these tumors will respond well to treatment with imatinib, in an analogous fashion to c-KIT positive GISTs (Heinrich et al., 2003).

In the lung, upregulation of PDGF ligands and PDGFRα is seen in pulmonary fibrosis (reviewed in Lasky and Brody, 2000), demonstrating the importance of PDGF signaling for mesenchymal cells in the lung.

The highly specific alveogenesis defect found in PDGF-A deficient mice makes it possible to hypothesize that regeneration of alveoli in the adult human might be feasible if PDGF-responsive mesenchymal progenitors still exist in the

lung. This scenario would be of major interest in the treatment of emphysema, one of the most common causes of death in the Western world.

Indeed, treatment with retinoic acid was shown to abrogate elastase-induced emphysema in rats (Massaro and Massaro, 1997), and it has been suggested that this effect at least to some extent is mediated by PDGF (Liebeskind et al., 2000).

# Acknowledgments

> ***"Acknowledgment (ák nol' éj mént), n.*** 1. *The act of acknowledging; an expression of gratitude; something given or done in return for a service or message.* 2. *The part of a doctoral thesis that people actually read."*
>
> The Concise English Dictionary, Scientific Edition (2003)

Thank you! It has been a privilege to spend a few (!) years at the **Department of Medical Biochemistry**, from both a scientific and a social perspective. I wish to express my sincere gratitude to all of you, and especially:

My fellow PDGF hunters and co-authors, none forgotten but only a few mentioned: **Ulf Eriksson** for doing that EST search at "15.30, September 29th, 1998", which lead to the discovery of PDGF-C, **Amel Gritli-Linde** for unsurpassed scientific enthusiasm and **Silvia Bianconi** for hard work with ill-tempered gene constructs.

**Per Lindahl** and **Holger & Nora Gerhardt**, for careful reading of my thesis summary, and much appreciated comments.

**Christer Betsholtz**, supervisor, friend, gourmet chef and singer/songwriter, for creating a unique and challenging, yet open and warm scientific atmosphere, which can only be described as "Betsholtzonian". Christer's lab is like Santa's workshop - everything is possible if you put your mind to it, and the exceptional intellectual freedom he bestows upon his PhD students helps foster truly independent researchers.

All Santa's little helpers: **Samuel Gebre-Medhin** and **Per Levéen** for teaching me everything when I was young and innocent, **Alexandra Abramsson** for always surrendering during our wrestling matches, **Mattias Bjarnegård** for whisky (not whiskey!) expertise and strange jokes, **Cecilia Bondjers** for being the only one left who remembers that -A comes before -B, **Monica Elmestam** for being such an animal... keeper, **Maria Enge** for being the mother this place so desperately needed, **Mikael Englund**, a post-doc in Christer Betsholtz' group(?) who provided me with quotes and Mekong (of which I had great use for the latter), **Helene Hjelm**, our second brave mouse-keteer, **Per Lindblom** - YHF (every day is a Friday when you're around!), **Andrea Lundqvist** for constantly making me feel just a little bit better, **Paula Morelli** for always providing a paper-bag full of candy, **Jenny Norlin**, the hope and future of the Betsholtzonians, for being the best room-mate a stressed-out PhD student could wish for, and

finally **Minoru Takemoto** and **Arianna Tocchetti**, our Japanese/Italian post-docs, for providing that certain je-ne-sais-quoi of international success to the lab.

A huge thanks to some very special ladies, who always have been there for me: **Marianne Eriksson** and **Kerstin Lundmark** for helping out with blastocyst injections, coffee and discussions about life; **Birgitta Ekström**, **Anita Jacobsson** and **Ulrika Molin** for being the best secretaries imaginable; and **Monica Olsson** for being the secret ruler of the department.

The crew at "**AngioGenetics - because kids matter**", and in particular: **Mattias Kalén**, CEO, BSc, for being my comrade-in-arms and for promising me that we will go get some Milanese suits when all this is over, **Mats Hellström** for answering all my questions even when his stomach told him otherwise and **Linda Karlsson** for tequila contests and for having a more passionate relationship to the PDGF-A deficient mice than I have.

**Albert Hietala**, my very dear and very bizarre friend. How would I have survived these years without our deeply intellectual and emotional discussions (usually about the advantages of processor multithreading, if Yanagisawa really is a match for Selmer, or if a 20GB iPod is more than twice as desirable as a 10GB version. The answer to the last two questions is "Yes!")? I will also take this opportunity to mention that you don't deserve your beautiful and charming wife, **Sofia**.

The groups of **Gunnar Hansson, Dan Baeckström, Henrik Semb, Per Lindahl, Sven Enerbäck, Milos Pekny** and **Marcela Pekna**, for providing a great working and partying atmosphere. Special thanks to: **Stefan Scheidl**, you will always be my Präses! **Rickard Westergren**, my fellow movie club organizer, for being a social hub at the department, **Linda Persson** for not wearing jeans too often, **Christian X. Andersson** for being the best looking PhD student in Göteborg, and all the other not-so-good-looking but happening members of the brat pack: **Fredrik Frick, Thomas Greiner, Henrik Lindskog, Fredrik Olson, Sven Nelander, Daniel Nilsson, Hilmar Vidarsson and Per Wasteson** (liberal).

I would also like to acknowledge my friends and fellow musicians in **2100 Big Band**, and especially **Tongångarne & Patriciabaletten**. Without you, this book would probably have been written a couple of years ago - but then again, it might not have been written at all.

Finally my parents, **Carin** and **Roland**, for endless encouragement - even though I suspect you sometimes wished that I had "a real job".

# References


Aase, K., Abramsson, A., Karlsson, L., Betsholtz, C., and Eriksson, U. (2002). Expression analysis of PDGF-C in adult and developing mouse tissues. Mech Dev *110*, 187-191.

Andersson, M., Ostman, A., Kreysing, J., Backstrom, G., Van de Poll, M., and Heldin, C. H. (1995). Involvement of loop 2 of platelet-derived growth factor-AA and -BB in receptor binding. Growth Factors *12*, 159-164.

Andersson, M., Ostman, A., Westermark, B., and Heldin, C. H. (1994). Characterization of the retention motif in the C-terminal part of the long splice form of platelet-derived growth factor A-chain. J Biol Chem *269*, 926-930.

Antoniades, H. N., Scher, C. D., and Stiles, C. D. (1979). Purification of human platelet-derived growth factor. Proc Natl Acad Sci U S A *76*, 1809-1813.

Ataliotis, P., and Mercola, M. (1997). Distribution and functions of platelet-derived growth factors and their receptors during embryogenesis. Int Rev Cytol *172*, 95-127.

Avery, O. T., MacLeod, C. M., and McCarty, M. (1944). Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Inductions of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III. J Exp Med *79*, 137-158.

Balk, S. D. (1971). Calcium as a regulator of the proliferation of normal, but not of transformed, chicken fibroblasts in a plasma-containing medium. Proc Natl Acad Sci U S A *68*, 271-275.

Bergsten, E., Uutela, M., Li, X., Pietras, K., Ostman, A., Heldin, C. H., Alitalo, K., and Eriksson, U. (2001). PDGF-D is a specific, protease-activated ligand for the PDGF beta-receptor. Nat Cell Biol *3*, 512-516.

Betsholtz, C. (1986) Role of Platelet-Derived Growth Factor in Neoplastic Transformation, Doctoral Dissertation, Uppsala University.

Betsholtz, C., Johnsson, A., Heldin, C. H., Westermark, B., Lind, P., Urdea, M. S., Eddy, R., Shows, T. B., Philpott, K., Mellor, A. L., and et al. (1986). cDNA sequence and chromosomal localization of human platelet-derived growth factor A-chain and its expression in tumour cell lines. Nature *320*, 695-699.

Betsholtz, C., Karlsson, L., and Lindahl, P. (2001). Developmental roles of platelet-derived growth factors. Bioessays *23*, 494-507.

Betsholtz, C., Rorsman, F., Westermark, B., Ostman, A., and Heldin, C. H. (1990). Analogous alternative splicing. Nature *344*, 299.

Blau, H. M., and Banfi, A. (2001). The well-tempered vessel. Nat Med *7*, 532-534.

Blume-Jensen, P., and Hunter, T. (2001). Oncogenic kinase signalling. Nature *411*, 355-365.

Bonthron, D. T., Morton, C. C., Orkin, S. H., and Collins, T. (1988). Platelet-derived growth factor A chain: gene structure, chromosomal location, and basis for alternative mRNA splicing. Proc Natl Acad Sci U S A *85*, 1492-1496.

Bork, P. (1991). Complement components C1r/C1s, bone morphogenic protein 1 and Xenopus laevis developmentally regulated protein UVS.2 share common repeats. FEBS Lett *282*, 9-12.

Buetow, B. S., Crosby, J. R., Kaminski, W. E., Ramachandran, R. K., Lindahl, P., Martin, P., Betsholtz, C., Seifert, R. A., Raines, E. W., and Bowen-Pope, D. F. (2001). Platelet-derived growth factor B-chain of hematopoietic origin is not necessary for granulation tissue formation and its absence enhances vascularization. Am J Pathol *159*, 1869-1876.

Calver, A. R., Hall, A. C., Yu, W. P., Walsh, F. S., Heath, J. K., Betsholtz, C., and Richardson, W. D. (1998). Oligodendrocyte population dynamics and the role of PDGF in vivo. Neuron *20*, 869-882.

Capdeville, R., Buchdunger, E., Zimmermann, J., and Matter, A. (2002). Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. Nat Rev Drug Discov *1*, 493-502.

Claesson-Welsh, L., Eriksson, A., Westermark, B., and Heldin, C. H. (1989). cDNA cloning and expression of the human A-type platelet-derived growth factor (PDGF) receptor establishes structural similarity to the B-type PDGF receptor. Proc Natl Acad Sci U S A *86*, 4917-4921.

Clements, J. M., Bawden, L. J., Bloxidge, R. E., Catlin, G., Cook, A. L., Craig, S., Drummond, A. H., Edwards, R. M., Fallon, A., Green, D. R., and et al. (1991). Two PDGF-B chain residues, arginine 27 and isoleucine 30, mediate receptor binding and activation. Embo J *10*, 4113-4120.

Cohen, P. (2002). Protein kinases--the major drug targets of the twenty-first century? Nat Rev Drug Discov *1*, 309-315.

Collett, M. S., Purchio, A. F., and Erikson, R. L. (1980). Avian sarcoma virus-transforming protein, pp60src shows protein kinase activity specific for tyrosine. Nature *285*, 167-169.

Crosby, J. R., Seifert, R. A., Soriano, P., and Bowen-Pope, D. F. (1998). Chimaeric analysis reveals role of Pdgf receptors in all muscle lineages. Nat Genet *18*, 385-388.

Crosby, J. R., Tappan, K. A., Seifert, R. A., and Bowen-Pope, D. F. (1999). Chimera analysis reveals that fibroblasts and endothelial cells require platelet-derived growth factor receptorbeta expression for participation in reactive connective tissue formation in adults but not during development. Am J Pathol *154*, 1315-1321.

Dalla-Favera, R., Gallo, R. C., Giallongo, A., and Croce, C. M. (1982). Chromosomal localization of the human homolog (c-sis) of the simian sarcoma virus onc gene. Science *218*, 686-688.

Devare, S. G., Reddy, E. P., Law, J. D., Robbins, K. C., and Aaronson, S. A. (1983). Nucleotide sequence of the simian sarcoma virus genome: demonstration that its acquired cellular sequences encode the transforming gene product p28sis. Proc Natl Acad Sci U S A *80*, 731-735.

Diliberto, P. A., Gordon, G. W., Yu, C. L., Earp, H. S., and Herman, B. (1992). Platelet-derived growth factor (PDGF) alpha receptor activation modulates the calcium mobilizing activity of the PDGF beta receptor in Balb/c3T3 fibroblasts. J Biol Chem *267*, 11888-11897.

Ding, H., Wu, X., Kim, I., Tam, P. P., Koh, G. Y., and Nagy, A. (2000). The mouse Pdgfc gene: dynamic expression in embryonic tissues during organogenesis. Mech Dev *96*, 209-213.

Doetschman, T., Gregg, R. G., Maeda, N., Hooper, M. L., Melton, D. W., Thompson, S., and Smithies, O. (1987). Targetted correction of a mutant HPRT gene in mouse embryonic stem cells. Nature *330*, 576-578.

Doolittle, R. F., Hunkapiller, M. W., Hood, L. E., Devare, S. G., Robbins, K. C., Aaronson, S. A., and Antoniades, H. N. (1983). Simian sarcoma virus onc gene, v-sis, is derived from the gene (or genes) encoding a platelet-derived growth factor. Science *221*, 275-277.

Downward, J., Yarden, Y., Mayes, E., Scrace, G., Totty, N., Stockwell, P., Ullrich, A., Schlessinger, J., and Waterfield, M. D. (1984). Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. Nature *307*, 521-527.

Duttlinger, R., Manova, K., Berrozpe, G., Chu, T. Y., DeLeon, V., Timokhina, I., Chaganti, R. S., Zelenetz, A. D., Bachvarova, R. F., and Besmer, P. (1995). The Wsh and Ph mutations affect the c-kit expression profile: c-kit misexpression in embryogenesis impairs melanogenesis in Wsh and Ph mutant mice. Proc Natl Acad Sci U S A *92*, 3754-3758.

Enge, M., Bjarnegard, M., Gerhardt, H., Gustafsson, E., Kalen, M., Asker, N., Hammes, H. P., Shani, M., Fassler, R., and Betsholtz, C. (2002). Endothelium-specific platelet-derived growth factor-B ablation mimics diabetic retinopathy. Embo J *21*, 4307-4316.

Enge, M., Wilhelmsson, U., Abramsson, A., Stakeberg, J., Kuhn, R., Betsholtz, C., and Pekny, M. (2003). Neuron-specific ablation of PDGF-B is compatible with normal central nervous system development and astroglial response to injury. Neurochem Res *28*, 271-279.

Eriksson, A., Siegbahn, A., Westermark, B., Heldin, C. H., and Claesson-Welsh, L. (1992). PDGF alpha- and beta-receptors activate unique and common signal transduction pathways. Embo J *11*, 543-550.

Esmaeli, B., Prieto, V. G., Butler, C. E., Kim, S. K., Ahmadi, M. A., Kantarjian, H. M., and Talpaz, M. (2002). Severe periorbital edema secondary to STI571 (Gleevec). Cancer *95*, 881-887.

Fambrough, D., McClure, K., Kazlauskas, A., and Lander, E. S. (1999). Diverse signaling pathways activated by growth factor receptors induce broadly overlapping, rather than independent, sets of genes. Cell *97*, 727-741.

Fenstermaker, R. A., Poptic, E., Bonfield, T. L., Knauss, T. C., Corsillo, L., Piskurich, J. F., Kaetzel, C. S., Jentoft, J. E., Gelfand, C., DiCorleto, P. E., and et al. (1993). A cationic region of the platelet-derived growth factor (PDGF) A-chain (Arg159-Lys160-Lys161) is required for receptor binding and mitogenic activity of the PDGF-AA homodimer. J Biol Chem *268*, 10482-10489.

Feyzi, E., Lustig, F., Fager, G., Spillmann, D., Lindahl, U., and Salmivirta, M. (1997). Characterization of heparin and heparan sulfate domains binding to the long splice variant of platelet-derived growth factor A chain. J Biol Chem *272*, 5518-5524.

Fruttiger, M., Karlsson, L., Hall, A. C., Abramsson, A., Calver, A. R., Bostrom, H., Willetts, K., Bertold, C. H., Heath, J. K., Betsholtz, C., and Richardson, W. D. (1999). Defective oligodendrocyte development and severe hypomyelination in PDGF-A knockout mice. Development *126*, 457-467.

Futreal, P. A., Kasprzyk, A., Birney, E., Mullikin, J. C., Wooster, R., and Stratton, M. R. (2001). Cancer and genomics. Nature *409*, 850-852.

Gilbertson, D. G., Duff, M. E., West, J. W., Kelly, J. D., Sheppard, P. O., Hofstrand, P. D., Gao, Z., Shoemaker, K., Bukowski, T. R., Moore, M.*, et al.* (2001). Platelet-derived growth factor C (PDGF-C), a novel growth factor that binds to PDGF alpha and beta receptor. J Biol Chem *276*, 27406-27414.

Gnessi, L., Basciani, S., Mariani, S., Arizzi, M., Spera, G., Wang, C., Bondjers, C., Karlsson, L., and Betsholtz, C. (2000). Leydig cell loss and spermatogenic arrest in platelet-derived growth factor (PDGF)-A-deficient mice. J Cell Biol *149*, 1019-1026.

Hammacher, A., Hellman, U., Johnsson, A., Ostman, A., Gunnarsson, K., Westermark, B., Wasteson, A., and Heldin, C. H. (1988a). A major part of platelet-derived growth factor purified from human platelets is a heterodimer of one A and one B chain. J Biol Chem *263*, 16493-16498.

Hammacher, A., Nister, M., Westermark, B., and Heldin, C. H. (1988b). A human glioma cell line secretes three structurally and functionally different dimeric forms of platelet-derived growth factor. Eur J Biochem *176*, 179-186.

Hart, C. E., Bailey, M., Curtis, D. A., Osborn, S., Raines, E., Ross, R., and Forstrom, J. W. (1990). Purification of PDGF-AB and PDGF-BB from human platelet extracts and identification of all three PDGF dimers in human platelets. Biochemistry *29*, 166-172.

Hearn, M. T., and Gomme, P. T. (2000). Molecular architecture and biorecognition processes of the cystine knot protein superfamily: part I. The glycoprotein hormones. J Mol Recognit *13*, 223-278.

Heidaran, M. A., Pierce, J. H., Yu, J. C., Lombardi, D., Artrip, J. E., Fleming, T. P., Thomason, A., and Aaronson, S. A. (1991). Role of alpha beta receptor heterodimer formation in beta platelet-derived growth factor (PDGF) receptor activation by PDGF-AB. J Biol Chem *266*, 20232-20237.

Heinrich, M. C., Corless, C. L., Duensing, A., McGreevey, L., Chen, C. J., Joseph, N., Singer, S., Griffith, D. J., Haley, A., Town, A.*, et al.* (2003). PDGFRA Activating Mutations in Gastrointestinal Stromal Tumors. Science *299*, 708-710.

Heldin, C. H., Eriksson, U., and Ostman, A. (2002). New members of the platelet-derived growth factor family of mitogens. Arch Biochem Biophys *398*, 284-290.

Heldin, C. H., Ostman, A., and Ronnstrand, L. (1998). Signal transduction via platelet-derived growth factor receptors. Biochim Biophys Acta *1378*, F79-113.

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

Heldin, C. H., Westermark, B., and Wasteson, A. (1979). Platelet-derived growth factor: purification and partial characterization. Proc Natl Acad Sci U S A *76*, 3722-3726.

Hellstrom, M., Kalén, M., Lindahl, P., Abramsson, A., and Betsholtz, C. (1999). Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. Development *126*, 3047-3055.

Heuchel, R., Berg, A., Tallquist, M., Ahlen, K., Reed, R. K., Rubin, K., Claesson-Welsh, L., Heldin, C. H., and Soriano, P. (1999). Platelet-derived growth factor beta receptor regulates interstitial fluid homeostasis through phosphatidylinositol-3' kinase signaling. Proc Natl Acad Sci U S A *96*, 11410-11415.

Hogg, P. J., Hotchkiss, K. A., Jimenez, B. M., Stathakis, P., and Chesterman, C. N. (1997). Interaction of platelet-derived growth factor with thrombospondin 1. Biochem J *326 ( Pt 3)*, 709-716.

Hosang, M., Rouge, M., Wipf, B., Eggimann, B., Kaufmann, F., and Hunziker, W. (1989). Both homodimeric isoforms of PDGF (AA and BB) have mitogenic and chemotactic activity and stimulate phosphoinositol turnover. J Cell Physiol *140*, 558-564.

Hunter, T. (2000). Signaling--2000 and beyond. Cell *100*, 113-127.

Hunter, T. (2002). Tyrosine phosphorylation in cell signaling and disease. Keio J Med *51*, 61-71.

Hunter, T., and Sefton, B. M. (1980). Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. Proc Natl Acad Sci U S A *77*, 1311-1315.

Jhiang, S. M. (2000). The RET proto-oncogene in human cancers. Oncogene *19*, 5590-5597.

Joosten, P. H., Toepoel, M., Mariman, E. C., and Van Zoelen, E. J. (2001). Promoter haplotype combinations of the platelet-derived growth factor alpha-receptor gene predispose to human neural tube defects. Nat Genet *27*, 215-217.

Kaminski, W. E., Lindahl, P., Lin, N. L., Broudy, V. C., Crosby, J. R., Hellstrom, M., Swolin, B., Bowen-Pope, D. F., Martin, P. J., Ross, R., *et al.* (2001). Basis of hematopoietic defects in platelet-derived growth factor (PDGF)-B and PDGF beta-receptor null mice. Blood *97*, 1990-1998.

Kantarjian, H., Sawyers, C., Hochhaus, A., Guilhot, F., Schiffer, C., Gambacorti-Passerini, C., Niederwieser, D., Resta, D., Capdeville, R., Zoellner, U., *et al.* (2002). Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. N Engl J Med *346*, 645-652.

Kapanci, Y., Assimacopoulos, A., Irle, C., Zwahlen, A., and Gabbiani, G. (1974). "Contractile interstitial cells" in pulmonary alveolar septa: a possible regulator of ventilation-perfusion ratio? Ultrastructural, immunofluorescence, and in vitro studies. J Cell Biol *60*, 375-392.

Karlsson, L., Bondjers, C., and Betsholtz, C. (1999). Roles for PDGF-A and sonic hedgehog in development of mesenchymal components of the hair follicle. Development *126*, 2611-2621.

Karlsson, L., Lindahl, P., Heath, J. K., and Betsholtz, C. (2000). Abnormal gastrointestinal development in PDGF-A and PDGFR-(alpha) deficient mice implicates a novel mesenchymal structure with putative instructive properties in villus morphogenesis. Development *127*, 3457-3466.

Kelly, J. L., Sanchez, A., Brown, G. S., Chesterman, C. N., and Sleigh, M. J. (1993). Accumulation of PDGF B and cell-binding forms of PDGF A in the extracellular matrix. J Cell Biol *121*, 1153-1163.

Kennelly, P. J., and Potts, M. (1996). Fancy meeting you here! A fresh look at "prokaryotic" protein phosphorylation. J Bacteriol *178*, 4759-4764.

King, N., and Carroll, S. B. (2001). A receptor tyrosine kinase from choanoflagellates: molecular insights into early animal evolution. Proc Natl Acad Sci U S A *98*, 15032-15037.

Klinghoffer, R. A., Hamilton, T. G., Hoch, R., and Soriano, P. (2002). An allelic series at the PDGFalphaR locus indicates unequal contributions of distinct signaling pathways during development. Dev Cell *2*, 103-113.

Klinghoffer, R. A., Mueting-Nelsen, P. F., Faerman, A., Shani, M., and Soriano, P. (2001). The two PDGF receptors maintain conserved signaling in vivo despite divergent embryological functions. Mol Cell *7*, 343-354.

Kohler, N., and Lipton, A. (1974). Platelets as a source of fibroblast growth-promoting activity. Exp Cell Res *87*, 297-301.

Koyama, N., Morisaki, N., Saito, Y., and Yoshida, S. (1992). Regulatory effects of platelet-derived growth factor-AA homodimer on migration of vascular smooth muscle cells. J Biol Chem *267*, 22806-22812.

Kozaki, K., Kaminski, W. E., Tang, J., Hollenbach, S., Lindahl, P., Sullivan, C., Yu, J. C., Abe, K., Martin, P. J., Ross, R.*, et al.* (2002). Blockade of platelet-derived growth factor or its receptors transiently delays but does not prevent fibrous cap formation in ApoE null mice. Am J Pathol *161*, 1395-1407.

Kristiansen, M., Kozyraki, R., Jacobsen, C., Nexo, E., Verroust, P. J., and Moestrup, S. K. (1999). Molecular dissection of the intrinsic factor-vitamin B12 receptor, cubilin, discloses regions important for membrane association and ligand binding. J Biol Chem *274*, 20540-20544.

LaRochelle, W. J., Jeffers, M., McDonald, W. F., Chillakuru, R. A., Giese, N. A., Lokker, N. A., Sullivan, C., Boldog, F. L., Yang, M., Vernet, C.*, et al.* (2001). PDGF-D, a new protease-activated growth factor. Nat Cell Biol *3*, 517-521.

LaRochelle, W. J., May-Siroff, M., Robbins, K. C., and Aaronson, S. A. (1991). A novel mechanism regulating growth factor association with the cell surface: identification of a PDGF retention domain. Genes Dev *5*, 1191-1199.

LaRochelle, W. J., Pierce, J. H., May-Siroff, M., Giese, N., and Aaronson, S. A. (1992). Five PDGF B amino acid substitutions convert PDGF A to a PDGF B-like transforming molecule. J Biol Chem *267*, 17074-17077.

Lasky, J. A., and Brody, A. R. (2000). Interstitial fibrosis and growth factors. Environ Health Perspect *108 Suppl 4*, 751-762.

Lee, B. A., Maher, D. W., Hannink, M., and Donoghue, D. J. (1987). Identification of a signal for nuclear targeting in platelet-derived-growth-factor-related molecules. Mol Cell Biol *7*, 3527-3537.

Lee, K. H., Bowen-Pope, D. F., and Reed, R. R. (1990). Isolation and characterization of the alpha platelet-derived growth factor receptor from rat olfactory epithelium. Mol Cell Biol *10*, 2237-2246.

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

Liebeskind, A., Srinivasan, S., Kaetzel, D., and Bruce, M. (2000). Retinoic acid stimulates immature lung fibroblast growth via a PDGF-mediated autocrine mechanism. Am J Physiol Lung Cell Mol Physiol *279*, L81-90.

Lindahl, P., Hellstrom, M., Kalén, M., Karlsson, L., Pekny, M., Pekna, M., Soriano, P., and Betsholtz, C. (1998). Paracrine PDGF-B/PDGF-Rbeta signaling controls mesangial cell development in kidney glomeruli. Development *125*, 3313-3322.

Lindahl, P., Johansson, B. R., Leveen, P., and Betsholtz, C. (1997a). Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. Science *277*, 242-245.

Lindahl, P., Karlsson, L., Hellstrom, M., Gebre-Medhin, S., Willetts, K., Heath, J. K., and Betsholtz, C. (1997b). Alveogenesis failure in PDGF-A-deficient mice is coupled to lack of distal spreading of alveolar smooth muscle cell progenitors during lung development. Development *124*, 3943-3953.

Lustig, F., Hoebeke, J., Ostergren-Lunden, G., Velge-Roussel, F., Bondjers, G., Olsson, U., Ruetschi, U., and Fager, G. (1996). Alternative splicing determines the binding of platelet-derived growth factor (PDGF-AA) to glycosaminoglycans. Biochemistry *35*, 12077-12085.

Maglione, D., Guerriero, V., Viglietto, G., Ferraro, M. G., Aprelikova, O., Alitalo, K., Del Vecchio, S., Lei, K. J., Chou, J. Y., and Persico, M. G. (1993). Two alternative mRNAs coding for the angiogenic factor, placenta growth factor (PlGF), are transcribed from a single gene of chromosome 14. Oncogene *8*, 925-931.

Maher, D. W., Lee, B. A., and Donoghue, D. J. (1989). The alternatively spliced exon of the platelet-derived growth factor A chain encodes a nuclear targeting signal. Mol Cell Biol *9*, 2251-2253.

Maimone, D., Dominici, R., and Grimaldi, L. M. (2001). Pharmacogenomics of neurodegenerative diseases. Eur J Pharmacol *413*, 11-29.

Manning, G., Plowman, G. D., Hunter, T., and Sudarsanam, S. (2002a). Evolution of protein kinase signaling from yeast to man. Trends Biochem Sci *27*, 514-520.

Manning, G., Whyte, D. B., Martinez, R., Hunter, T., and Sudarsanam, S. (2002b). The protein kinase complement of the human genome. Science *298*, 1912-1934.

Massaro, G. D., and Massaro, D. (1997). Retinoic acid treatment abrogates elastase-induced pulmonary emphysema in rats. Nat Med *3*, 675-677.

Matoskova, B., Rorsman, F., Svensson, V., and Betsholtz, C. (1989). Alternative splicing of the platelet-derived growth factor A-chain transcript occurs in normal as well as tumor cells and is conserved among mammalian species. Mol Cell Biol *9*, 3148-3150.

Matsui, T., Heidaran, M., Miki, T., Popescu, N., La Rochelle, W., Kraus, M., Pierce, J., and Aaronson, S. (1989a). Isolation of a novel receptor cDNA establishes the existence of two PDGF receptor genes. Science *243*, 800-804.

Matsui, T., Pierce, J. H., Fleming, T. P., Greenberger, J. S., LaRochelle, W. J., Ruggiero, M., and Aaronson, S. A. (1989b). Independent expression of human alpha or beta platelet-derived growth factor receptor cDNAs in a naive hematopoietic cell leads to functional coupling with mitogenic and chemotactic signaling pathways. Proc Natl Acad Sci U S A *86*, 8314-8318.

Mercola, M., Melton, D. A., and Stiles, C. D. (1988). Platelet-derived growth factor A chain is maternally encoded in Xenopus embryos. Science *241*, 1223-1225.

Morishita, R. (2002). Recent progress in gene therapy for cardiovascular disease. Circ J *66*, 1077-1086.

Muller, U. (1999). Ten years of gene targeting: targeted mouse mutants, from vector design to phenotype analysis. Mech Dev *82*, 3-21.

Nagy, A. (2000). Cre recombinase: the universal reagent for genome tailoring. Genesis *26*, 99-109.

Nakamura, F., Tanaka, M., Takahashi, T., Kalb, R. G., and Strittmatter, S. M. (1998). Neuropilin-1 extracellular domains mediate semaphorin D/III-induced growth cone collapse. Neuron *21*, 1093-1100.

Oefner, C., D'Arcy, A., Winkler, F. K., Eggimann, B., and Hosang, M. (1992). Crystal structure of human platelet-derived growth factor BB. Embo J *11*, 3921-3926.

Ohlsson, R., Falck, P., Hellstrom, M., Lindahl, P., Bostrom, H., Franklin, G., Ahrlund-Richter, L., Pollard, J., Soriano, P., and Betsholtz, C. (1999). PDGFB regulates the development of the labyrinthine layer of the mouse fetal placenta. Dev Biol *212*, 124-136.

Oldmixon, E. H., Carlsson, K., Kuhn, C., 3rd, Butler, J. P., and Hoppin, F. G., Jr. (2001). alpha-Actin: disposition, quantities, and estimated effects on lung recoil and compliance. J Appl Physiol *91*, 459-473.

Osornio-Vargas, A. R., Lindroos, P. M., Coin, P. G., Badgett, A., Hernandez-Rodriguez, N. A., and Bonner, J. C. (1996). Maximal PDGF-induced lung fibroblast chemotaxis requires PDGF receptor-alpha. Am J Physiol *271*, L93-99.

Ostman, A., Andersson, M., Backstrom, G., and Heldin, C. H. (1993). Assignment of intrachain disulfide bonds in platelet-derived growth factor B-chain. J Biol Chem *268*, 13372-13377.

Pollock, R. A., and Richardson, W. D. (1992). The alternative-splice isoforms of the PDGF A-chain differ in their ability to associate with the extracellular matrix and to bind heparin in vitro. Growth Factors *7*, 267-277.

Raines, E. W., Lane, T. F., Iruela-Arispe, M. L., Ross, R., and Sage, E. H. (1992). The extracellular glycoprotein SPARC interacts with platelet-derived growth factor (PDGF)-AB and -BB and inhibits the binding of PDGF to its receptors. Proc Natl Acad Sci U S A *89*, 1281-1285.

Raines, E. W., and Ross, R. (1982). Platelet-derived growth factor. I. High yield purification and evidence for multiple forms. J Biol Chem *257*, 5154-5160.

Raines, E. W., and Ross, R. (1992). Compartmentalization of PDGF on extracellular binding sites dependent on exon-6-encoded sequences. J Cell Biol *116*, 533-543.

Raines, E. W., and Ross, R. (1993). Platelet-Derived Growth Factor in vivo. In Biology of Platelet-Derived Growth Factor, B. Westermark, and C. Sorg, eds. (Basel, Karger), pp. 74-114.

Rodt, S. A., Ahlen, K., Berg, A., Rubin, K., and Reed, R. K. (1996). A novel physiological function for platelet-derived growth factor-BB in rat dermis. J Physiol *495 ( Pt 1)*, 193-200.

Rorsman, F., and Betsholtz, C. (1992). Characterization of the mouse PDGF A-chain gene. Evolutionary conservation of gene structure, nucleotide sequence and alternative splicing. Growth Factors *6*, 303-313.

Rorsman, F., Bywater, M., Knott, T. J., Scott, J., and Betsholtz, C. (1988). Structural characterization of the human platelet-derived growth factor A-chain cDNA and gene: alternative exon usage predicts two different precursor proteins. Mol Cell Biol *8*, 571-577.

Ross, R., Glomset, J., Kariya, B., and Harker, L. (1974). A platelet-dependent serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro. Proc Natl Acad Sci U S A *71*, 1207-1210.

Rupp, E., Siegbahn, A., Ronnstrand, L., Wernstedt, C., Claesson-Welsh, L., and Heldin, C. H. (1994). A unique autophosphorylation site in the platelet-derived growth factor alpha receptor from a heterodimeric receptor complex. Eur J Biochem *225*, 29-41.

Sasahara, A., Kott, J. N., Sasahara, M., Raines, E. W., Ross, R., and Westrum, L. E. (1992). Platelet-derived growth factor B-chain-like immunoreactivity in the developing and adult rat brain. Brain Res Dev Brain Res *68*, 41-53.

Sasahara, M., Fries, J. W., Raines, E. W., Gown, A. M., Westrum, L. E., Frosch, M. P., Bonthron, D. T., Ross, R., and Collins, T. (1991). PDGF B-chain in neurons of the central nervous system, posterior pituitary, and in a transgenic model. Cell *64*, 217-227.

Schlessinger, J. (2000). Cell signaling by receptor tyrosine kinases. Cell *103*, 211-225.

Shure, D., Senior, R. M., Griffin, G. L., and Deuel, T. F. (1992). PDGF AA homodimers are potent chemoattractants for fibroblasts and neutrophils, and for monocytes activated by lymphocytes or cytokines. Biochem Biophys Res Commun *186*, 1510-1514.

Siegbahn, A., Hammacher, A., Westermark, B., and Heldin, C. H. (1990). Differential effects of the various isoforms of platelet-derived growth factor on chemotaxis of fibroblasts, monocytes, and granulocytes. J Clin Invest *85*, 916-920.

Simon, M. A. (2000). Receptor tyrosine kinases: specific outcomes from general signals. Cell *103*, 13-15.

Somasundaram, R., and Schuppan, D. (1996). Type I, II, III, IV, V, and VI collagens serve as extracellular ligands for the isoforms of platelet-derived growth factor (AA, BB, and AB). J Biol Chem *271*, 26884-26891.

Soriano, P. (1994). Abnormal kidney development and hematological disorders in PDGF beta-receptor mutant mice. Genes Dev *8*, 1888-1896.

Soriano, P. (1997). The PDGF alpha receptor is required for neural crest cell development and for normal patterning of the somites. Development *124*, 2691-2700.

Sun, T., and Storb, U. (2001). Insertion of phosphoglycerine kinase (PGK)-neo 5' of Jlambda1 dramatically enhances VJlambda1 rearrangement. J Exp Med *193*, 699-712.

Tallquist, M. D., Klinghoffer, R. A., Heuchel, R., Mueting-Nelsen, P. F., Corrin, P. D., Heldin, C. H., Johnson, R. J., and Soriano, P. (2000a). Retention of PDGFR-beta function in mice in the absence

of phosphatidylinositol 3'-kinase and phospholipase Cgamma signaling pathways. Genes Dev *14*, 3179-3190.

Tallquist, M. D., and Soriano, P. (2003). Cell autonomous requirement for PDGFRalpha in populations of cranial and cardiac neural crest cells. Development *130*, 507-518.

Tallquist, M. D., Weismann, K. E., Hellstrom, M., and Soriano, P. (2000b). Early myotome specification regulates PDGFA expression and axial skeleton development. Development *127*, 5059-5070.

Thomas, K. R., and Capecchi, M. R. (1987). Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. Cell *51*, 503-512.

Ullrich, A., Coussens, L., Hayflick, J. S., Dull, T. J., Gray, A., Tam, A. W., Lee, J., Yarden, Y., Libermann, T. A., Schlessinger, J., and et al. (1984). Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature *309*, 418-425.

Uutela, M., Lauren, J., Bergsten, E., Li, X., Horelli-Kuitunen, N., Eriksson, U., and Alitalo, K. (2001). Chromosomal location, exon structure, and vascular expression patterns of the human PDGFC and PDGFD genes. Circulation *103*, 2242-2247.

van Heyningen, P., Calver, A. R., and Richardson, W. D. (2001). Control of progenitor cell number by mitogen supply and demand. Curr Biol *11*, 232-241.

Waterfield, M. D., Scrace, G. T., Whittle, N., Stroobant, P., Johnsson, A., Wasteson, A., Westermark, B., Heldin, C. H., Huang, J. S., and Deuel, T. F. (1983). Platelet-derived growth factor is structurally related to the putative transforming protein p28sis of simian sarcoma virus. Nature *304*, 35-39.

Veikkola, T., and Alitalo, K. (1999). VEGFs, receptors and angiogenesis. Semin Cancer Biol *9*, 211-220.

Yarden, Y., Escobedo, J. A., Kuang, W. J., Yang-Feng, T. L., Daniel, T. O., Tremble, P. M., Chen, E. Y., Ando, M. E., Harkins, R. N., Francke, U., and et al. (1986). Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors. Nature *323*, 226-232.

Yeh, H. J., Ruit, K. G., Wang, Y. X., Parks, W. C., Snider, W. D., and Deuel, T. F. (1991). PDGF A-chain gene is expressed by mammalian neurons during development and in maturity. Cell *64*, 209-216.

Zambrowicz, B. P., and Sands, A. T. (2003). Knockouts model the 100 best-selling drugs--will they model the next 100? Nat Rev Drug Discov *2*, 38-51.