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GROWTH FACTOR RECEPTORS

From signal transduction to human diseases

Growth factors are secreted proteins that stimulate cell proliferation via transmembrane receptors. Our research interests are focused on the mechanisms of activation of these receptors. We are particularly interested in receptor-tyrosine kinases that are binding to platelet-derived growth factors (PDGF) and fibroblasts growth factors (FGF) (Fig. 1). These proteins play an important role in the development of the embryo and in wound healing, as well as in cancer and in fibrosis.

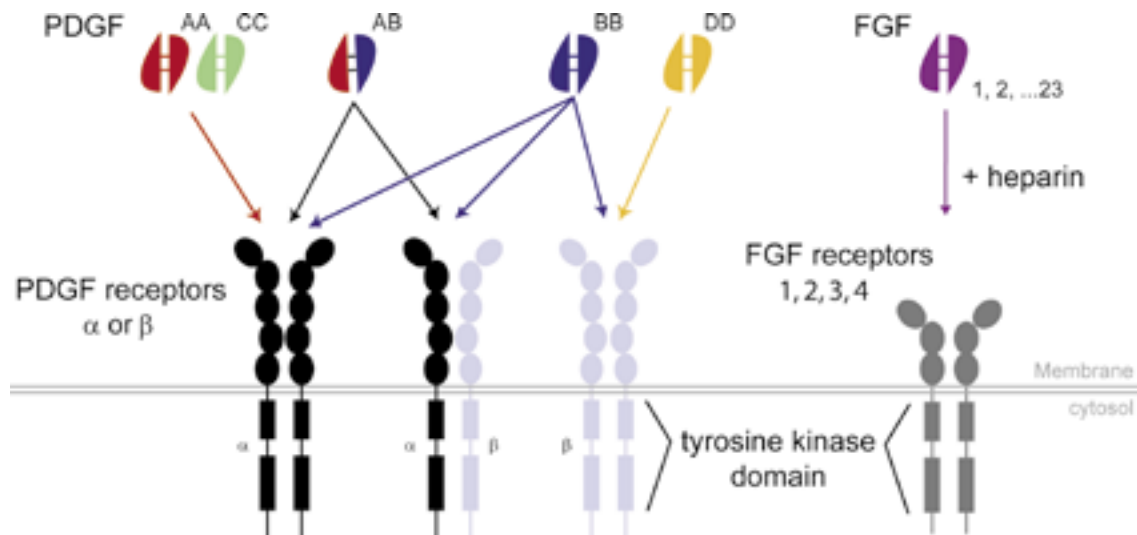


Figure 1. PDGF and FGF receptors and ligands

REARRANGEMENTS OF THE RECEPTOR TYROSINE KINASE GENES ASSOCIATED WITH LEUKEMIA

F. Toffalini, S. Medves, C. Montano, A. Velghe, J.B. Demoulin

Although PDGF receptors are expressed on platelets and macrophages, PDGF receptor-deficient mice show no primary hematopoietic or immune defect. *In vitro*, PDGF is a poor mitogen for hematopoietic cells. However, modifications of PDGF receptor genes, as a result of chromosomal translocation or deletion, are found in chronic myeloid neoplasms associated with eosinophilia (formerly classified as chronic eosinophilic leukemia, atypical chronic myeloid leukemia or chronic myelomonocytic leukemia). In all cases, the rearranged gene produces a hybrid protein comprising the PDGF receptor tyrosine kinase domain and an oligomerisation domain. In most cases, they also retain the receptor transmembrane domain, which plays a particular role in the activation of these oncoproteins (3). Similar hybrid oncogenes derive from FGF receptors.

TEL-PDGFR β (TP β , also called ETV6-PDGFR β) is a hybrid protein produced by the t(5;12) translocation. FIP1L1-PDGFR α (FP α) results from a deletion on chromosome 4q12 in patients with chronic eosinophilic leukemia. These oncogenes were studied in Ba/F3 cells, a mouse hematopoietic cell line that is easy to culture and transfect. In contrast to wild-type PDGF receptors α and β , which are quickly degraded upon activation, we observed that TP β and FP α escaped down-regulation resulting in stabilization of the proteins. High stability of these hybrid oncogenes was confirmed in leukocytes from two patients. Similar data were obtained in cells expressing ZNF198-FGFR1, another fusion protein associated with the 8p11 myeloproliferative syndrome. Cbl-mediated monoubiquitination of receptor lysines targets them for lysosomal degradation. Ubiquitination of TP β and FP α was much re-

duced compared to wild-type receptors, despite marked Cbl phosphorylation in cells expressing hybrid receptors. Deletion of the pointed (PNT) domain, impairing TP β polymerization, strongly destabilized the protein. In conclusion, chimeric receptor tyrosine kinases escape efficient ubiquitination and down-regulation through lysosomes and proteasomes (4).

In order to develop a model that is more relevant for the human disease, we introduced TP β and FP α in human CD34+ cells, which were purified from cord blood and are enriched in hematopoietic stem cells. These cells are able to differentiate *in vitro* into various blood cell types, depending on the cytokine cocktail that is added in the culture medium. We observed that TP β and FP α induce the proliferation and differentiation of cytokine-independent cells. We are now analyzing this process in detail.

It is particularly important to identify PDGF receptor alterations in cancer patients, as they can benefit from tyrosine kinase inhibitor therapy. Imatinib mesylate, for instance, is very efficient in patients with leukemia that present a PDGF receptor translocation. In collaboration with the hematology unit of the Saint-Luc university hospital, we identified a novel fusion of the PDGF receptor β with the KANK1 gene in a leukemia patient harboring a t(5;9) translocation (Fig. 2 and reference 2). We are now looking for other mutations in tyrosine kinase genes.

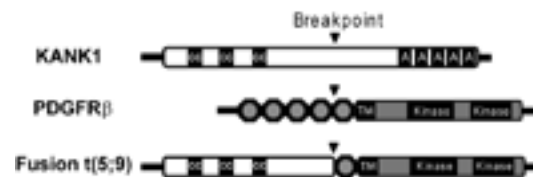


Figure 2. Structure of the KANK1-PDGFR β fusion protein created by the t(5;9) translocation.

CC: coiled-coil domain; A: ankyrin repeat; TM: transmembrane domain

ACTIVATION OF PDGF RECEPTORS IN SYSTEMIC SCLEROSIS

S. Charni, J.-F. Classen, J.B. Demoulin

Systemic sclerosis (also called scleroderma) is a severe connective tissue disease of unknown etiology, characterized by fibrosis of the skin and multiple internal organs. A recent report suggested that the disease is driven by stimulatory autoantibodies to the platelet-derived growth factor receptors (PDGFR), which stimulate the production of reactive oxygen species (ROS) and collagen by fibroblasts (Baroni et al, *New Engl. J. Med* 2006; 254:2667). These results opened novel research avenues for the diagnosis and treatment of systemic sclerosis. Several clinical trials using imatinib mesylate, a drug that inhibits PDGF receptors, were promptly initiated following this publication. In order to confirm this important observation, we purified immunoglobulins from 37 patients with systemic sclerosis by protein A/G chromatography. PDGFR activation was tested using four different sensitive bioassays, namely cell proliferation, ROS production, signal transduction and receptor phosphorylation. Purified IgG from patients with scleroderma comprised a panel of antinuclear autoantibodies, but did not specifically activate the PDGFR α or β in any of our tests, compared to controls. As positive control, cell stimulation with PDGF itself consistently produced a strong signal. Our results question the existence of agonistic autoantibodies to PDGFR in scleroderma (5). Two independent research centers have reported similar negative results. We are now trying to identify other factors that activate PDGF receptors in systemic sclerosis and other related fibrotic conditions, such as extensive chronic graft-versus-host disease.

SIGNAL TRANSDUCTION AND GENE REGULATION BY GROWTH FACTORS: ROLE OF THE TRANSCRIPTION FACTORS FOXO, STAT AND SREBP

A. Essaghir, A. Coomans de Brachène, J.B. Demoulin

Most cellular effects of growth factor occur through reprogramming gene expression within the cell nucleus. Each signal transduction cascade controls a number a transcription factors that will activate or repress the expression of many genes. We analyzed the transcriptional program elicited by stimulation of normal human fibroblasts with growth factors (PDGF or FGF) using microarrays. In several successive analyses, we identified hundreds of regulated transcripts that had not previously been linked to PDGF signaling (6, 10). We also analyzed gene expression in neural stem cells, glioma, carcinoid tumors and leukemic cells (1, 7-10).

One key transcription factor group that is regulated by growth factors is FOXO. These factors induce a cell cycle arrest, increase resistance toward oxidative stress and regulate metabolism. They are inactivated by growth factors via AKT, which phosphorylates three conserved sites within FOXO proteins. Phosphorylated FOXO is excluded from the nucleus and targeted for degradation by proteasomes (Fig. 3). We observed that FOXO mRNA expression is also decreased upon stimulation with growth factors (6). We showed that the promoter of FOXO genes is stimulated by FOXO themselves, a process that is disrupted by growth factors, most likely via AKT, and regulates cell growth. We are now analyzing whether this mechanism could play a role in the proliferation of tumor cells.

In our microarray analysis, a cluster of genes involved in fatty acid and cholesterol biosynthesis, including stearoyl-CoA desaturase (SCD), fatty acid synthase and hydroxy-methylglutaryl-CoA synthase (HMGCS), was up-regulated by

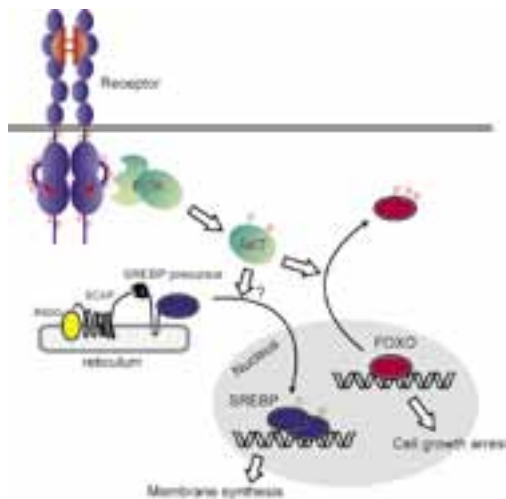


Figure 3. Activation of SREBP and inactivation of FOXO by PDGF

PDGF after 24 h of treatment. Their expression correlated with an increase in membrane lipid biosynthesis. All these genes are known to be controlled by sterol regulatory element-binding proteins (SREBP). PDGF increased the amount of mature SREBP-1, and regulated the promoters of SCD and HMGCS in a SREBP-dependent manner. In line with these results, blocking SREBP processing by addition of 25-hydroxycholesterol blunted the effects of PDGF on lipogenic enzymes and PDGF-driven proliferation. SREBP activation was dependent on the phosphatidylinositol 3-kinase (PI3K) pathway, as judged from the effects of the inhibitor LY294002 and mutation of the PDGF receptor b tyrosine residues that bind the regulatory PI3K subunit p85. In conclusion, our results suggest that PDGF induces membrane lipid synthesis via PI3K and the activation of SREBP (Fig 3. and reference 10). The role of SREBP in PDGF and tumor development will be further analyzed. We went on by identifying novel target genes for the SREBP transcription factors. We found that these transcription factors regulate p55 γ , a subunit of the PI3K complex and heme oxygenase, which plays an important role in stress responses (8). These results expand the list of genes regulated by SREBP to targets that are not directly involved in lipid metabolism. We are now trying to understand more precisely the role of these SREBP target genes in growth factor responses.

TFACTS: A BIOINFORMATICS TOOL TO PREDICT TRANSCRIPTION FACTOR REGULATION FROM MICROARRAY DATA

A. Essaghir, J.B. Demoulin, in collaboration with Jacques van Helden (Université Libre de Bruxelles)

Deciphering transcription factor networks from microarray data remains difficult. We have developed a simple method to infer the regulation of transcription factors from microarray data based on well-characterized target genes (1). We generated a catalogue containing 352 transcription factors associated with 2,721 target genes and 6,422 regulations. When it was available, a distinction between transcriptional activation and inhibition was included for each regulation. Next, we built a tool (www.TFactS.org) that compares new submitted gene lists with target genes in the catalogue to detect regulated transcription factors. We validated TFactS with our own microarray experiments and with published lists of regulated genes in various models and compared it to tools based on in silico promoter analysis. We also analyzed the NCI60 cancer microarray dataset and showed the regulation of SOX10, MITF and JUN in melanomas. Our results show that the expression level of transcription factor target genes constitute a robust signature for transcription factor regulation, and can be efficiently used for microarray data mining. We are now using this tool to analyse cancer genome data.

SELECTED PUBLICATIONS

1. Essaghir A, Toffalini F, Knoops L, Kallin A, van Helden J, Demoulin JB. *Transcription factor regulation can be accurately predicted from the presence of target gene signatures in microarray gene expression data.* **Nucleic Acids Res** 2010; In press.
2. Medves S, Duhoux FP, Ferrant A, Toffalini F, Ameye G, Libouton JM, Poirel HA, Demoulin JB. *KANK1, a candidate tumor suppressor gene, is fused to PDGFRB in an imatinib-responsive myeloid neoplasm with severe thrombocythemia.* **Leukemia** 2010;24:1052-5.
3. Toffalini F, Hellberg C, Demoulin JB. *Critical role of the platelet-derived growth factor receptor (PDGFR) beta transmembrane domain in the TEL-PDGFRbeta cytosolic oncoprotein.* **J Biol Chem** 2010;285:12268-78.
4. Toffalini F, Kallin A, Vandenberghe P, Pierre P, Michaux L, Cools J, Demoulin JB. *The fusion proteins TEL-PDGFRbeta and FIP1L1-PDGFRalpha escape ubiquitination and degradation.* **Haematologica** 2009;94:1085-93.
5. Classen JF, Henrohn D, Rorsman F, Lenartsson J, Lauwerys BR, Wikström G, Rorsman C, Lenglez S, Franck-Larsson K, Tomasi JP, Kämpe O, Vanthuyne M, Houssiau FA, Demoulin JB. *Lack of evidence of stimulatory autoantibodies to platelet-derived growth factor receptor in patients with systemic sclerosis.* **Arthritis Rheum** 2009;60:1137-44.
6. Essaghir A, Dif N, Marbehant CY, Coffey PJ, Demoulin JB. *The transcription of FOXO genes is stimulated by FOXO3 and repressed by growth factors.* **J Biol Chem** 2009;284:10334-42.
7. Leja J, Essaghir A, Essand M, Wester K, Oberg K, Tötterman TH, Lloyd R, Vasmatzis G, Demoulin JB, Giandomenico V. *Novel markers for enterochromaffin cells and gastrointestinal neuroendocrine carcinomas.* **Mod Pathol** 2009;22:261-72.
8. Kallin A, Johannessen LE, Cani PD, Marbehant CY, Essaghir A, Foufelle F, Ferré P, Heldin CH, Delzenne NM, Demoulin JB. *SREBP-1 regulates the expression of heme oxygenase 1 and the phosphatidylinositol-3 kinase regulatory subunit p55 gamma.* **J Lipid Res** 2007;48:1628-36.
9. Demoulin JB, Enarsson M, Larsson J, Essaghir A, Heldin CH, Forsberg-Nilsson K. *The gene expression profile of PDGF-treated neural stem cells corresponds to partially differentiated neurons and glia.* **Growth Factors** 2006;24:184-96.
10. Demoulin JB, Ericsson J, Kallin A, Rorsman C, Rönstrand L, Heldin CH. *Platelet-derived growth factor stimulates membrane lipid synthesis through activation of phosphatidylinositol 3-kinase and sterol regulatory element-binding proteins.* **J Biol Chem** 2004;279:35392-402.

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