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GROWTH FACTOR RECEPTORS

From signal transduction to human diseases

Growth factors such as platelet-derived growth factors (PDGF) are secreted proteins that stimulate cell proliferation via transmembrane receptors. PDGF binds to a receptor-tyrosine kinase which signals by phosphorylating various intracellular proteins on tyrosine residues, leading to the regulation of multiple transcription factors and profound changes in genes expression. Understanding how this network of signaling cascades and transcriptional regulations controls cell growth is the first goal of our team.

The uncontrolled activation of PDGF receptors has been linked to several diseases, such as cancer, leukemia and fibrosis. Our second objective is to clarify the role of PDGF receptors in these diseases and to identify the patients who could benefit from a therapy based on the potent, well-tolerated inhibitors of PDGF receptors.

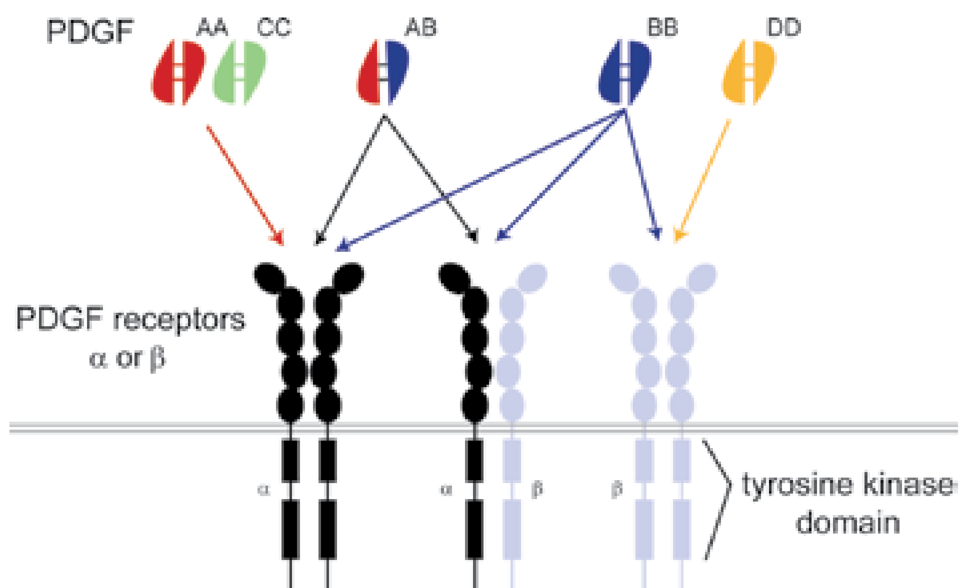

Fig. 1. PDGF receptors and ligands

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

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

Most of the cellular effects of growth factors are mediated by reprogramming gene expression within the cell nucleus. Each signal transduction cascade controls a number a transcription factors, which 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 (1-5). We also analyzed gene expression in neural stem cells, glioma, carcinoid tumors and leukemic cells.

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. 2). We observed that FOXO mRNA expression is also decreased upon stimulation with growth factors (2). 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. We also identified several mediators of the effects of FOXO on the cell cycle.

In our microarray analysis, a cluster of genes involved in fatty acid and cholesterol

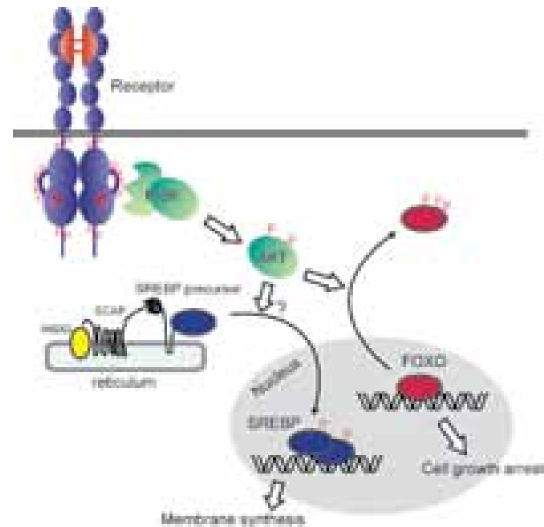

Figure 2. Activation of SREBP and inactivation of FOXO by PDGF

biosynthesis, including stearoyl-CoA desaturase (SCD), fatty acid synthase and hydroxymethylglutaryl-CoA synthase (HMGCS), was up-regulated by 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 β 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 2. and reference 4). 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 p55γ, a subunit of the PI3K complex and heme oxygenase, which plays an important role in stress

responses (3). 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 the 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 J. 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 introducing new features into this tool and we are using it to analyse cancer genome data.

REARRANGEMENTS OF RECEPTOR TYROSINE KINASE GENES ASSOCIATED WITH LEUKEMIA

C. Montano, L. Noël, A. Velghe, J.B. Demoulin, in collaboration with F. Duhoux, V. Havelange and H. Antoine-Poirel (Cliniques Universitaires Saint-Luc, UCL).

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, alterations of PDGF receptor genes, as a result of chromosomal translocation or deletion, are found in chronic myeloid neoplasms associated with hypereosinophilia, a disease which affects mostly male patients. In most cases, the rearranged gene produces a hybrid protein comprising the PDGF receptor tyrosine kinase domain and an oligomerisation domain. They also retain the receptor transmembrane domain, which plays a particular role in the activation of these oncoproteins (6, 8). Similar hybrid oncogenes derive from FGF receptors.

TEL-PDGFRβ (TPβ, also called ETV6-PDGFRB) is a hybrid protein produced by the t(5;12) translocation. FIP1L1-PDGFRα (FPα) results from a deletion on chromosome 4q12 (6). 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, which are quickly degraded upon activation, we observed that TPβ and FPα escaped down-regulation resulting in the accumulation of these oncoproteins in cells. This was confirmed in leukocytes from patients. Similar data were obtained in cells expressing ZNF198-FGFR1, another fusion protein associated with the 8p11 myeloproliferative syndrome. Ubiquitination of TPβ and FPα was much reduced compared to wild-type receptors. We showed that the accumulation of TPβ is required to activate STAT5 efficiently and transform Ba/F3 cells. Thus, chimeric receptor tyrosine kinases escape efficient ubi-

quitination and degradation through lysosomes and proteasomes (9). This is a new mechanism that contributes to cell transformation by fusion kinase.

TPβ and FPα do not induce eosinophilia in mice. In order to develop a model that is more relevant for the human disease, we introduced TPβ and FPα in human CD34+ cells, which were purified from cord blood and are enriched in hematopoietic stem cells. These cells are able to differentiate normally in vitro into various blood cell types, depending on the cytokine cocktail that is added in the culture medium. We observed that TPβ and FPα induce the proliferation of these cells and their differentiation into eosinophils in the absence of cytokine. 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 fusion. 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. 3 and reference 7). We are now looking for other mutations in tyrosine kinase genes.

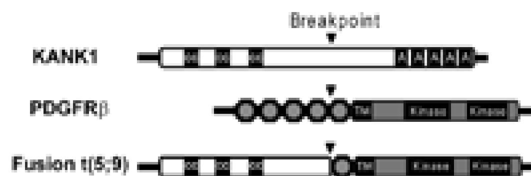

Fig. 3. Structure of the KANK1-PDGFRβ fusion protein created by the t(5;9) translocation.

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

ACTIVATION OF PDGF RECEPTORS IN SYSTEMIC SCLEROSIS

S. Charni, J.B. Demoulin, in collaboration with B. Lauwerys and F. Houssiau (Cliniques Universitaires Saint-Luc, UCL).

Systemic sclerosis (also called scleroderma) is a severe connective tissue disease of unknown etiology characterized by vascular alterations, autoimmunity and fibrosis of the skin and multiple internal organs, which is potentially fatal. 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 receptors 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 (10). Four 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.

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