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Introduction

The aim of the work at the Uppsala Branch is to elucidate the signaling pathways in cells that control cell growth, survival and migration. As malignant cells are characterized by perturbations in such pathways, we hope that our work will reveal suitable target molecules for the development of signal transduction modulators, which can be used in the treatment of cancer patients.

We have a longstanding interest in two growth regulatory molecules, *i.e.* platelet-derived growth factor (PDGF) and transforming growth factor- β (TGF- β). PDGF is a family of dimeric isoforms of A-, B-, C- and D-polypeptide chains, which exert their effects on target cells by binding to α - and β -tyrosine kinase receptors. PDGF isoforms are major mitogens for connective tissue cells and certain other cell types, and are implicated in autocrine as well as paracrine stimulation in tumors. Our aim is to elucidate the molecular mechanisms of signal transduction downstream of PDGF receptors, and to explore the clinical utility of PDGF antagonists.

The TGF- β family consists of 33 members, including TGF- β isoforms, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs) and activins. They all act via heteromeric complexes of type I and type II serine/threonine kinase receptors, and have important roles during the embryonal development. TGF- β most often inhibits the growth of cells, and also promotes apoptosis, matrix production and cell differentiation. TGF- β has a complicated role in cancer. Initially, it is a tumor suppressor through its ability to inhibit cell growth and stimulate apoptosis. At later stages of tumor progression, however, TGF- β becomes a tumor promoter by induction of epithelial-mesenchymal transition, which makes cancer cells more invasive, and by stimulating angiogenesis and suppressing the immune system. Our aim is to elucidate the molecular mechanism whereby TGF- β acts, and to explore whether selective TGF- β antagonists can be developed, which would inhibit the tumor promoting effects of TGF- β while leaving the tumor suppressive effects unperturbed. Our ultimate goal is to investigate the clinical usefulness of such antagonists.

In addition to the work on PDGF and TGF- β , groups at our Branch study other signaling mechanisms of direct relevance for cancer, including members of the Rho family of GTP binding proteins, the transcription factor SREBP, and the polysaccharide hyaluronan and its receptor CD44.

During the year Rainer Heuchel, the Group Leader for the Gene Targeting Group, left the Branch for a position at the Karolinska Institute in Stockholm. Rainer has been instrumental in the introduction of mouse models at our Branch, which has been of utmost importance for many of our groups. We wish Rainer the best success in his future work and are looking forward to future interactions with him.

Our Branch is located at the Biomedical Center in Uppsala, using laboratory space provided by Uppsala University. Some of our progress during last years is described on the following pages.

C.-H. Heldin

PDGF Signaling Group

Introduction

Tyrosine phosphorylation of proteins is essential in signal transduction pathways that regulate cell growth, survival and death, as well as adhesion, migration and differentiation. Both protein tyrosine kinases and protein tyrosine phosphatases control cellular phosphotyrosine levels. Amplification of genes encoding tyrosine kinase receptors have been described in several forms of tumors. Moreover, increased expression of both ligand and receptor occur within certain tumors, causing autocrine receptor activation. It has also been suggested that growth factors synthesized by activated stromal cells participate in the growth and survival of tumor cells. In addition, tyrosine kinase receptors are involved in the signals inducing tumor vascularization, a process that is necessary for both growth and metastasis of tumors.

The appreciation of the role of tyrosine kinases in the generation and progression of cancer has led to development of a number of anti-tumor drugs that specifically target tyrosine kinases. For example Gleevec, which targets the Bcr-Abl kinase, the PDGF receptors and c-Kit, has been successfully used in treatment of chronic myeloid leukemia and gastrointestinal stromal tumors. Elucidation of the molecular mechanisms underlying tumor formation should generate new target-specific drugs. Moreover, improved screening of patient material to determine the pathogenic alterations in the cancer cell signaling network for each tumor, may make it possible to individualize tumor treatment.

Section for Translational Research

The research in the Translational Research section is focused on the following two aims:

- Evaluation of PDGF receptors as cancer drug targets
- Elucidation of mechanisms for termination of PDGF β -receptor signal transduction

PDGF receptors as cancer drug targets

PDGF receptor signaling is implicated in various cancer associated processes, including autocrine stimulation of growth, stimulation of tumor fibroblasts and promotion of tumor angiogenesis. During angiogenesis, PDGF receptor signaling is important for the

recruitment of pericytes to the newly formed vessels, a process needed for the stabilization and function of the newly formed vasculature. The presence of pericytes on vessels has been proposed to protect endothelial cells from anti-angiogenic tumor therapy targeting the VEGF pathway. Thus, PDGF receptors on pericytes could provide a novel target for anti-angiogenic therapy. To investigate this possibility, we used the B16 mouse melanoma tumor model where PDGF-dependent pericyte recruitment contributes to tumor growth. Treatment of B16 tumors with a combination of PDGF and VEGF receptor inhibitors, but not single agent treatment, significantly reduced tumor growth (9). This effect was more pronounced in the fast-growing tumors with a pericyte-rich vasculature, and coincided with a reduction of the number of immature pericytes. Pericytes expressing the differentiation marker desmin were unaffected, identifying these cells as a subset of pericytes resistant to combined anti-PDGF and anti-VEGF therapy. The growth inhibition was associated with vascular remodeling, including an increase in tumor cell apoptosis and a decrease in proliferation. This suggests that PDGF receptor antagonists could potentiate anti-angiogenic treatment in mature tumors, which are relatively insensitive to VEGF receptor inhibitors.

In experimental models, inhibition of PDGF receptors on stromal cells increases tumor uptake of low molecular weight chemotherapy drugs concurrently with lowering of the interstitial fluid pressure (IFP) in tumors. VEGF receptor inhibitors also lowers tumor IFP, and we are currently investigating the effects of the combination of PDGF and VEGF receptor inhibitors on Kat4 tumor IFP. Both agents lowered the tumor IFP when given as monotherapies, with increasing effects over a four day treatment period. Surprisingly, combination therapy given for four days resulted in tumors displaying the same IFP as vehicle treated tumors. However, when PDGF receptor inhibitor was given for four days and the VEGF receptor inhibitor for the last two days of therapy, the tumor IFP was further reduced compared to either monotherapy. These data clearly demonstrate the need of identifying the optimal treatment regimes for combination therapies. We are currently investigating the effects of these therapies on vessel perfusion and leakiness.

In addition, we are continuing the studies on the mechanism(s) underlying the role of PDGF in the regulation of tumor IFP, focusing on the roles of the PI3-kinase and $\beta 3$ integrins (in collaboration with K. Rubin, Uppsala University). Furthermore, the possibilities that targeting of PDGF receptors on stromal cells could increase the tumor uptake of therapeutic antibodies, as well as lowering tumor hypoxia, are under investigation.

Ongoing clinical studies with targeted therapies have emphasized the need for activation-specific reagents to monitor target presence and activity. We are therefore developing novel methods for monitoring PDGF receptor status in tissues. As a consequence of the discovery of PDGF-CC and -DD, we have also generated neutralizing PDGF-CC antisera to evaluate the role of PDGF-CC in disease processes.

Termination of PDGF β -receptor signal transduction

Following ligand stimulation, the PDGF β -receptor signaling is terminated by dephosphorylation of the receptor autophosphorylation sites in parallel with receptor internalization and subsequent degradation. We have identified the T-cell protein tyrosine phosphatase as a site-selective regulator of PDGF β -receptor phosphorylation. The screening for tyrosine phosphatases that are regulating PDGF β -receptor phosphorylation and signal transduction is being continued, with a current focus on the LAR phosphatase. Initial experiments showed that LAR, unexpectedly, was needed for maximum PDGF β -receptor phosphorylation to occur. It is possible that this phosphatase is required for activation of other kinases, such as Src family kinases, that participate in PDGF β -receptor phosphorylation.

The increased PDGF β -receptor phosphorylation observed in mouse embryo fibroblasts deficient in T-cell phosphatase is paralleled by a pronounced decrease in clearance of activated receptors from the cell surface and delayed receptor degradation. We found that this is due to an induction of PDGF β -receptor recycling, which occurs through Rab 4 positive recycling endosomes (Karlsson, *et al.*, Mol. Biol. Cell 17, 4846-4855, 2006). Recycling of the PDGF β -receptor is also induced by stably knocking down the T-cell phosphatase in HeLa cells, providing a model system for studying which domains of the receptor that are required for sorting towards recycling. We are currently investigating the molecular mechanisms underlying the regulation of PDGF β -receptor trafficking, and have identified activation of protein kinase C (PKC) alpha as a critical component in the sorting of the PDGF β -receptor into Rab 4 positive recycling endosomes. PKC activation was found to be both necessary and sufficient for the induction of receptor recycling in both these model systems. The downstream target of PKC is currently being investigated.

Section for PDGF Signal Transduction

A primary function of growth factors, such as PDGF, is to transmit signals into the cell which are critical for cell division, and consequently, perturbations of these signals may

result in cancer. The aim of the work performed in the PDGF Signal Transduction Section is to elucidate the molecular mechanisms employed in PDGF-induced signal transduction.

MAP kinase phosphatase 3 in PDGF-induced Erk MAP kinase activation

MAP kinase phosphatases (MKPs) are dual specificity phosphatases that dephosphorylate and thereby inactivate MAP kinases. We found that MKP3 has an important role in the regulation of Erk phosphorylation after PDGF stimulation. In an early phase after PDGF stimulation Mek is activated and targets MKP3 for proteasomal degradation, which is necessary for Erk activation (Jurek *et al.*, submitted for publication). At a later stage, the *mkp3* gene is induced and MKP3 synthesized leading to efficient Erk dephosphorylation. Both the degradation and subsequent synthesis of MKP3 are dependent on activation of the Erk pathway indicating that they represent feed-forward and feed-back mechanisms, respectively. siRNA mediated silencing of MKP3 results in enhanced Erk phosphorylation and DNA synthesis in response to PDGF. Furthermore, we have preliminary data suggesting that differential recruitment of RasGAP to the α - and β -receptors for PDGF may, at least in part, contribute to the different kinetics of Erk phosphorylation downstream of these two receptor isoforms; PDGF α -receptor induces a transient and biphasic Erk phosphorylation whereas PDGF β -receptor leads to sustained Erk activation. In summary, RasGAP is important for the kinetics of Erk activation and MKP3 primarily for the intensity; differences in the regulation of RasGAP and MKP3 downstream of the α - and β -receptor may explain the differences in Erk activation downstream of these receptor isoforms.

The role of Alix in PDGF β -receptor downregulation

The process of receptor downregulation is important for the cell to respond in a controlled manner to growth factors, and malfunctions in this process may lead to disturbances in signal transduction which may result in cancer. We are currently investigating the role of an adaptor or scaffolding protein denoted Alix in the process of PDGF β -receptor downregulation. We found that siRNA-mediated Alix downregulation resulted in a stronger and more sustained receptor ubiquitination compared to mock-transfected controls. However, when performing a similar experiment under conditions that prevent receptor internalization we could no longer observe a significant difference in receptor ubiquitination. Thus, we hypothesize that Alix may function after internalization, potentially on the early endosomes in conjunction with the ESCRT machinery, and may recruit deubiquitinating enzymes to the receptor. Furthermore, we

found that when Alix was silenced PDGF could no longer efficiently induce tyrosine phosphorylation of the ubiquitin ligase c-Cbl and the complex formation between PDGF β -receptor and Cbl was reduced. Our current data suggests an important function of Alix in regulation of PDGF β -receptor ubiquitination.

PDGF-induced changes in mRNA levels

Growth factors may increase protein production either by enhancing gene transcription or mRNA stability. We are investigating the role of G3BP in PDGF signal transduction. G3BP has an RNase activity, intrinsic or associated, and has been suggested to participate in degradation of c-myc mRNA. Thus, G3BP may connect the PDGFR to mRNA stability. This possibility has been addressed by downregulating G3BP using siRNA and evaluating the consequence on PDGF-induced expression of c-myc, MKP1, and MKP3 using quantitative real-time PCR. Hitherto we have observed different effects on the various genes tested; downregulation of G3BP expression enhanced PDGF-induced *c-myc* expression and suppressed *mkp1* expression, but had no significant effect on the induction of *mkp3*. Current work focuses on understanding the details on how G3BP affects mRNA levels. The signaling pathways leading to PDGF-induced *c-myc* induction are also under investigation.

Involvement of Vav2 in PDGF mediated cell migration

Using a synthetic peptide corresponding to the sequence containing Tyr771 in the PDGF β -receptor we were able to identify Vav2 as an interacting protein using mass spectroscopy. The interaction between the PDGF β -receptor and Vav2 was confirmed by co-immunoprecipitation. Vav2 has been described as an exchange factor for Rac and studies have linked Rac to activation of the JNK MAP kinases. Earlier studies from our group have shown that JNK is a critical component of the machinery that mediates PDGF-induced cell migration. Current work aims to clarify the role of Vav2 in chemotaxis toward PDGF and if the differential recruitment of Vav2 between the PDGF α -receptor, which lacks a tyrosine residue corresponding to Tyr771, and the PDGF β -receptor may contribute the different abilities of these receptor isoforms to mediate cell migration.

Cytoskeletal Regulation Group

The work within the Cytoskeletal Regulation Group is aimed at elucidating signalling pathways that control cell growth and cell migration during normal physiological conditions as well as during disease.

Atypical Rho GTPases

The Rho GTPases are key regulators of cell morphogenesis and cell migration. Current cladistics analysis has determined that the family consists of 20 members. We have established that the Rho GTPases can be further divided into two classes, the classical and the atypical Rho GTPases (36). The atypical Rho GTPases either have reduced GTP hydrolysis activity or they have increased nucleotide exchange activity. As a result, they are constitutively in the active, GTP-bound, conformation. We have focused our attention to the atypical Rho GTPases *Wrch-1* and *RhoBTB*.

Wrch1

We have previously shown that ectopic expression of *Wrch1* in fibroblasts resulted in an altered cell morphology visible as a formation of filopodia, a loss of stress fibres and a reduction in focal adhesions. A yeast two-hybrid system screen identified the non-receptor tyrosine kinase *Pyk2* as a binding partner for the activated form of *Wrch1* (34). *Wrch1* requires *Pyk2* in imposing the cytoskeletal effects, seen as the formation of filopodia, since treatment of cells with a *Pyk2*-specific siRNA abrogated this response. We found that the presence and activity of *Src* was needed for the formation of a *Wrch1*-*Pyk2* complex as well as for the *Wrch1*-induced formation of filopodia.

RhoBTB

There are three human *RhoBTBs* and they each contain two BTB (Broad-Complex, Tramtrack and Bric à brac) domains. The *RhoBTBs* have been proposed to function as tumor suppressors. We noticed that transiently transfected *RhoBTBs* localised to proteasomes and we have hypothesised that they might be involved in regulating proteasomal degradation of specific target proteins. *RhoBTB2* has previously been found to interact with cullin-3 and we have seen that all three *RhoBTBs* bind cullin-3 and the ring-finger domain-containing protein *ROC1*. These observations suggest that the *RhoBTBs* can function as an adapter protein bringing cullin-3 (and maybe other cullins) and *ROC1* together creating a functional ubiquitin E3 ligase. This *RhoBTB*/cullin/*ROC1* complex could then target specific substrates, ubiquitinate them

and target them for degradation. We are currently in the process to identify substrates for the RhoBTB/cullin/ROC1 ubiquitin ligase.

Miro GTPases - regulators of mitochondrial homeostasis

Miro (mitochondrial Rho) GTPases are localised to the outer mitochondrial membrane. Ectopic expression of a constitutively active Miro resulted in a collapse of the mitochondrial network, indicating that the GTP-loaded status of Miro proteins is of importance for the heterogeneity of the mitochondria. Mitochondria are under a constant and dynamic reorganisation and Miro appears to be needed for the transport of mitochondria along microtubules by binding to the kinesin binding proteins OIP106 and GRIF-1. By using the yeast two-hybrid system, we have identified a number of Miro-binding proteins. We are studying how these proteins affect Miro function.

F-BAR proteins - linking the actin polymerisation machinery and membrane dynamics

The Cdc42-binding protein 4 (CIP4) was shown to have a role in the Cdc42-dependent regulation of the actin filament system. There are three members of the CIP4-like proteins: CIP4, Formin-binding protein 17 (FBP17) and Transducer of Cdc42-dependent actin assembly-1 (Toca-1). These proteins have N-terminal F-BAR domains and C-terminal SH3 domain. Between these two domains resides a HR1 domain, which in CIP4 and Toca-1 has been shown to bind Cdc42. The F-BAR domain has been shown to bind phospholipids in membranes and to induce a curvature of the membranes resulting in an invagination of the lipid bilayers. The membrane dynamics induced by the CIP4-like proteins can be seen as the formation of long, F-actin rich, tubules in the cytoplasm. We are currently studying the impact of CIP4 like proteins on cell migration and internalisation and signalling by receptor tyrosine kinases.

Gene Targeting Group

This group makes use of gene targeting in the mouse to explore the *in vivo* importance of specific signaling pathways initiated by different growth factors.

Analysis of PDGF β -receptor function by the use of knock-in mice

Increased PDGF signaling is a hallmark of many diseases, such as fibrosis, atherosclerosis and solid tumors. In order to investigate the aberrant signaling

mechanisms of constitutively increased PDGF β -receptor activity in disease development, we generated a mouse with a gain of function point mutation in the activation loop of the second kinase domain (D849N). Analogous mutations in the hepatocyte growth factor receptor and the stem cell factor receptor have been found in patients with hereditary papillary renal carcinoma and mastocytosis, respectively. The D849N mutation conferred increased transforming characteristics to ligand-stimulated mouse embryonic fibroblasts derived from mutant mice. By comparing the enzymatic properties of the wildtype versus the mutant receptor protein, we demonstrated that the D849N mutation lowers the threshold for kinase activation, causes a dramatic alteration in the pattern of tyrosine phosphorylation kinetics following ligand-stimulation but induces also significant ligand-independent phosphorylation of several tyrosine residues. These changes resulted in deregulated recruitment of specific signal transducers. For instance, the p85 regulatory subunit of the phosphatidylinositol-3'-kinase (PI3K) is constitutively associated with the mutant receptor and this ligand-independent activation of the PI3K pathway results in protection against apoptosis and increased motility in cellular wounding assays. Moreover, we have observed enhanced ligand-independent ERK1/2 activation and an increased proliferation of mutant cells. Although the D849N-mutant mice did not display any overt phenotype, we observed interesting differences compared to wildtype mice upon specific challenges, underlining the important function of PDGF in the stromal compartment. In an orthotopic tumor mode, we found that B16 melanoma established faster in D849N-mutant mice compared to wildtype mice and were more rich in stroma, whereas the final tumor size was not affected (23). Similarly, the early responses in two different wound healing models (CCl₄-induced liver fibrosis, skin wound healing) were accelerated in D849N-mutant versus wildtype mice, without showing any differences in the late response phases (Krampert *et al.*, submitted for publication).

Recently, an activation loop mutation in the PDGF α -receptor was reported to be responsible for a certain percentage of gastrointestinal stromal tumors in human. We have generated ES-cells with the same D-to-V mutation in the β -receptor (D849V). In sharp contrast to the previously generated D849N mutant mice, which are fully viable, the D849V mutation has considerably stronger *in vivo* effect. Targeted ES-cells carrying the D849V mutant PDGF β -receptor do not generate viable chimeras. Chimeric placentas, for instance displayed a largely disorganized labyrinthine layer, unable to support nutrient waste exchange between the maternal and fetal vascular systems (16). In order to examine the differentiation potential of D849V-mutant ES-cells, we set up a collaboration with the group of Dr. Lena Claesson-Welsh, Uppsala University. We found that the D849V mutant PDGF β -receptor exerts a strong

vasculogenic and angiogenic effect in an *in vitro* assay for differentiating embryonic stem cells, as well as *in vivo* (teratoma generated from embryonic stem cells injected into nude mice). This effect could be explained by increased VEGF-A expression in differentiating embryonic stem cells. In addition, we found evidence that also the D849V mutant PDGF β -receptor resulted in increased endothelial/perivascular lineage commitment of hemangio-precursors and thus contributed to the increased vasulogenic/angiogenic effects observed *in vitro* and *in vivo* (17).

Regulation and *in vivo* function of Smad7

The TGF- β family members, which include TGF- β s, activins and BMPs, are secreted molecules that regulate a plethora of cellular responses, such as proliferation, differentiation, migration and apoptosis. Deregulated TGF- β family signaling has been implicated in multiple disorders and in various human diseases, including cancer, fibrosis and autoimmune diseases. TGF- β family members signal through specific type I and type II serine/threonine kinase receptors, which in turn activate a subset of Smad proteins. These molecules relay signals into the nucleus where they direct transcriptional responses in concert with other proteins. The mRNA expression of a particular member of this family, namely Smad7, is induced by TGF- β itself. Overexpression of Smad7 lead to downregulation of TGF- β signaling, suggesting an auto-regulatory feedback mechanism. We investigated the mouse Smad7 promoter and found not only an essential DNA binding site for the TGF- β activated Smads 2, 3 and 4, but also the requirement for cooperation of these Smads with Sp1 and Ap1 transcription factors, in order to guarantee an efficient TGF- β response of the Smad7 promoter.

In order to learn more about the *in vivo* function of Smad7, we targeted the Smad7 gene in mice in collaboration with Dr. Tony Pawson's lab in Toronto, Canada. In line with the role of TGF- β as a major player in the immune system we observed increased immunoglobulin class switching activity towards IgA, as well as an elevated growth suppressing effect of TGF- β on B-cells, both of which can easily be explained by lack of Smad7 function. Likewise, in collaboration with the lab of Steven Dooley we observed strongly increased liver fibrosis in Smad7-mutant mice following chronic CCl₄-stimulation (26, 29). Recently, we uncovered an unexpected and potentially important function of Smad7 in adult tissue stem cells (Krampert *et al.*, in preparation). Together, our observations underscore the important function Smad7 plays in modulating TGF- β signalling activity *in vivo*.

TGF- β Signaling Group

The TGF- β Signaling Group centers its focus on epithelial cell biology and investigates how transforming growth factor β (TGF- β) regulates epithelial cell growth, differentiation and cellular processes of tumor invasiveness and metastasis. Current activities also branch towards normal and cancer stem cell biology.

Regulatory mechanisms in the TGF- β /Smad signaling pathway

TGF- β family members, including bone morphogenetic proteins (BMPs), signal via receptor serine/threonine kinases (52). TGF- β receptors phosphorylate Smads and alternative signaling proteins in the cytoplasm. The TGF- β receptor-activated Smads (R-Smads) pair together with the Co-Smad (Smad4), and rapidly accumulate in the nucleus. The nuclear Smads bind to chromatin and to many other transcription factors and regulate expression of a few hundred genes. A primary TGF- β -responsive gene is Smad7 that codes for an inhibitory Smad (I-Smad). Smad7 blocks the transcriptional function of the other Smads and also binds to the TGF- β receptors. When bound to the receptors, Smad7 inhibits R-Smad phosphorylation by the receptors, recruits receptor phosphatases and ubiquitin ligases of the Smurf family that regulate TGF- β receptor internalization and turnover.

We currently analyze nuclear proteins that interact with the Smads and regulate the function of chromatin. We also concentrate on factors that regulate how long time Smads stay in the nucleus. Such factors include proteins that regulate the addition of ubiquitin to Smads. Via large scale gene expression analysis, we identified a new TGF- β -responsive gene, SNF1-like kinase (SNF1LK, also called salt-inducible kinase SIK), whose serine/threonine kinase product regulates turnover of the TGF- β receptor after ligand binding (Kowanetz *et al.*, submitted for publication). SNF1LK binds to the inhibitory Smad7 and cooperates with the Smurf ubiquitin ligases to downregulate the TGF- β type I receptor. SNF1LK/SIK belongs to the family of AMP-regulated kinases (AMPKs), which are thought to be regulated by the master kinase and tumor suppressor LKB1.

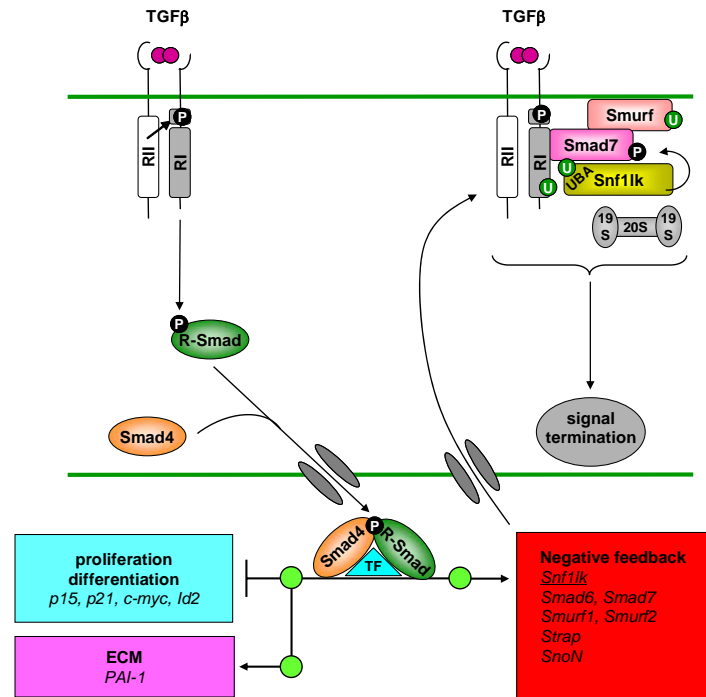


Figure 1. Model of the role of SNF1LK as a negative regulator of TGF- β signaling. TGF- β receptor-mediated activation of nuclear Smad complexes with transcription factors (TF) mediate transcriptional responses (green circles). Negative feedback loop protein products assemble the machinery that inhibits signaling. SNF1LK protein associates with the TGF β type I receptor (RI) and Smad7 and phosphorylates Smad7 (P). SNF1LK, via its UBA domain, binds to Smad7 which is ubiquitinated (U) by Smurf2, while recruiting proteasomes (19S/20S complexes), thus promoting internalization and termination of receptor signaling.

The gene encoding LKB1 is a tumor suppressor in humans, and is mutated in people with the Peutz-Jeghers Syndrome (PJS). PJS patients develop benign hamartomatous polyps and are predisposed to intestinal or other forms of cancer. PJS resembles the Juvenile Polyposis Syndrome (JPS), in which children inherit mutations that inactivate Smad4 or the BMP type I receptor, BMPR1A. We investigate the molecular links between the Smad4, BMPR1A and LKB1 pathways. Similar to SNF1LK/SIK, our current evidence supports mechanisms whereby LKB1 negatively regulates TGF- β and BMP signaling components in human epithelial cells. We have therefore uncovered the first molecular links between TGF- β , BMP and LKB1 signaling.

Regulation of epithelial cell proliferation and epithelial-mesenchymal transition by TGF- β

The TGF- β pathway acts as a tumor suppressor based on the ability of TGF- β to inhibit cell proliferation and induce apoptosis (41). TGF- β also induces epithelial-mesenchymal transition (EMT) that facilitates carcinoma cell invasiveness and metastasis (40, 53; Savary et al., in preparation). In addition, TGF- β regulates the

function of cancer-associated fibroblasts in the tumor stroma, thus modulating the tumor suppressor and pro-metastatic actions of this pathway (51).

An important factor that explains the tumor suppressor activities of TGF- β is the cell cycle inhibitor p21. Based on previous transcriptomic screens, we identified the homeobox transcription factor Meox2, which is responsible for the sustained and prolonged induction of *p21* gene expression by TGF- β (24). In addition, we found that TGF- β regulates expression of Notch pathway ligands and receptors. Notch regulation by TGF- β is important during epithelial cell cycle arrest, as inhibition of Notch signaling abrogates growth inhibition by TGF- β (19). This cross-talk mechanism depends on cooperative regulation of *p21* gene expression by the TGF- β and Notch pathways.

In an effort to understand the process of EMT deeper, we established a new transcriptional mechanism based on which, TGF- β , via Smads and via the transcription factor HMGA2, induces expression of the transcriptional repressor Snail, a master regulator of EMT (Thuault *et al.*, submitted for publication). We extend our analysis of *Snail* gene regulation by TGF- β and other pathways that cause EMT. We also examine the role of another EMT mediator, Twist, downstream of TGF- β . We also found that long-term exposure of mammary epithelial cells to TGF- β promotes EMT and enhances tumor growth (28). In addition, we analyze the function of LKB1 and SNF1LK/SIK in the process of EMT. Finally, we try to demonstrate that EMT plays important roles in regulating cancer stem cell differentiation. We have therefore unraveled a complex regulatory network that defines the cytostatic and EMT programs of epithelial cells in response to TGF- β . Our findings have direct relevance to the mechanisms by which TGF- β acts as a tumor suppressor or a pro-metastatic factor and direct our research towards novel therapeutic approaches.

Mathematical modeling of TGF- β signaling

Using bioinformatics and mathematical modeling we analyze the kinetics of TGF- β signaling and downstream target gene regulation in epithelial cells. In addition, we analyze the evolutionary origins of the TGF- β superfamily pathways based on new algorithms and sequence data of all available metazoan genomes.

Apoptotic Signaling Group

TGF- β plays an important role in the regulation of cell fate, *i.e.* proliferation, migration, differentiation and apoptosis, during embryogenesis. TGF- β inhibits proliferation and induces apoptosis in most normal cell types, and therefore acts as a tumor suppressor, while at late stages of tumor progression TGF- β instead promotes tumorigenesis by promoting tumor cell invasiveness, stimulating angiogenesis and suppressing the immuno-surveillance. Smad7 is a target gene for TGF- β and we have demonstrated that Smad7 is required for TGF- β -induced apoptosis in prostate cancer cells as well as in human keratinocytes. Furthermore, we have reported that Smad7 facilitates activation of the TGF- β activating kinase 1 (TAK1), mitogen activated protein kinase kinase 3 (MKK3) and p38 mitogen activated protein (MAP) kinase pathway, presumably by acting as an adaptor protein bringing the kinases close to each other. The aim of our work is to in detail investigate the molecular mechanisms for the activation of the TAK1 – p38 MAPK pathway by cytokines like TGF- β , TNF- α and IL-1, as well as the potential cross-talks between these inflammatory cytokines. We also continue to investigate the targets for Smad7 and p38 in the TGF- β -induced apoptotic signalling pathway.

Molecular mechanisms for TGF- β -induced apoptosis in prostate cancer cells

We have observed increased expression of the receptor-activated Smads (Smad2 and Smad3), the common-mediator Smad4, as well as the inhibitory Smad6 and Smad7, in normal and malignant prostate epithelial cells *in vivo*, preceding apoptosis induced by androgen withdrawal. The presence of Smad7 in apoptotic cells *in vivo*, together with our previous observation that Smad7 is required for induction of apoptosis, encourage us to continue our search for the detailed molecular mechanisms for how Smad7 can act as a mediator for TGF- β -induced apoptosis in epithelial cells.

We have identified the tumor suppressor p53 as one target downstream of the active Smad7-p38 complex. We found that Smad7 as well as p38 MAPK are required for TGF- β -induced phosphorylation of p53 on Ser15 in a number of human epithelial cells preceding apoptosis. Interestingly, Smad7 was also required for TGF- β -induced activation of the ataxia teleangiectasia mutated (ATM) kinase. As we observed that Smad7 interacts both with p53 and the ATM kinase, which is known to activate p53, we suggest that Smad7 can act as an adaptor protein in this pathway (Zhang, *et al.*, Cell Cycle 5, 2787-2795, 2006). We also observed that Smad7 might play an important role to prevent genomic instability in prostate cancer cells. We plan to further investigate the

role for Smad7 in prostate cancer by investigating the expression of Smad7 in human prostate cancer tissues as well as the role for Smad7 in prostate cancer cell growth and survival.

We have found that the TGF- β receptor interacts with TAK1, and that activation of TAK1 requires Lys63-dependent ubiquitination regulated by a specific E3-ligase. This molecular modification of TAK1 determines the signaling pathway activities downstream of the active receptor complex (Sorrentino *et al.*, submitted for publication). This novel finding provides a molecular mechanism for non-Smad signaling downstream of the TGF- β receptors.

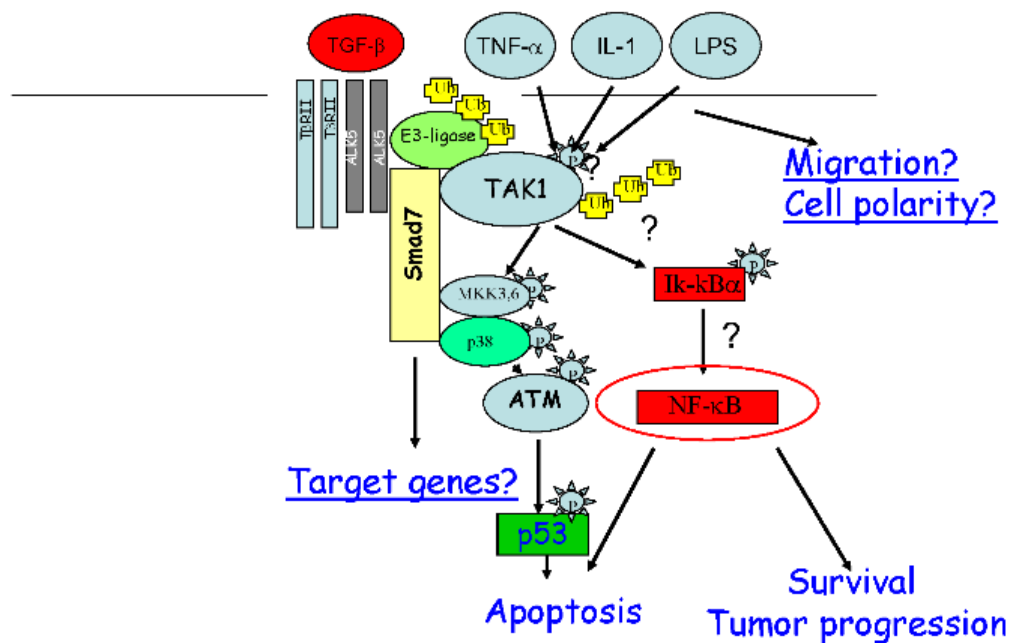


Figure 2. Role of an E3-ligase in TGF- β signaling

Does Smad7 act in a cross-talk between TGF- β and Wnt signaling?

Signaling molecules downstream of TGF- β and Wnt receptors regulate both cell fate and proliferation during development and tissue homeostasis. We have recently reported that Smad7 interacts with β -catenin and lymphoid enhancer binding factor 1/T-cell-specific factor (LEF1/TCF), transcriptional regulators in Wnt signaling in a TGF- β -dependent manner. Furthermore, by the use of siRNA and anti-sense techniques, we have shown that Smad7 expression is required for TGF- β -induced stabilization of β -catenin, increase of c-Myc and subsequent apoptosis in human prostate cancer cells as

well as in immortalized human keratinocytes. Interestingly, we observed that Smad7 together with p38 regulates the activity of Akt and glycogen synthetase kinase-3 β (GSK-3) which in turn leads to the stabilization of β -catenin (Ekman M. *et al.*, submitted for publication). We are now exploring by which molecular mechanisms TGF- β regulates the activity of Akt and GSK-3 β . We will also continue to further examine the underlying molecular mechanisms by which Smad7 and p38 MAPK affect the components in the Wnt-signalling pathway.

We have found that Smad7 expression is required for TGF- β -mediated cytoskeletal regulation, which is important for migration of cells and occurs mainly via the small GTP-ase Cdc42, in collaboration with Dr. Aspenström, Cytoskeleton Regulation Group LICR, Uppsala. Smad7 interacts with components in the Wnt-signalling pathway and is crucial for TGF- β -induced migration of prostate cancer cells (Ekman M. *et al.*, submitted for publication).

Smad7 target genes

Smad7 is a nuclear protein, which upon TGF- β stimulation of cells is exported to the cytoplasm as previously reported by us. We are very interested in the potential effects of Smad7 on gene regulation and have therefore performed microarray analyses on cells overexpressing Smad7. Candidate genes are presently being validated by RT-PCR and ChIP analysis (Thakur *et al.*, manuscript in preparation).

Shb in apoptosis

In collaboration with Michael Welsh and Padideh Davoodpour, Uppsala University, have we investigated the role for the adaptor-protein Shb in apoptosis. Interestingly, we found that overexpression of Shb causes activation of an apoptotic signalling pathway which involves activation of the tyrosine kinase c-Abl (6).

Gene Expression Group

Our group is interested in how post-translational modifications regulate the activity of transcription factors. As model proteins, we have selected certain transcription factors involved in the regulation of cell growth and proliferation.

SREBPs – Key regulators of lipid metabolism and the cell cycle

Members of the SREBP family of transcription factors control cholesterol and lipid homeostasis and play important roles during adipocyte differentiation (37). We hypothesize that compounds that enhance the stability of SREBPs should increase the levels of transcriptionally active SREBP in cells. Such compounds could potentially be used to enhance the cholesterol-lowering activities of statins.

Transcriptionally active SREBP molecules are rapidly degraded by the ubiquitin-proteasome pathway. The ubiquitin ligase Fbw7 has been shown to control several proteins critical for cell cycle control, and is inactivated in cancers of the breast, endometrium, ovary and colon. We previously demonstrated that Fbw7 enhances the ubiquitination and degradation of SREBP following GSK-3-mediated phosphorylation of two specific residues in SREBP, Thr426 and Ser430. We have also found that the GSK-3 β -dependent phosphorylation of these residues in SREBP1 is enhanced in response to DNA binding. DNA binding enhances the direct interaction between the C-terminal domain of SREBP1 and GSK-3 β . Accordingly, we could demonstrate that GSK-3 β was recruited to the promoters of SREBP target genes *in vivo*. As a result of the phosphorylation of Thr426 and Ser430 in response to DNA binding, Fbw7 was recruited to SREBP molecules associated with target promoters. Using a reconstituted ubiquitination system, we could demonstrate that Fbw7-mediated ubiquitination of SREBP1 was dependent on its DNA binding activity. Thus, DNA binding could provide a link between the phosphorylation, ubiquitination and degradation of active transcription factors. Fbw7 and its interaction with SREBPs may be attractive targets for developing new cholesterol-lowering therapies to prevent cardiovascular disease.

The SREBP family of transcription factors regulates genes involved in the synthesis of lipids, which are required for the formation of cell membranes. We recently found that SREBP-mediated transcription is regulated during the cell cycle as a result of specific modifications of the SREBP proteins. We found that the mature forms of SREBP1a and SREBP1c are hyperphosphorylated in mitotic cells, giving rise to a phosphoepitope recognized by the mitotic protein monoclonal-2 (MPM-2) antibody. We also found that mature SREBP1 was stabilized in a phosphorylation-dependent manner during mitosis. We have now mapped the major MPM-2 epitope to a serine residue, S439, in the C-terminus of mature SREBP1. Using phosphorylation-specific antibodies, we could demonstrate that endogenous SREBP1 is phosphorylated on S439 during mitosis. Our results suggest that Cdk1-mediated phosphorylation of S439 is required for the hyperphosphorylation and stabilization of mature SREBP1 during mitosis, thereby

preserving a critical pool of active transcription factors to support lipid synthesis. This possibility was supported by our observation that siRNA-mediated inactivation of SREBP1 arrested cells in the G1 phase of the cell cycle, thereby attenuating cell growth. It may be important to stabilize nuclear SREBP1 during mitosis, since the membrane systems that regulate the cleavage of the precursor form of the protein is dispersed during this phase of the cell cycle. We hypothesize that the hyperphosphorylated, stable, form of SREBP1 is associated with specific target genes during mitosis, ready to activate these genes as cells leave mitosis and an active chromatin structure is reformed. Thus, we speculate that SREBP1 could be involved in an epigenetic mechanism that retains the expression of specific genes after cell division. Such a mechanism could be of utmost importance for cell cycle progression and cell growth. We now propose to test this hypothesis and identify the factors and mechanisms involved in this process. Altogether, our data support the hypothesis that deregulation of lipid synthesis could facilitate the growth and proliferation of cancer cells.

Matrix Biology Group

Our research is focused on understanding how the stromal microenvironment influences the proliferative and invasive behavior of malignant cells. In particular, we investigate the growth factor regulation of hyaluronan biosynthesis and the biological significance of the interaction between hyaluronan and its receptor CD44 in promoting breast tumor progression. Furthermore, we investigate how hyaluronan-CD44 conveys signals into the cells and how they cooperate with growth factor receptors, including PDGF and TGF- β receptors.

Hyaluronan-CD44 interactions promote the malignant phenotype of tumor cells

Because of its physicochemical and hygroscopic nature, hyaluronan has under normal conditions important physiological properties including tissue organization and hydration. However, in fast remodeling tissues, *e.g.* tumor tissues, signaling molecules, for example PDGF-BB, promote aberrant accumulation of hyaluronan of different sizes. The levels of hyaluronan in rapidly remodeling tissues are due to the concerted action of hyaluronan synthesizing (HAS1, 2 and 3) and hyaluronan degrading (HYAL) enzymes expressed by the tumor cells themselves or by the stromal cells commandeered by the cancer cells.

Recently, we have delineated the downstream signaling pathways through which PDGF-BB stimulates hyaluronan production in human dermal fibroblasts; PDGF-BB

potently induced hyaluronan synthesis through the induction of the *HAS2* gene. The downstream signaling pathways through which PDGF-BB modulate hyaluronan synthesis involve PKC, ERK MAPK and PI3K pathways. In addition, NF- κ B is also central in the PDGF-BB-mediated hyaluronan production (14). Interestingly, PDGF-BB-mediated proliferation of human dermal fibroblasts is promoted by the binding of hyaluronan to its receptor CD44 (Fig. 3).

To investigate the importance of *HAS2* in the maintenance of the malignant and invasive phenotype of the Hs578T breast cancer cells, we investigated the consequences of *HAS2* gene suppression by specific silencing using siRNAs. Interestingly, suppression of *HAS2* slows down the growth as well as the migratory and invasive capacities of Hs578T cells (15). Our data show that *HAS2* expression and endogenously synthesized hyaluronan promote breast cancer progression whereas exogenously added hyaluronan cannot restore the growth, migration and invasion of tumor cells. Furthermore, *HAS2* cooperates with CD44 and *HYAL2*, and most likely these molecules are crucial for the aggressive character of breast tumor cells. Notably, breast cancer cells exhibiting low aggressive phenotype, express very low amounts of *HAS2*, but relatively high levels of the *HAS3* isoform and even higher levels of *HYAL2* mRNA. Recently, we have developed multicellular 3D models to mimic the *in vivo* 3D milieu of breast tumor / basement matrices and breast tumor / endothelial barrier. Such models will make it possible to elucidate the roles of hyaluronan - CD44 associations in the progression of the malignant phenotype of breast cancer cells.

Currently, experiments are in progress where we elucidate the regulation of *HAS* activities through posttranslational modifications and interaction with other proteins of functional importance for *HAS* activity and stability (Karousou *et al.*, in preparation).

Cross-talk between cell surface growth factor receptors and CD44

During tumorigenesis and wound healing, growth factors released by the tumor cells and inflammatory cells can "activate" stromal cells to synthesize more extracellular macromolecules (desmoplasia). Additionally, these regulatory signals can activate adhesive receptors, for example the hyaluronan receptor CD44, to promote metastasis. Recently, we investigated the importance of high amounts of hyaluronan, which is common during wound healing and in desmoplastic carcinoma tissues, for PDGF-BB-mediated fibroblast growth and migration. The analysis revealed that hyaluronan-stimulated CD44 suppresses the activation state of the PDGF β -receptor, in PDGF-BB-stimulated human dermal fibroblasts, by the activation of a CD44-associated tyrosine

phosphatase acting on the receptor, decreasing PDGF-BB-mediated fibroblast migration. Additionally, hyaluronan binding to CD44 is important for the mitogenic PDGF-BB response (Li, *et al.*, J. Biol. Chem. 281, 26512-26519, 2006). Thus, dermal fibroblast CD44 binding to exogenous hyaluronan negatively regulates PDGF β -receptor-mediated migration, but positively regulate its mitogenic response (Fig. 3). Further studies are needed in order to elucidate the physiological importance of these observations during normal and abnormal tissue remodeling.

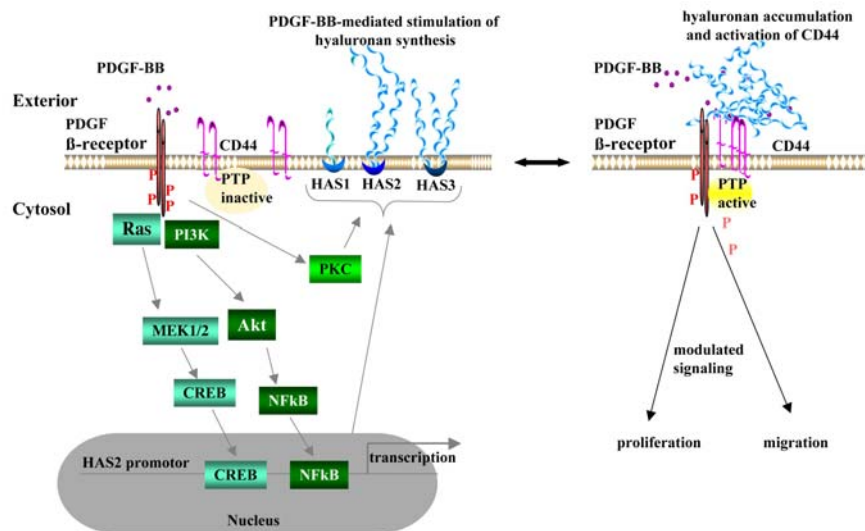


Figure 3. Cross-talk between CD44 and PDGF β -receptor in human dermal fibroblasts.

Our current work is aiming at elucidating the functional consequences of the cross-talk between hyaluronan-CD44 and the PDGF β -receptor or the TGF- β receptor, and their importance in cancer and inflammation. Increased understanding of the molecular mechanisms involved may help to design therapeutic interventions to prevent tumor invasiveness and metastasis.

Protein Structure Group

Our main mission is to support the activities within the Branch with our specialized techniques, and when possible, to interact with other Branches in Sweden and abroad. Also, when time allows, we collaborate with other research groups. This has over the years provided a broad experience with applications onto various types of projects.

The Protein Structure Group has a solid experience in peptide synthesis, radio-labeled amino acid sequencing and mass spectrometry. For the latter activity, we use a top of the line matrix-assisted-laser-desorption-ionization-time-of-flight-mass-spectrometer (MALDI-TOF/TOF-MS). The present instrument (Bruker Ultraflex III), installed in March 2007, offers a dramatic increase in sensitivity, accuracy for both MS and especially for MS/MS, user friendliness and peptide sequencing possibilities compared to our previous instrument. Our three main activities are described in more detail below.

Peptide synthesis

Contrary to the trend of outsourcing this highly specialized skill, we prefer to take advantage of our long gained know-how. Our synthesizer, an eight-year-old Applied Biosystems 433A instrument, is operated with Fmoc chemistry, and produces high quality peptides. The synthesis of peptides modified with *e.g.* phosphorylations, acetylations or oxidations, at given amino acid residues, has been most useful for the different groups at our Branch. All peptides are worked up manually and often, depending on the intended use, purified to homogeneity by liquid chromatography. The products are quality controlled using MALDI-TOF/TOF-MS. The peptides produced are used to generate anti-peptide antibodies, as substrates or inhibitors, or as ligands in affinity chromatography experiments. An important extension has developed over the last years – we now also carry out affinity purification of anti-peptide antibodies in collaboration with other groups of our Branch. Another recent development in our Group is that we, in collaboration with other Groups, perform affinity based searches for interacting partners using immobilized ligand, *i.e.* a modified synthetic peptide.

Radiolabeled phosphopeptide mapping

The introduction in our group of MALDI-TOF-MS ten years ago has made peptide sequencing faster, more sensitive and less expensive, compared to classical chemical amino acid sequence analysis ("Edman degradation"). We therefore do not use our ABI 494 peptide sequencer (from Applied Biosystems) for classical sequencing anymore. However, it is still being used for the important positioning of phosphorylated Tyr, Ser and Thr residues in phosphorylated proteins.

Sample preparation for mass spectrometry

Over 95% of the samples for analysis by MALDI-TOF/TOF-MS come as bands or spots from one- or two-dimensional gels. With Coomassie-visible material only a few

percent is needed for analysis; with silver-stained material, often the entire sample must be applied after concentration and desalting on micro RPC columns, *i.e.* μ ZipTips. We have recently introduced a novel approach to enrich for acidic, especially phosphorylated peptides, notorious for low sensitivity by MALDI TOF MS. This is based on home-made micro-columns comprising TiO_2 particles. This has proven to increase the sensitivity significantly for this group of highly relevant peptides.

Peptide mass fingerprinting (PMF)

Determining protein identity by PMF is a routine procedure for known proteins. After generation of a proteolytic digest and determination of peptide masses by MALDI-TOF/TOF-MS, we utilize a search engine (ProFound or MASCOT are preferred) to make a match with a known protein. If a significant mass spectrum is obtained, we are practically always able to identify the protein with high confidence. Should this not be the case, *i.e.* if the searched protein is not found in any database, the identity may be established by obtaining the amino acid sequence of one or more tryptic peptides (see below). A sequence homology search is, in contrast to PMF, tolerant to amino acid substitutions. We have taken up a technique of Lys-modification (by an imidazol derivative), which renders lysine-containing peptides more basic and therefore increases the sensitivity resulting in higher sequence coverage. As a bonus, such peptides are easy to fragment for sequence analysis. Furthermore, the Lys-Tag reagent may be used to label a set of proteins with deuterium for relative quantifications. Over the last year, we have carried out interesting proteomics projects with groups the Karolinska Hospital and at the Department of Pathology, Uppsala University, looking for proteins that are significantly changed in various tumors (7, 21).

Post Source Decay (PSD) sequencing by MALDI-TOF/TOF-MS

Fragment analysis of peptides by MALDI-TOF/TOF-MS using PSD is a straightforward technique. The resulting fragment spectra are commonly used for protein identification, but are difficult to use for *de novo* sequencing. The TOF/TOF technology allows complete PSD spectra to be generated in seconds, so it is possible to scan through several peptides from one digest in a short time. This easy and quick PSD combines extremely well with the Chemically Assisted Fragmentation (CAF) derivatization protocol, leading to easily interpreted spectra, as they comprise a unique series of γ -ions. Hence, an amino acid sequence can unambiguously (with the single exception of the isobaric Leu/Ile) be determined faster, cheaper and more sensitive than is possible using chemical Edman degradation. An example is given in Fig. 4 below.

We use CAF-PSD for identification of un-characterized species, as well as for analysis of modified peptides (10). As the CAF reagent will be discontinued, we have started to replace it by SPITC (4-sulfo-phenyl-isothiocyanate), which is a much cheaper compound with similar positive effects on peptide fragmentation by MALDI-TOF/TOF.

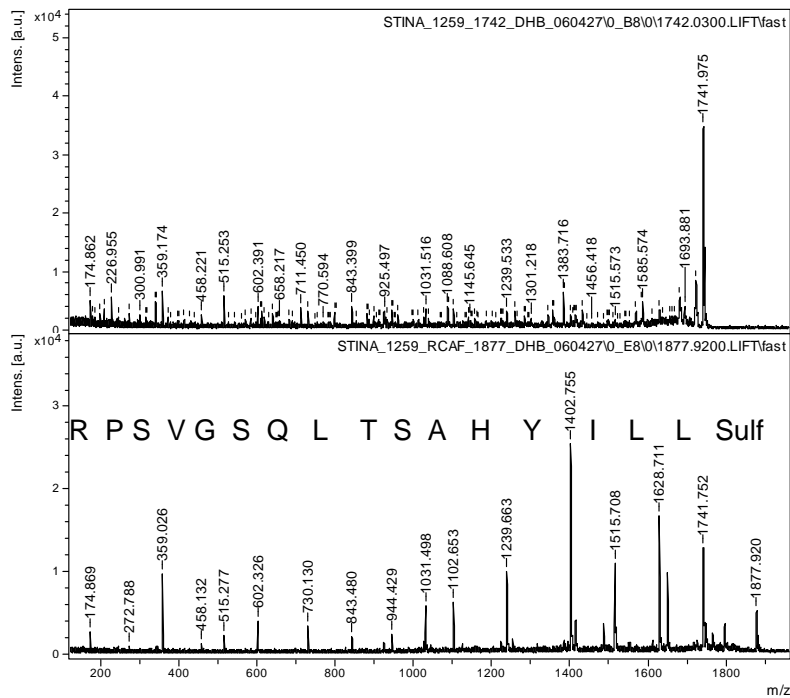


Figure 4. A peptide from a tryptic digest was analyzed by Post Source Decay before (top panel) and after sulfonation. In the latter case, an amino acid sequence is easily read.

PRIMARY RESEARCH PAPERS

Published

1. **Aase K, Ernkvist M, Ebarasi L, Jakobsson L, Majumdar A, Yi C, Birot O, Ming Y, Kvanta A, Edholm D, Aspenström P, Kissil J, Claesson-Welsh L, Shimono A and Holmgren L.** Angiomotin regulates endothelial cell migration during embryonic angiogenesis. *Genes & Development* 21: 2055-2068, 2007.
2. **Aspenström-Fagerlund B, Ring L, Aspenström P, Tallkvist J, Ilbäck N-G and Glynn AW.** Oleic acid and docosahexaenoic acid cause an increase in the paracellular absorption of hydrophilic compounds in an experimental model of human absorptive enterocytes. *Toxicology* 237: 12-23, 2007.
3. **Bardales JR, Hellman U and Villamarín JA.** CK2-mediated phosphorylation of a type II regulatory subunit of cAMP-dependent protein kinase from the mollusk *Mytilus galloprovincialis*. *Archives of Biochemistry and Biophysics* 461: 130-137, 2007.
4. **Conrotto P, Yakymovych I, Yakymovych M and Souchelnytskyi S.** Interactome of transforming growth factor- β type I receptor (T β RI): Inhibition of TGF β signaling by Epac1. *Journal of Proteome Research* 6: 287-297, 2007.
5. **Cortez L, Marino-Buslje C, de Jiménez Bonino MB and Hellman U.** Interactions between α -conotoxin MI and the *Torpedo marmorata* receptor α - δ interface. *Biochemical and Biophysical Research Communications* 355: 275-279, 2007.
6. **Davoodpour P, Landström M and Welsh M.** Reduced tumor growth in vivo and increased c-Abl activity in PC3 prostate cancer cells overexpressing the Shb adapter protein. *BMC Cancer* 7: 161, 2007.
7. **Enqvist S, Sletten K, Stevens FJ, Hellman U and Westermarck P.** Germ line origin and somatic mutations determine the target tissues in systemic AL-amyloidosis. *PLoS ONE* 2: e981, 2007.
8. **Gianoukakis AG, Jennings TA, King CS, Sheehan CE, Hoa N, Heldin P and Smith TJ.** Hyaluronan accumulation in thyroid tissue: evidence for contributions from epithelial cells and fibroblasts. *Endocrinology* 148: 54-62, 2007.
9. **Hasumi Y, Klosowska-Wardega A, Furuhashi M, Östman A, Heldin C-H and Hellberg C.** Identification of a subset of pericytes that respond to combination therapy targeting PDGF and VEGF signaling. *International Journal of Cancer* 121: 2606-2614, 2007.
10. **Ivarsson Y, Norrgård MA, Hellman U and Mannervik B.** Engineering the enantioselectivity of glutathione transferase by combined active-site mutations and chemical modifications. *Biochimica et Biophysica Acta* 1770: 1374-1381, 2007.
11. **Kallin A, Johannessen LE, Cani PD, Marbehant CY, Essaghir A, Foufelle F, Ferré P, Heldin C-H, Delzenne NM and Demoulin J-B.** SREBP-1 regulates the expression of heme oxygenase 1 and the phosphatidylinositol-3 kinase regulatory subunit p55 γ . *Journal of Lipid Research* 48: 1628-1636, 2007.
12. **Kappert K, Paulsson J, Sparwel J, Leppänen O, Hellberg C, Östman A and Mücke P.** Dynamic changes in the expression of DEP-1 and other PDGF receptor-antagonizing PTPs during onset and termination of neointima formation. *The FASEB Journal* 21: 523-534, 2007.

13. **Larsson A, Söderberg L, Westermark GT, Slettend K, Engström U, Tjernberg LO, Näslund J and Westermark P.** Unwinding fibril formation of medin, the peptide of the most common form of human amyloid. *Biochemical and Biophysical Research Communications* 361: 822-828, 2007.
14. **Li L, Asteriou T, Bernert B, Heldin C-H and Heldin P.** Growth factor regulation of hyaluronan synthesis and degradation in human dermal fibroblasts: Importance of hyaluronan for the mitogenic response of PDGF-BB. *Biochemical Journal* 404: 327-336, 2007.
15. **Li Y, Li L, Brown TJ and Heldin P.** Silencing of hyaluronan synthase 2 suppresses the malignant phenotype of invasive breast cancer cells. *International Journal of Cancer* 120: 2557-2567, 2007.
16. **Looman C, Sun T, Yu Y, Zieba A, Åhgren A, Feinstein R, Forsberg H, Hellberg C, Heldin C-H, Zhang X-Q, Forsberg-Nilsson K, Khoo N, Fundele R and Heuchel R.** An activating mutation in the PDGF receptor-beta causes abnormal morphology in the mouse placenta. *International Journal of Developmental Biology* 51: 361-370, 2007.
17. **Magnusson PU, Looman C, Åhgren A, Wu Y, Claesson-Welsh L and Heuchel RL.** Platelet-derived growth factor receptor- β constitutive activity promotes angiogenesis in vivo and in vitro. *Arteriosclerosis, Thrombosis, and Vascular Biology* 27: 2142-2149, 2007.
18. **Micke P, Kappert K, Ohshima M, Sundqvist C, Scheidl S, Lindahl P, Heldin C-H, Botling J, Pontén F and Östman A.** *In situ* identification of genes regulated specifically in fibroblasts of human basal cell carcinoma. *Journal of Investigative Dermatology* 127: 1516-1523, 2007.
19. **Niimi H, Pardali K, Vanlandewijck M, Heldin C-H and Moustakas A.** Notch signaling is necessary for epithelial growth arrest by TGF- β . *The Journal of Cell Biology* 176: 695-707, 2007.
20. **Nishitsuka K, Kashiwagi Y, Tojo N, Kanno C, Takahashi Y, Yamamoto T, Heldin P and Yamashita H.** Hyaluronan production regulation from porcine hyalocyte cell line by cytokines. *Experimental Eye Research* 85: 539-545, 2007.
21. **Rahman-Roblick R, Roblick UJ, Hellman U, Conrotto P, Liu T, Becker S, Hirschberg D, Jörnvall H, Auer G and Wiman KG.** p53 targets identified by protein expression profiling. *Proceedings of the National Academy of Sciences USA* 104: 5401-5406, 2007.
22. **Singh U, Sun T, Looman C, Heuchel R, Elliott R, Freichel M, Meissner M, Flockerzi V and Fundele R.** Expression and function of the gene encoding the voltage-dependent calcium channel β 3-subunit in the mouse placenta. *Placenta* 28: 412-420, 2007.
23. **Suzuki S, Heldin C-H and Heuchel RL.** Platelet-derived growth factor receptor- β , carrying the activating mutation D849N, accelerates the establishment of B16 melanoma. *BMC Cancer* 7: 224, 2007.
24. **Valcourt U, Thuault S, Pardali K, Heldin C-H and Moustakas A.** Functional role of Meox2 during the epithelial cytotstatic response to TGF- β . *Molecular Oncology* 1: 55-71, 2007.
25. **Wallez Y, Cand F, Cruzalegui F, Wernstedt C, Souchelnytskyi S, Vilgrain I and Huber P.** Src kinase phosphorylates vascular endothelial cadherin in response to vascular endothelial growth factor: identification of tyrosine 685 as the unique target site. *Oncogene* 26: 1067-1077, 2007.

26. **Weng H-L, Ciucan L, Liu Y, Hamzavi J, Godoy P, Gaitantzi H, Kanzler S, Heuchel R, Ueberham U, Gebhardt R, Breitkopf K and Dooley S.** Profibrogenic transforming growth factor- β /activin receptor-like kinase 5 signaling via connective tissue growth factor expression in hepatocytes. *Hepatology* 46: 1257-1270, 2007.
27. **Yoshizaki L, Troncoso MF, Lopes JLS, Hellman U, Beltramini LM and Wolfenstein-Todel C.** *Calliandra selloi* Macbride trypsin inhibitor: Isolation, characterization, stability, spectroscopic analyses. *Phytochemistry* 68: 2625-2634, 2007.

In press

28. **Gal A, Sjöblom T, Fedorova L, Imreh S, Beug H and Moustakas A.** Sustained TGF β exposure suppresses Smad and non-Smad signalling in mammary epithelial cells, leading to EMT and inhibition of growth arrest and apoptosis. *Oncogene*.
29. **Hamzavi J, Ehnert S, Godoy P, Ciucan L, Mertens PR, Heuchel R and Dooley S.** Disruption of the Smad7 gene enhances CCl4-dependent liver damage and fibrogenesis in mice. *Journal of Cellular and Molecular Medicine*.
30. **Hegazy UM, Tars K, Hellman U and Mannervik B.** Modulating catalytic activity by unnatural amino acid residues in a GSH-binding loop of GST P1-1. *Journal of Molecular Biology*.
31. **Kurtovic S, Grehn L, Karlsson A, Hellman U and Mannervik B.** Glutathione transferase activity with a novel substrate mimics the activation of the prodrug azathioprine. *Analytical Biochemistry*.
32. **Li Y, Zhang F, Nagai N, Tang Z, Zhang S, Scotney P, Lennartsson J, Zhu C, Qu Y, Fang C, Hua J, Matsuo O, Fong G-H, Ding H, Cao Y, Becker KG, Nash A, Heldin C-H and Li X.** VEGF-B inhibits apoptosis via VEGFR-1-mediated suppression of the expression of BH3-only protein genes in mice and rats. *Journal of Clinical Investigation*.
33. **Maslyk M, Kochanowicz E, Zielinski R, Kubinski K, Hellman U and Szyszka R.** Yeast surviving factor Svf1 as a new interacting partner, regulator and in vitro substrate of protein kinase CK2. *Molecular and Cellular Biochemistry*.
34. **Ruusala A and Aspenström P.** The atypical Rho GTPase Wrch1 collaborates with the non-receptor tyrosine kinases Pyk2 and Src in regulating cytoskeletal dynamics. *Molecular and Cellular Biology*.
35. **Yin BW, Kiyamova R, Chua R, Caballero OL, Gout I, Gryshkova V, Bhaskaran N, Souchelnytskyi S, Hellman U, Filonenko V, Jungbluth AA, Odunsi K, Lloyd KO, Old LJ and Ritter G.** Monoclonal antibody MX35 detects the membrane transporter NaPi2b (SLC34A2) in human carcinomas. *Cancer Immunology*.

REVIEWS

Published

36. **Aspenström P, Ruusala A and Pacholsky D.** Taking Rho GTPases to the next level: The cellular functions of atypical Rho GTPases. *Experimental Cell Research* 313: 3673-3679, 2007.
37. **Bengoechea-Alonso MT and Ericsson J.** SREBP in signal transduction: cholesterol metabolism and beyond. *Current Opinion in Cell Biology* 19: 215-222, 2007.

38. **Ekman S, Bergqvist M, Heldin C-H and Lennartsson J.** Activation of growth factor receptors in esophageal cancer – implications for therapy. *The Oncologist* 12: 1165-1177, 2007.
39. **Heldin C-H and ten Dijke P.** Cell regulation: Cellular signaling. *Current Opinion in Cell Biology* 19: 109-111, 2007.
40. **Moustakas A and Heldin C-H.** Signaling networks guiding epithelial–mesenchymal transitions during embryogenesis and cancer progression. *Cancer Science* 98: 1512–1520, 2007.
41. **Pardali K and Moustakas A.** Actions of TGF- β as tumor suppressor and pro-metastatic factor in human cancer. *Biochimica et Biophysica Acta* 1775: 21-62, 2007.
42. **Simonsson M.** Protein Acetylation - A Multifunctional Regulator of TGF- β Signaling. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. Medicine TDoFo. Acta Universitatis Upsaliensis, Uppsala, 232: pps. 1-63, 2007.
43. **Östman A and Heldin C-H.** PDGF receptors as targets in tumor treatment. *In: Adv. Cancer Res., Vande Woude GF and Klein G, eds. Academic Press, 97: pps. 247-274, 2007.*

In press

44. **Bergström R.** Epigenetic Regulation of Replication Timing and Signal Transduction. Acta Universitatis Upsaliensis. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. Medicine TDoFo. Uppsala.
45. **Heldin C-H.** The European Research Council - a new opportunity for European Science. *Nature Reviews Molecular and Cellular Biology*.
46. **Heldin C-H.** TGF- β signaling from receptors to Smads. *In: The TGF- β Family, Derynck R and Miyazono K, eds. Cold Spring Harbor Laboratory Press, Woodbury, N.Y.*
47. **Heldin C-H.** Preface. Transforming growth factor- β in cancer. *In: Transforming Growth Factor- β in Cancer Therapy, Jakowlew SB, ed. The Human Press.*
48. **Heldin C-H.** Platelet-derived growth factor. *In: Encyclopedia of Cancer, Schwab M, ed.*
49. **Heldin C-H.** Protein tyrosine kinase receptor signaling overview. *In: Handbook of Cell Signaling, Bradshaw RA and Dennis EA, eds. Academic Press, San Diego.*
50. **Heldin C-H, Lennartsson J and Hellberg C.** Negative feedback mechanisms control signal transduction. *European Journal of Human Genetics.*
51. **Micke P, Moustakas A, Ohshima M and Kappert K.** Cancer-associated fibroblasts and the role of TGF β . *In: Transforming Growth Factor- β in Cancer Therapy, Cancer Drug Discovery and Development, Jakowlew SB, ed.*
52. **Moustakas A and Heldin C-H.** Dynamic control of TGF- β signaling and its links to the cytoskeleton. *FEBS Letters.*
53. **Thuault S, Valcourt U, Kowanetz M and Moustakas A.** TGF- β and Smad signaling in transcriptome reprogramming during EMT. *In: Transforming Growth Factor- β in Cancer Therapy, Cancer Drug Discovery and Development, Jakowlew SB, ed. The Human Press Inc.*