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Signal Transducer and Activator of Transcription 3β in pancreatic cancer

Communication

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Abstract: Signal transducer and activator of transcription 3 (STAT3) has four isoforms: α, β, γ and δ. STAT3α and STAT3β are the transcriptionally active isoforms, while STAT3γ and STAT3δ are the products of STAT3 proteolytic degradation. STAT3 plays an important role in angiogenesis, cell proliferation and apoptosis. High levels of STAT3β in blood cells from acute leukaemia patients have been reported, suggesting that STAT3β may play an important role in cancerogenesis. Fourteen pancreatic cancer patients and six chronic pancreatitis patients were included in this pilot study. Levels of STAT3 isoforms from samples with pancreatic cancer and in adjacent histologically-normal pancreatic tissue were analysed. Pancreas from chronic pancreatitis patients served as a non-neoplastic tissue. Western-blot analysis of STAT3 proteins with the use of anti-STAT3 antibodies was performed. STAT3α and STAT3β isoforms in both cancerous and in adjacent normal tissues were found in 10 of 14. In chronic pancreatitis patients, only STAT3α and STAT3δ were detected. STAT3β was absent in pancreas from chronic pancreatitis patients, in contrast to pancreatic cancer patients. The presence of STAT3β in pancreatic cancer and in adjacent histologically-normal tissues, but not in inflamed tissues suggests that STAT3β may play a key role in cancer development.

Keywords: *Pancreatic cancer • Chronic pancreatitis • Differentiating marker • STAT3 • Isoforms*

1. Introduction

Signal transducers and activators of transcription (STAT) proteins are latent transcription factors. The STAT family consists of at least seven members: STAT1, 2, 3, 4, 5A, 5B and 6 [1]. STATs play important roles in angiogenesis, proliferation and apoptosis [2]. With the exception of STAT 2, all STATs have isoforms generated by the alternative splicing of single gene mRNA transcript. Moreover, STAT3, STATA, STAT5B and STAT6 have isoforms generated by proteolysis. STAT3 is activated by phosphorylation catalyzed by JAK kinases (usually JAK1, JAK2, Tyk2) or receptor tyrosine kinases in response to IL-6 and related cytokines such as leukaemia inhibitory factor (LIF) or oncostatin M (OSM) [3-5]. After the phosphorylation of residues Tyr705/Ser727, STAT3 dimerizes and translocates from the cytoplasm into the nucleus. Phosphorylation of Tyr705 is critical for the transcriptional activation of STAT3 [6]. Phosphorylated STAT3 is transcriptionally active and binds to two kinds of DNA sequence elements: hSIE (human Serum Inducible Element) and GAS (Gamma-interferon Activated Sequence). STAT3α and STAT3β are the transcriptionally active isoforms of STAT3, while STAT3γ and STAT3δ are the products of STAT3 proteolytic degradation products.

High levels of STAT3β in peripheral blood cells from acute leukaemia patients have been observed [7]. Furthermore, constitutively active STAT3β can act like constitutively active growth factors receptors in

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neoplasia [8]. T cells transformed with HTLV-1 (Human T-cell Leukaemia Virus type I) or with the Src oncoprotein express DNA-binding proteins that are immunologically similar to STAT3 [9,10].

Collectively, existing literature suggests that STAT3β might play an important role in cancerogenesis, and it was interesting to examine the levels of STAT3β in pancreas from patients with pancreatic cancer. In the present pilot study we analyzed STAT3 isoform patterns in cancer tissues and in adjacent normal pancreas from patients with pancreatic cancer. Pancreas sampled from chronic pancreatitis patients served as non-neoplastic pancreatic tissues.

The results presented in this paper indicate that STAT3α and STAT3β isoforms were present in both cancer and in adjacent histologically normal tissue sampled from 10 of 14 patients with pancreatic cancer. In pancreas from chronic pancreatitis patients STAT3α and STAT3δ were detected, but not STAT3β.

2. Experimental Procedures

2.1 Tissues

The study was performed in accordance with the Declaration of Helsinki of the World Medical Association and was approved by the Medical University of Gdansk Ethics Committee. Informed written consent was obtained from all subjects before the study, according to the guidelines in Helsinki Declaration II. Pancreas was obtained during surgery in the Department of General, Endocrine and Transplant Surgery, Medical University of Gdansk from pancreatic cancer and chronic pancreatitis patients. Tissues underwent histopathological evaluation. STAT3 isoform patterns were analysed in tissues from six patients with chronic pancreatitis (5 males, 1 female) and 14 pancreatic cancer patients (12 males, 2 females). All cases were diagnosed as primary pancreatic ductal adenocarcinoma by histopathology (well-differentiated in 1 case, moderately-differentiated in 12 cases, and poorly-differentiated in 1 case). After macroscopic and microscopic examination, tissues were frozen in liquid nitrogen and stored at -80ºC until the extraction of protein.

2.2 Western-blot analysis

Tissue extracts were prepared with the use of RIPA buffer (10 mM Tris/HCl [tris(hydroxymethyl)aminomethane/HCl], pH 7.4; 150 mM NaCl; 1% Triton X−100; 0.5% deoxycholate; 0.1% sodium dodecylsulfate [SDS], 5 mM ethylenediaminetetraacetic acid [EDTA]) containing protease inhibitors (complete tablets; Roche, Basel, Switzerland) and phosphatase inhibitors (complete mixture: Phosphatase Inhibitor Cocktail 2, Sigma, Saint Louis, USA). Tissues were homogenized with the use of liquid nitrogen. After homogenization ice-cold RIPA buffer with protease and phosphatase inhibitors was added. After 5 minutes of incubation on ice with slight mixing, lysates were clarified by centrifugation at 8,000 x g for 10 minutes at 4°C to pellet the tissue debris. Supernatant (100 µg of protein) was mixed with the same volume of Laemmli buffer (125 mM Tris–HCl pH 6.8, 4% SDS, 20% glycerol, 10% 2–mercaptoethanol, and 0.002% bromophenol blue). Samples were boiled for 5 min and subjected to SDS-polyacrylamide gel electrophoresis (PAGE) (4% stacking, 10% resolving gel), and transferred to nitrocellulose membrane with semi-dry blotter (Sigma, Saint Louis, USA). Immunoblotting was performed using polyclonal antibodies against P-STAT3 Tyr705 (Tyr705 Phosphorylated STAT3, sc-7993, Santa Cruz Biotechnology, USA) and β-actin (A 5441, Sigma, Saint Louis, USA). Negative control cells with silenced STAT3 gene: Stat3 HeLa/si (sc-29550 Santa Cruz Biotechnology) were used. Secondary antibodies were conjugated with horseradish peroxidase (rabbit anti-goat IgG or rabbit anti-mouse IgG, horseradish peroxidase linked whole antibodies, sc-2768 Santa Cruz Biotechnology and A 9044 Sigma) and an enhanced chemiluminescence substrate for detection of HRP (34079, Thermo Scientific, USA) involving exposure to CL-XPosure film (34090, Pierce, USA). Blots were analyzed using Quantity One program, version 4,0 (Bio Rad).

3. Results

In pancreas from chronic pancreatitis patients only STAT3α and STAT3δ isoforms were detected (Figure 1). No bands corresponding to isoforms STAT3β and STAT3γ were found in pancreases from chronic pancreatitis patients (Figure 1). Surprisingly, in both cancer and adjacent microscopically-normal tissue, all isoforms of phosphorylated STAT3 were detected. Figure 1 displays two (of 10) typical blots obtained from patients with pancreatic cancer. In four pancreatic cancer patients no bands corresponding to STAT3β and STAT3γ were found (not shown). This suggests that STAT3β and STAT3γ were present in pancreas from most sampled patients with pancreatic cancer. In pancreas from ten pancreatic cancer patients, the STAT3β isoform was detected, usually together with proteolitically formed STAT3δ. In pancreas from four pancreatic cancer patients, not only the presence, but also the dominant (*versus* STAT3α) band of STAT3β was observed (compared with β-actin internal standard).

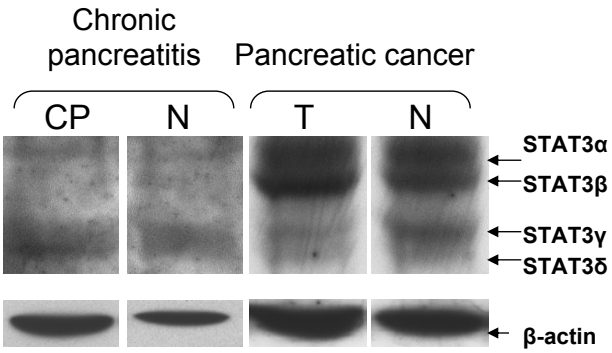

Figure 1. Western-blotting analysis of tissues from: chronic pancreatitis patient, pancreatic cancer patient. For every patient two tissues were examined: cancer (T) /inflamed (CP) and adjacent normal tissue (N).

4. Discussion

In this paper we show for the first time that STAT3β is present in pancreas from patients with pancreatic cancer but not in pancreas from patients with chronic pancreatitis. The presence of the STAT3β isoform in 10 of 14 pancreases sampled from pancreatic cancer patients together with the absence of this isoform in pancreases of chronic pancreatitis patients suggests that STAT3β may play a key role in pancreatic cancer development. Moreover, the presence of the STAT3β isoform in adjacent, microscopically normal tissue of the same patient suggests that STAT3β might differentiate in a potentially preneoplastic state. A similar pattern of STAT3 isoforms in pancreatic cancer and adjacent tissue may be a systemic organ response to cytokines not confined to neoplastic tissue, but also present in normal adjacent tissue [11]. Previously reported data strongly support these suggestions. For instance, high levels of STAT3β have been observed in peripheral blood cells from acute leukaemia patients [7]. Furthermore, in neoplasia, constitutively active STAT3β has been shown to act like constitutively active growth factors receptors [8]. T cells transformed with HTLV-1 (Human T-cell Leukaemia Virus type I) or with the Src oncoprotein expresse STAT related proteins that are immunologically similar to STAT3 [9,10]. Thus STAT3β isoforms seem to play an important role in myeloid differentiation [12].

The results presented in this paper also suggest that an imbalance between STAT3 isoforms in pancreas might play an important role in cancerogenesis. Changes in STAT3 isoforms patterns, especially an imbalance in the STAT3α : STAT3β ratio, can be connected with cancer genesis and development [12]. STAT3α and STAT3β are the alternatively spliced products of the same gene, differing only at their C-terminus, where the transactivation domain present in isoform α is replaced by seven unique amino acids residues (CT7 domain). The isoforms of STAT3 also have different levels of half-maximal cytoplasmic reaccumulation after cytokine withdrawal: STAT3α – 15 minutes, and STAT3β – 180 minutes [13]. STAT3α has greater transcriptional activity than STAT3β, but lower DNA-binding activity. The C-terminal sequence of STAT3α normally destabilizes active dimers, resulting in lower DNA binding activity [14,15]. It was observed that STAT3α was observed to suppress *FAS (Fatty Acid Synthase)* gene transcription, while STAT3β opposed this effect. STAT3α and STAT3β can form homo- and heterodimers. The α/α homodimer is less stable than the α/β heterodimer, which in turn is less stable than the β/β homodimer. Accordingly, the balance between isoforms is able to change overall STAT3 transcriptional activity [16]. Overexpression of STAT3β can break the balance further increasing *FAS* expression [17]. It should be noted that *FAS* gene expression is elevated in many neoplastic tissues [18]. Because of the lack of an intrinsic transactivation domain STAT3β can act as a dominant-negative form of STAT3α [19]. STAT3β can constitutively bind DNA and activate transcription in the absence of extracellular factors, such as cytokines and growth factors. STAT3γ and STAT3δ are the products of STAT3α/β proteolytic degradation, and although their precise function is unknown, they may be involved with development and differentiation [20].

In conclusion, the results presented in this paper indicate that there are significant differences between levels of STAT3 isoforms in pancreas from pancreatic cancer patients and pancreas from chronic pancreatitis patients. It is possible that STAT3β - present in pancreas of pancreatic cancer patients but absent in pancreas of chronic pancreatitis patients - may play an important role in cancer development. However, the lack of results concerning STAT3 in normal pancreas does not allow us to draw unequivocal conclusions regarding the cause of the observed significant difference in STAT3 levels

between pancreas from pancreatic cancer and chronic pancreatitis patients. Forthcoming studies should clarify both the cause and the function, if any, of the different levels.

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