Inflammation and pancreatic cancer: molecular and functional interactions between S100A8, S100A9, NT-S100A8 and TGFβ1

Andrea Padoan, Stefania Moz, Dania Bozzato, Daniela Basso

Department of Medicine (DIMED), University of Padova, Padova, Italy

Correspondence: Andrea Padoan
E-mail: andrea.padoan@unipd.it
Received: August 07, 2014
Published online: September 25, 2014

In pancreatic ductal adenocarcinoma (PDAC), the crosstalk between pancreatic cancer cells and stromal cells is both contact dependent and independent. The latter is mediated by molecules released by tumor and stromal cells in the tumour microenvironment and it is increasingly recognized that the establishment of a pro-inflammatory milieu has important consequences in these interactions. In order to gain further insights on this mechanism we investigated whether the interactions that occur between TGFβ1 and the inflammatory proteins S100A8, S100A9 and NT-S100A8, a PDAC-associated S100A8 derived peptide, affect Akt, NF-κB and mTOR intracellular signalling and epithelial to mesenchymal transition (EMT) in well and poorly differentiated PDAC cell lines. We found that S100A8, S100A9 and NT-S100A8 have a main inhibitory effect on NF-κB and Akt in the presence of intact SMAD4, while these molecules stimulate Akt when SMAD4 is homozygously deleted. NT-S100A8 induces mTOR phosphorylation in all PDAC cell lines, while the effects of S100A8, S100A9 and S100A8/S100A9 complex on this signalling pathway depend on the cell type. TGFβ1 counteracts S100A8, S100A9 and NT-S100A8 effects in Smad4 expressing, not in SMAD4 negative cells, while S100A9 antagonizes the effects of TGFβ1 on EMT (Twist and N-Cadherin expression). We further demonstrated that S100A9, in the presence of Calcium, is a cognate binding molecule of TGFβ1. Overall we found that the effect of S100A8, S100A9, NT-S100A8 and TGFβ1 molecules on PDAC cell signalling appeared to be cell-type and SMAD4 dependent and that the molecular interaction between TGFβ1 and S100A9 leading to macrocomplexes formation may cause a reciprocal antagonism on cell signalling and EMT.

Keywords: Akt; Calcium binding proteins; Epithelial to mesenchymal transition (EMT); Mass spectrometry (MS); Matrix metalloproteinase (MMP); mTOR; Pancreatic cancer; SMAD4

To cite this article: Andrea Padoan, et al. Inflammation and pancreatic cancer: molecular and functional interactions between S100A8, S100A9, NT-S100A8 and TGFβ1. Inflamm Cell Signal 2014; 1: e293. doi: 10.14800/ics.293.

Copyright: © 2014 The Authors. Licensed under a Creative Commons Attribution 4.0 International License which allows users including authors of articles to copy and redistribute the material in any medium or format, in addition to remix, transform, and build upon the material for any purpose, even commercially, as long as the author and original source are properly cited or credited.

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal human malignancies and this aggressiveness has been ascribed to the biological nature of PDAC, to the ineffectiveness of therapies and to the inability of the immune system to eradicate malignant cells [11]. Pancreatic carcinogenesis has been described as a multistage process,
characterised by the accumulation of genetic alterations. Twelve core cellular signalling pathways and processes are altered in the majority of PDAC [2]. These genetic alterations involve important pathways such as KRAS, TGFβ1 (and SMAD proteins), apoptosis and DNA repair. KRAS signalling, altered in more than 90% PDAC, is highly complex, and involves many downstream effectors, such as Raf, Mek, Erk, phosphatidylinositol 3-kinase (PI3K), mTOR and Akt, which participate to a complex intracellular signalling framework, being most of them intimately linked with each other [3]. Another relevant feature of PDAC is the surrounding dense stroma, a hypovascular microenvironment, with desmoplastia, fibrosis and an extensive inflammatory status. PDAC surrounding stroma is very heterogeneous, being composed of cellular (e.g. fibroblasts, pancreatic stellate cells and immune cells) and acellular components, such as extracellular matrix (EMC), matrix metalloproteinases (MMPs) and other soluble proteins. The MMP-2 and MMP-9 may enhance tumour migration and invasion by degrading ECM, which synthesis is mainly induced by TGFβ1, produced not only by tumor-associated fibroblasts, but also by tumor cells. TGFβ1 induces desmoplastia, but it exerts also several and different effects on tumor cells which complexity lead to consider TGFβ1 as a Janus-Faced molecule. It may exert growth promoting and growth inhibitory effects, in different cell types and at different stages of tumorigenesis and it was demonstrated to activate Akt and, in pair, NF-κB, while mTOR acts as downstream and upstream effector on Akt [4]. These alternating effects were correlated to the status of SMAD4 mutation [5]. TGFβ1 may also play a predominant role in stimulating EMT, enhancing the invasiveness and migratory potential of PDAC [6,7].

PDAC derived MMPs fragment S100A8

We demonstrated that PDAC tissue is enriched of a biologically active fragment of the calcium binding protein S100A8, NT-S100A8 [8,9]. Since the amino acid sequence of NT-S100A8 fits with the group II consensus motif of MMP2, we hypothesized that MMPs may be a potential source of other protein-derived biologically active fragments. We proved that the molecule S100A8 when incubated with Capan-1 conditioned media (CM) resulted in a series of low molecular weight peptides. Differently, S100A8 incubated in Capan-1 CM treated with Ukrain, which down regulate MMPs activities, did not result in any fragmentation pattern. Moreover, the degradation kinetic of S100A8 by CM depended on the SMAD4 status: the SMAD4 deleted BxPC3 cells presented a slower kinetic than the SMAD4 wild type Capan-1 cells [10].

Smad4 expression is permissive for NF-κB and Akt inhibition by TGFβ1, S100A8 and S100A9

Sheik et al. found that S100A8 and S100A9 are highly expressed in pancreatic stroma, compared to malignant or benign pancreatic tissue [11]. S100A8 and S100A9 form stable heterodimeric complex (calprotectin) at sites of inflammation and have pro-inflammatory effect involved in recruitment and migration of inflammatory cells [12]. Moreover, S100A8 and S100A9 were shown to be implicated in the recruitment of myeloid derived suppressive cells (MDSCs) [13].

To understand the possible interaction between PDAC and stroma we investigated if S100A8, S100A9, S100A8/S100A9 complex but also NT-S100A8, alone or in combination with TGFβ1, are able to activate PI3K-Akt, NF-κB and mTOR signalling pathways and thereby enhance cell proliferation and metastasis and whether this activation depend on: 1) the differentiation grade and 2) the genetic set-up (Smad4 in particular) of PDAC cell lines.

S100A8, S100A9, S100A8/S100A9 shared an inhibitory effect on NF-κB in PDAC cells in the presence of intact Smad4 (Capan1, MiaPaCa2 and Panc1), while these molecules did not affect NF-κB in BxPC3 cells with SMAD4 homozygous deletion. S100A8, S100A9 and S100A8/A9 dephosphorylated the two Akt activation sites Thr308 and Ser473 in Smad4 expressing cells, while Akt Thr308 was activated and Ser473 unaffected by all these molecules in Smad4 negative BxPC3 cells. NT-S100A8 had similar effects of its parent molecule S100A8: the phosphorylation grade of the Akt Thr308 site was induced in BxPC3 and reduced in both Capan1 and MiaPaCa2. TGFβ1 combined with S100A8, S100A9, S100A8/A9 and NT-S100A8 partly counteracted the effects of these S100s on NF-κB and Akt in cells with Smad4 expression. Moreover, we verified by mass spectrometry that S100A9, in presence of calcium ions, might form not only homo-dimers but also hetero-dimers with TGFβ1.

An increasing interest is currently rising on mTORC1 pathway in PDAC, because some clinical studies demonstrated the opportunity of using rapamycin analogues to block the assembly of mTORC complex [14]. Although mTORC1 blockage was demonstrated to strongly over-activate (by mTORC2) different pro-oncogenic pathways [15], high-dose rapamycin induce cell death in absence of TGFβ1 signalling [16].

We found that S100A8, S100A9 and the S100A8/A9 complex induced mTOR Ser2481 phosphorylation in BxPC3 and MiaPaCa2 cells, while S100A9 and S100A8/A9 complex had opposite effects on Capan1 and Panc1. NT-S100A8 induced mTOR phosphorylation in all PDAC cell lines studied. Interestingly, in cells with intact Smad4, TGFβ1 didn’t counteract the effects of S100A8, S100A9 and NT-S100A8 on mTOR signalling.
**TGFβ1 and S100 molecules are EMT inducers**

In light of the interaction found between S100A8/A9 proteins and TGFβ1 and the fact that TGFβ1 play an important role in EMT, we studied the mRNA expression of Snail, Slug, Zeb-1 and Zeb-2 as well as Twist, in response of S100A8/A9, NT-S100A8 and TGFβ1 stimuli. In fact, this transcription factors have all been implicated as important regulators of EMT in PDAC [17,18]. TGFβ1, as expected, induced an increased expression of all the EMT markers, while S100A8/A9 induced only Twist mRNA expression. In addition, S100A8/A9 enforced the expression of N-Cadherin that has been recognized as a feature of the more aggressive of PDAC tumors. We confirmed these results also by immunocytochemistry.

**Conclusive remarks**

In our study we focused on the PDAC-stroma microenvironment, because it may play a role in the PDAC anti-tumour immunity response and in the PDAC aggressiveness. We underlined important interactions between the inflammatory molecules S100A8 and S100A9 and TGFβ1, which are notably dependent on the Smad4 mutation status of PDAC cells. In particular, we verified that S100A9 might be a binding cognate molecular partner of TGFβ1. At least in some early stages of PDAC, the binding of TGFβ1 by S100A9 may counteract the tumour-inhibitory effect exerted by this molecule, allowing the tumour growth. Further, EMT was enhanced after S100A8/A9 stimuli. Overall, these results suggest a link between the inflammatory S100s molecules, TGFβ1 and cancer progression. MMPs proteases, which are produced by tumour and are essential to cell metastatic spreading, may target the inflammatory S100A8 protein causing the release of small peptides. One of these peptides, NT-S100A8, is biologically active and was demonstrated to exert TGFβ1-like effects on PDAC cell NF-κB, Akt and mTOR signalling and to synergize with TGFβ1 in stimulating cell proliferation.

**Conflicting interests**

The authors have declared that no competing interests exist.

**References**