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Ca²⁺ signaling is critical for pancreatic stellate cell's pathophysiology : from fibrosis to cancer hallmarks

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Abstract :

~~Considerated~~ **Known** as the key effector of pancreatic fibrosis and pancreatic cancer's ~~stroma~~ desmoplastic ~~stromal~~ reaction development, activated pancreatic stellate cells (aPSCs) are attracting more and more the attention of pancreatic research. Since ~~a~~ few years, a crucial role of Ca^{2+} signalling in PSC's activation processes has ~~been~~ emerged. Although most of the work on PSCs and Ca^{2+} signalling has been carried on aPSCs, because once seated in culture, they become activated within 2-5 days, there are also some studies focusing on the role of Ca^{2+} in quiescent pancreatic stellate cells (qPSCs). In this mini-review, we reported all findings on Ca^{2+} signalling in qPSCs and aPSCs, in relation ~~with~~ to pancreatic fibrosis and cancer promotion.

Key words : calcium signalling, pancreatic stellate cells, ion channels, fibrosis, cancer

Introduction

Pancreatic stellate cell's research is receiving, since several years, an increased attention due to the crucial role of PSCs in the development of pancreatic fibrosis (PF) and cancer (PC) [1]. Initially characterized as resident quiescent vitamin A storing cells of the exocrine pancreas, they undergo transformations during pancreatic injury in order to become activated [2]. The aPSCs are responsible for the extracellular matrix (ECM) deposition, leading to the creation of a dense fibrotic stroma, presented during PF and PC [2,3]. Despite, the well studied hitherto mechanisms participating in PSC's activation, still much has to be learned, especially regarding the processes triggering their transition from the quiescent to the activated state.

Ca^{2+} acts as a universal intracellular second messenger implicated in the regulation of myriad biological processes, including protein secretion, gene expression, cell proliferation, migration, contraction and cell death, as well as cell differentiation [4,5]. Several studies have already elucidated the importance of Ca^{2+} signals in PSCs pathophysiology, suggesting its role in the modulation of PSC's phenotype transition. Here, we summarized what is already known about Ca^{2+} signaling in qPSCs and aPSCs, focusing on extracellular agent's and ion channel's mediated- Ca^{2+} signals, in the PF and PC context.

How important pancreatic stellate cells are for pancreatic pathophysiology ?

The role of PSCs in the healthy and diseased pancreas has been underestimated for a long time. In the normal healthy pancreas, PSCs represent only 4-7% of the pancreas' total cell number, existing in a so-called quiescent state [6]. Under physiological conditions, qPSCs are characterized by their ability to store retinoid-containing cytoplasmic lipid droplets, having periacinar space location but also founded in perivascular and periductal regions [2,6]. The presence of retinoids participates in their quiescent state maintenance by inhibiting α -SMA expression [7]. Even though to be in an inactive state, qPSCs play a crucial role in maintaining pancreatic tissue architecture by modulating the ECM turnover through ECM protein degradation and synthesis [8]. Furthermore, the expression of toll-like receptors (TLRs 2-5) in rat qPSCs, suggests that they are also implicated in innate immunity [9]. However, it is still unknown whether human qPSCs possess TLRs as well, since they are hardly accessible.

Nevertheless, in response to pancreatic injury or inflammation, qPSCs undergo morphological and functional transformations to become activated. This transition is highlighted by the

acquisition of a myofibroblast-like phenotype. This latter is characterized by an increased expression of α -SMA and excessive ECM component's production and secretion, especially type I collagen synthesis, as well as intensive proliferative, migratory, and contractile abilities. All these properties define aPSCs as the major actor involved in the development of PF, resulting from the presence of ECM large protein amount [10]. PF constitute is the main feature of three pancreatic diseases : acute (AP), and chronic pancreatitis (CP) and as well as pancreatic ductal adenocarcinoma (PDAC), and is caused by the exacerbated activation of PSCs, inducing an increased deposition and reduced degradation of ECM [11,12]. Nonetheless, during pancreatic damage, a variety of extracellular stimuli including cytokines (e.g : interleukin [IL]-1, IL-6, IL-8 and tumor necrosis factor- α), and growth factors (e.g : platelet-derived growth factor [PDGF] and transforming growth factor β [TGF β]) trigger PSC' activation [13–16]. Alcohol metabolites [17], trypsin, thrombin, fatty and bile acids [18*,19*,20*,21**], endothelin-1 (ET-1) [22], angiotensin II (ATII) [23] and hypoxic conditions [24] participate in PSC's activation as well. In turn, aPSCs are capable of an autocrine stimulation by secreting cytokines and growth factors, in order to maintain their activated state [10,13,14,25]. Moreover, aPSCs are in permanent dialogue with their neighbor cells, involving pancreatic acinar cells (PACs) during pancreatitis [18*] and pancreatic cancer cells (PCCs) during PDAC [26]. PSC's interplay with PACs and PCCs strengthens the poor prognosis of pancreatitis and PDAC, respectively. Throughout AP, aPSCs are responsible for the perpetual PACs necrosis leading to the replacement of the pancreatic cellular component by non-cellular fibrotic tissue, caused by the necrotic amplification loop between PACs and aPSCs [27]. This phenomenon conducts to extended AP resulting in CP, that significantly increases the risk of PDAC development [18*,19*,20*,28,29]. Furthermore, in PDAC, and in hepatocellular carcinoma, activated stellate cells have the particularity of constituting the chief source of Cancer-Associated Fibroblasts (CAFs). During PDAC, the pancreatic CAFs (pCAF) or aPSCs are liable for more than 80% of the pancreatic tumor mass, representing the main component of the dense fibrotic stroma leading to the desmoplastic reaction, tightly surrounding the PCCs [30]. aPSCs enhance PDAC spreading by raising PCCs proliferation, migration et and invasion so as to form early distant metastases. In this line, aPSCs not only provide the ideal microenvironment for pancreatic tumor development and progression, but they also feed the tumor by the paracrine stimulation of PCCs through cytokines and growth factors secretion. Since recently, few studies have identified the existence of more than one distinct aPSC/pCAF subtypes, underlining their inter- and intra-tumor heterogeneity. This latter is defined from the cell surface marker expression, cell signaling, cytokine production,

aPSC-PCC interactions, and gene expression, which differs between each subtype [31]. Among others, Öhlund *et al.*'s study, is of important interest, proposing two main aPSC subpopulations according to their spatial location. One subpopulation that interacts directly with the PCCs, characterized by high α -SMA expression, called myofibroblastic CAFs, and one located more distantly from the PCCs, defined by high IL-6, IL-11 and leukemia inhibitory factor expression and low α -SMA expression, called inflammatory CAFs [32]. Neuzillet *et al.* highlighted the importance of such aPSC heterogeneity by identifying four more pCAF subpopulations, classified depending on their : α -SMA and vimentin expression, ECM production, proliferation rate, immunogenic profile and role in PDAC prognosis [33]. All these recent studies brought a new feature of aPSC functioning, showing that according to the subtype, they can promote more or less PDAC progression. In addition, they restrict oxygen availability maintaining the ideal hypoxic conditions for the tumor, as well as they limit inflammatory infiltration and delivery of chemotherapeutic agents increasing PDAC chemoresistance. Contrary to PACs, there is a positive amplification loop between aPSCs and PCC in order to stimulate PDAC propagation [34–38]. It seems that aPSCs are the key link between these three pancreatic diseases and therefore understanding and limiting PSC's activation may be the key for their medical treatment.

Distinguished Ca^{2+} signalling between quiescent and activated PSCs

Impaired Ca^{2+} homeostasis is one of the critical events triggering pancreatic failure, leading to AP, CP, or PDAC. However, the involvement of Ca^{2+} signalling in PSC's pathophysiology remains relatively little explored hitherto. Won *et al.* [21**] were the first to investigate the Ca^{2+} signature in mouse PSCs from healthy pancreas as well as from a model of chronic pancreatitis, showing evidence of distinguished intracellular Ca^{2+} signaling between qPSCs and aPSCs.

Believed for a longtime to exist in a « quiescent » state under physiological conditions, qPSCs are far away from being really « inactive », since they can generate Ca^{2+} signals in response to extracellular stimuli. Won *et al.* [21**] nicely demonstrated that qPSCs can produce Ca^{2+} signals in response to the muscarinic agonist carbachol, A23187, ATP, and bradykinin (BK). This latter finding was supported by Gryshchenko *et al.* [19*], showing that, **in fact**, qPSCs can respond to BK concentrations (100 pM), close to the ones present during AP (140 pM), through the activation of BK type 2 receptors. **Indeed, Once linked to its receptor**, BK generates a biphasic Ca^{2+} response, in qPSCs, represented by an initial large cytosolic Ca^{2+} transient **signal followed by a sustained Ca^{2+} plateau. The Ca^{2+} transient signal**

is caused by Ca^{2+} release from the endoplasmic reticulum (ER) stores, after phospholipase C (PLC) and inositol triphosphate receptor (InsP3R) activation, followed by a sustained while the Ca^{2+} plateau is induced by Ca^{2+} entry through Ca^{2+} release-activated Ca^{2+} (CRAC) channels. BK-elicited Ca^{2+} signals in qPSCs may play a key role in PSC transition transformation into the ~~from the quiescent to~~ activated state, since once activated, they respond to a much higher concentration of BK, involving BK type 1 receptor, which is a hallmark of inflammatory diseases, such as pancreatitis [18*,19*]. Moreover, BK-mediated Ca^{2+} signaling changes in aPSCs, inducing only a single cytosolic Ca^{2+} transient signal. Furthermore, qPSCs but also aPSCs ~~as well~~, are capable of producing sustained toxic cytosolic Ca^{2+} signals in response to bile acids (BAs), such as sodium cholate and taurocholate, activating intracellular InsP3R- Ca^{2+} release and thus Ca^{2+} entry via CRAC channels [20*]. In turn, BK and BAs-induced cytosolic Ca^{2+} ~~as well as~~ and hydrogen peroxide-mediated Ca^{2+} , activates the Ca^{2+} /calmodulin-dependent enzyme nitric oxide synthase in order to produce a diffusible nitric oxide-mediated inflammatory response, acting on the PAC neighbor cells. This phenomenon triggers an amplification loop by promoting PAC's death and increasing PSC-stimulating factor's secretion from dying PACs and therefore aggravate AP, rising the risk to transform into CP [39*]. Another example of different Ca^{2+} signaling toolkit between qPSCs and aPSCs, is their responsiveness to ATP. Indeed, aPSCs are approximately threefold more sensitive to ATP than qPSCs. Won *et al.* [21**] established that ATP-mediated Ca^{2+} signals are initiated near to the nucleus and propagated into it, increasing nucleoplasmic Ca^{2+} concentrations in aPSCs, which stimulates their proliferation during CP. In accordance with these data, some other authors reported that ATP ~~and~~ but also UTP and UDP-induced intracellular Ca^{2+} signals ~~are mediated~~ through several types of purinergic receptors P2X and P2Y in aPSCs. Hennigs *et al.* [40] ~~showed~~ ~~demonstrated~~ that P2X₄ and P2Y₂ (shown to be upregulated in CP) are the most abundantly expressed isoforms in aPSCs. Interestingly, Haanes *et al.* [41] completed these findings, by reporting the importance of ATP-induced Ca^{2+} signals mediated by P2X₇, shown to be overexpressed and playing an important role in aPSCs proliferation, in CP. The authors concluded that depending on ATP concentration, P2X₇ can drive PSC's proliferation (at low ATP concentration) or death (at high ATP doses). These could explain, in part, the high sensitivity of aPSCs to ATP compared to qPSCs, so they can maintain their activated state by promoting their ~~own~~ proliferation in a Ca^{2+} -dependent manner, leading to pancreatic fibrosis development. Giannuzzo *et al.* [42] highlighted the significance of P2X₇ also in collagen's deposition during the desmoplastic reaction in PDAC. Nevertheless, contrary to qPSCs, only

aPSCs produce prominent nuclear Ca^{2+} signals in response to protease-activated receptors 1 driven by thrombin and PDGF, stimulating PSC proliferation [21**]. Furthermore, ET-1 and ATII, have been reported to be implicated in fibrosis progression by increasing Ca^{2+} -dependent contractility and proliferation of aPSCs. Moreover, only aPSCs can evoke intracellular Ca^{2+} signals in response to ET-1 through ET_A receptor [22], while both qPSCs and aPSCs respond to ATII via AT_1 receptor [23]. In contrast, some other agents, like melatonin [43,44] or the pesticide dimethoate [45], impair PSC's viability even though they promote Ca^{2+} mobilization from the ER.

Ca^{2+} signaling and ion channels in aPSCs

Ca^{2+} entry is controlled by a large number of Ca^{2+} -permeable ion channels localized at the plasma membrane and PSCs have been shown to also express a number of Ca^{2+} channels.

Ion channels and pancreatitis

Zhang *et al.* [46**] were the first to report the presence of transient receptor potential (TRP) channels, ~~et~~ particularly ~~of~~ TRPV4 in aPSCs from rat model of CP. The authors **found demonstrated** an upregulated TRPV4 expression in CP **conditions** compared to control, which is in concordance with the observed increased and sustained intracellular Ca^{2+} mobilization. Their study revealed that TRPV4-mediated Ca^{2+} mobilization is enhanced by tumor necrosis factor- α stimulation, showing the importance of cytokines in Ca^{2+} signaling modulation in the aPSCs activation. ~~A Few~~ years after, Gryshchenko *et al* [19*], pharmacologically identified the presence of CRAC channels, suggesting a CRAC-mediated Ca^{2+} entry in **both** qPSCs and aPSCs. Their findings were clearly supported **recently** by Waldron *et al.* [47**], who highlighted the expression of Orai1, Orai2, and Stim1 proteins, the main constituent of CRAC channels, in aPSCs of AP model. Using a CRAC channel inhibitor, they observed an inhibition of TLR-mediated fibro-inflammatory signals, leading to a decrease expression, among others, of IL-6, $\text{TGF}\beta_1$, type I collagen, and α -SMA, proposing a crucial role of CRAC-mediated Ca^{2+} entry in cytokine expression from aPSCs in AP.

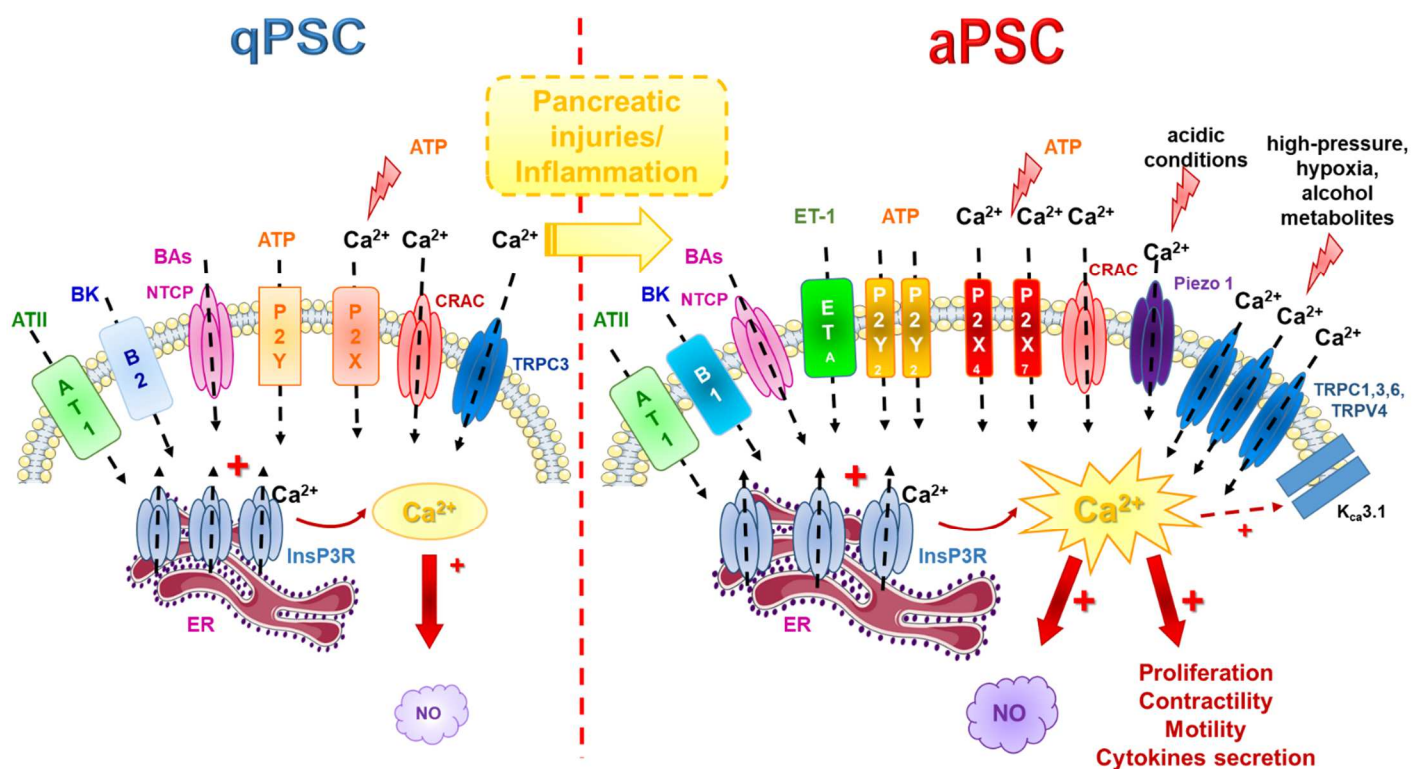
Ion channels and PDAC

Several reports have been focusing on the role of PSC-expressed ion channels in the context of PDAC as well. Fels *et al.* [48**] firstly established a role of TRPC1 ion channel in the activation of PSCs, under high-pressure application, which is one of the PDAC microenvironment features and one of PSC's activator stimuli, thereby perpetuating fibrosis

formation. They demonstrated that pressure induces TRPC1 activation allowing Ca^{2+} influx into PSCs in order to regulate their migration and enhance their activated behaviour. Similar to pressure, it has been shown by Nielsen *et al.* [49**] that hypoxia, another hallmark of PDAC microenvironment, known to activate PSCs, induces activation of TRPC6 leading to an increase of Ca^{2+} influx, which promotes aPSC's migration and promigratory factor's secretion by aPSCs. The report of Storck *et al.* [50**] brought evidence of cooperative regulation of PSC's migration, mediated by TRPC3 (shown to be upregulated in the PDAC stroma) and the calcium-activated potassium channel $\text{K}_{\text{Ca}3.1}$, under PDGF stimulation, known to activate PSCs. The authors intelligently demonstrated that Ca^{2+} entry through TRPC3 is involved in neighboring $\text{K}_{\text{Ca}3.1}$ channel's activation, resulting in increased PDGF-stimulated migration of PSCs. These data confirm, once more, the importance of cytokines in the regulation of PSC's Ca^{2+} signalling. ~~To complete~~ The key role of PDAC microenvironment on PSC's ion channels-mediated Ca^{2+} influx in the perpetuation of cancer progression, was also documented by Kuntze *et al.* [51**]. Their work determined the impact of Piezo1-mediated Ca^{2+} influx on PSC's pathophysiology, under acidification conditions, also characteristic of PDAC microenvironment. ~~It appears that~~ Indeed Piezo1 activity is ~~modified~~ reduced upon acidification, ~~causing leading to~~ Ca^{2+} influx decrease. ~~The reduction of Piezo 1 activity would be probably~~ due to the protonation-caused ~~inhibition~~, in order to protect aPSCs from cell death and promote their migration into well-perfused invasive tumor regions where Piezo1 can be fully functional. Therefore, under physiological pH, the increased Piezo1 mediated- Ca^{2+} influx enhances PSC's migration ~~as well as and~~ myosin light chain isoform 9 phosphorylation, leading to aPSC's cytoskeletal architecture modification, which, in turn, increases aPSC's motility.

Conclusion

To summarize, pancreatic injury or inflammation induces a disequilibrium in PSC's composition, leading to an increased number of aPSCs, due to the transition of qPSCs to aPSCs. Taken together, it seems that in both qPSCs and aPSCs, Ca^{2+} signalling is initiated through the canonical pathway, involving InsP3R -mediated Ca^{2+} release from the ER, under diverse extracellular stimuli. Moreover, in aPSCs, ion channels-mediated Ca^{2+} influx appears to be a key effector for the development and progression of PF and PDAC, as summarized in the conclusion scheme (Figure 1). ~~And what if managing PSC's overloaded intracellular Ca^{2+} , by a combination of two or more ion channel inhibitors, is the answer for controlling PSC's transition and so PF and PDAC?~~ Hence, controlling the transition of qPSCs to aPSCs



will limit pancreatic fibrosis formation and so PF and PDAC promotion. May the answer to this challenge is laying on the management of PSC's intracellular Ca^{2+} by using a combination of two or more ion channel inhibitors.

Figure 1 : Concluding model that distinguish the Ca^{2+} signature between quiescent (qPSCs) and activated PSCs (aPSCs). In response to pancreatic injury or inflammation qPSC undergo transformations to become activated. During this transition, the cell surface expression and function of several receptors and ion channels are modified. qPSCs are characterized by bradykinin type 2 receptor activation, while in aPSCs, bradykinin activates type 1 receptor. Moreover, aPSCs are defined by an overexpression of P2Y_2 receptor, P2X_4 and P2X_7 channels, as well as TRPC3 and TRPV4 channels. However, both qPSCs and aPSCs express AT1 receptors, NTCP transporters and CRAC channels but only aPSCs express ET_A receptor.

ATII = angiotensin II, AT1 = angiotensin II type 1 receptor, BK = bradykinin, B1 = bradykinin type 1 receptor, B2 = bradykinin type 2 receptor, BAS = bile acids, CRAC = Ca^{2+} -release-activated Ca^{2+} channels, ER = endoplasmic reticulum, ET-1 = endothelin-1, ET_A = endothelin type A receptor, InsP3R = inositol trisphosphate receptor, NO = nitric oxide, NTCP = sodium-taurocholate cotransporting polypeptide, P2Y_2 = purinergic receptors, P2X_4 = purinergic channels, TRPC = transient receptor potential-canonical channels, TRPV = transient receptor potential-vanilloid channels

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CONFLICT OF INTERESTS STATEMENT

The authors declare no conflicts of interest

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