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Interaction of tumour cells with their microenvironment: ion channels and cell adhesion molecules. A focus on pancreatic cancer

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Cancer must be viewed as a 'tissue', constituted of both transformed cells and a heterogeneous microenvironment, the 'tumour microenvironment' (TME). The TME undergoes a complex remodelling during the course of multistep tumourigenesis, hence strongly contributing to tumour progression. Ion channels and transporters (ICTs), being expressed on both tumour cells and in the different cellular components of the TME, are in a strategic position to sense and mediate signals arising from the TME. Often, this transmission is mediated by integrin adhesion receptors, which are the main cellular receptors capable of mediating cell-to-cell and cell-to-matrix bidirectional signalling. Integrins can often operate in conjunction with ICT because they can behave as functional partners of ICT proteins. The role of integrin receptors in the crosstalk between tumour cells and the TME is particularly relevant in the context of pancreatic cancer (PC), characterized by an overwhelming TME which actively contributes to therapy resistance. We discuss the possibility that this occurs through integrins and ICTs, which could be exploited as targets to overcome chemoresistance in PC.

1. Introduction

Tumour biology can be understood only taking into account both the individual transformed cells and the 'tumour microenvironment' (TME). The TME is a complex array of cells and extracellular matrix (ECM) proteins that tumour cells construct during the course of multistep tumourigenesis [1] and strongly influences the behaviour and malignancy of the transformed cells. Moreover, the TME may change during tumour progression, hence it may differ (structurally and functionally) from the primary tumour to its metastases [2,3]. The TME greatly varies among cancers of different histogenesis. For example, in leukaemias, it is mainly represented by the bone marrow, with the complex array of stromal and vascular cells which constitute the bone marrow nike, where leukaemia stem cells reside [4]. In carcinomas, a clear distinction is made between the neoplastic cells, named as the 'parenchyma', and the TME, indicated as the 'tumour stroma'. An active and overwhelming tumour stroma (in this case, addressed as 'desmoplastic reaction') characterizes some specific carcinomas, such as breast, prostate or pancreatic cancer (PC) [5]. In particular, the desmoplastic reaction is one of the histopathological and functional hallmarks of PC: histopathological analysis reveals the presence of dense collagen (types I and III) bundles associated with fibroblasts, with loss of basement integrity and invasion of malignant cells into the interstitial matrix with exposure of collagens. The ECM in PC also contains fibronectins, tenascin-C, laminins, mainly secreted by pancreatic stellate cells (PSCs), a cellular phenotype peculiar of PC [6]. The desmoplastic reaction in PC is associated with an abnormal vasculature with numerous circuitous small leaky blood

Table 1. Examples of ion channels expressed by different cell types participating to the TME complexity.

TME cells	ion channel type	evidence of ion channel expression
human mesenchymal cells		
endothelial cells	TRPC1, TRPC4, TRPC6, TRPM7, TRPV1, and TRPV4	[12]
endothelial cells	Orai1ans Stim1 (CRAC channels)	[12]
endothelial cells	K_{Ca}	[11]
bone marrow mesenchymal cells	K_{ATP}	[13]
bone marrow – umbilical cord vein mesenchymal cells	$K_V1.1$, $K_V4.2$, $K_V1.4$, Kir2.1, heag1, MaxiK, hNE-Na, and TWIK-1	[14]
mesenchymal cells	nicotinic and muscarinic receptors	[14]
innate immune cells		
neutrophils	TRP, K_{Ca}, Cl	[11]
macrophages	K_{IR}, ligand-gated cation channel P2X7	[11]
specific immune cells		
lymphocytes	Kv1.3, $K_{Ca}3.1$, TRP	[15 – 17]

vessels and capillaries [7]. On the whole, the desmoplastic reaction is one of the major contributors to PC malignancy (see below).

Taking into account the relevance of the tumour stroma, antineoplastic therapeutic strategies must be tuned to target the 'cancer tissue', e.g. not only tumour cells, but also the cellular constituents of the TME [8,9]. In this context, deciphering the role of ion channel and transporter (ICT) proteins in the crosstalk between the tumour cells and the various constituents of the TME merits particular attention, also from a therapeutic viewpoint.

In this review, we briefly describe the TME as well as ICTs present in the different cells of the TME. For molecular and functional description of ICTs, we refer to other papers [10]. Then, we focus on adhesion receptors of the integrin family, and on their functional interaction with ICT. Because most of these data have been reported elsewhere [11], in this review, we mainly focus on PC, where the TME drives tumour progression and resistance to therapy.

2. The tumour microenvironment and its ion channels and transporters profile

The TME comprises both cells (endothelial cells and their precursors, fibroblasts and specialized mesenchymal cells as well as cells of the innate and specific immunity) and the proteins of the ECM. The main ICTs expressed in the cells of the TME have been detailed in [11] and are summarized in table 1. We must remember the prevalent role of Ca^{2+}-permeable channels (both voltage-dependent and non-selective channels of the TRP family) in endothelial cells (ECs; reviewed in [18,19]). The electrochemical driving force for Ca^{2+} entry is provided by Ca^{2+}-dependent K^+ channels (K_{Ca}), and the cooperation between Ca^{2+}-permeable channels and K_{Ca} can serve to sustain the Ca^{2+}-dependent secretion of growth and vasodilating factors by ECs. Because many recent studies are revealing distinctive gene expression profiles and cell-surface markers of tumour-associated versus normal ECs [20], it is possible that ICT can contribute to determine such difference. Owing to

the relevant role of ECs and tumour angiogenesis in tumour progression, ICTs could be exploited to develop novel anti-angiogenesis therapies to selectively target the ECs inside the cancer tissue.

Inside the TME, both 'cancer-associated fibroblasts' [21] and specialized mesenchymal cells, such as myofibroblasts or the PSCs [22], are present. Although cancer-associated fibroblasts and specialized mesenchymal cells are not malignant, in that they do not bear cancerogenic mutations, they can exhibit epigenetic changes, which affect their behaviour [23]. Moreover, they are actively secreting ECM proteins, behaving as the main determinant of the desmoplastic reaction. The latter, directly or through the creation of a hypoxic environment, regulates tumour progression and dictates therapy resistance [24].

Finally, the TME is densely infiltrated by cells of both the innate and adaptive arms of the immune system, whose exact role in controlling tumour progression is still debated [25,26]. Neutrophil ion channels (mainly TRPs, K_{Ca} and Cl^- channels; reviewed in [27]) are exploited to accomplish the antimicrobial activity which characterizes these cells of innate immunity. Macrophages express K_{IR} channels, which are involved in cell adhesion, and in turn affect the Ca^{2+}-dependent macrophage activation [28]. Macrophages also express P2X7 receptors, which mediate the release of lysosomal cathepsin [29]. This fact could contribute to ECM remodelling, with a strong impact on malignant progression. The complex array of ion channels which contribute to T lymphocyte activation has been thoroughly described [15,16]: a coordinated influx of Ca^{2+} is indeed essential to trigger T lymphocyte activation, and an unique contingent of ion channels (including Kv1.3 and $K_{Ca}3.1$ K^+ channels) orchestrate the duration and intensity of the Ca^{2+} signals. Moreover, the balance of these channel types constitutes a specific functional marker of activated lymphocytes, thus providing a possible novel therapeutic target [11].

The ECM of the TME comprises proper matrix proteins, a multitude of growth factors and cytokines that, more or less directly, affect malignant cells [30]. Type I collagen, fibronectin and thrombospondins are the ECM proteins which

characterize the TME, with collagen I being the main determinant of the desmoplastic reaction. ECM proteins can affect tumour progression by controlling cell motility as well as by dictating tumour cell differentiation programmes [30]. The ECM of the TME is produced by both tumour and mesenchymal cells, which also modulate their ECM by secreting proteases [31]. TME remodelling can lead to the release of molecules sequestered in the ECM, such as the vascular endothelial growth factors [32], and many cleavage products of ECM proteins [2], which further control tumour angiogenesis and metastasis formation.

The crosstalk between the ECM and tumour cells, as well as of the cellular components of the TME, is mainly mediated through the intervention of adhesion receptors of the integrin family.

3. Cell adhesion molecules: the integrin family

(a) Integrin structure and function

Integrin receptors are transmembrane proteins formed by non-covalent association of α- and β-subunits. To date, 18 α- and eight β-subunits are known in mammals. All subunits are type I transmembrane glycoproteins with a short cytoplasmic tail (20–70 amino acids), a membrane-spanning helix and a large multidomain extracellular portion [33]. The β4-subunit is an exception because its cytoplasmic domain contains around 1000 amino acids [34]. Integrin subunits can combine to form at least 24 functional heterodimers, each of which binds a specific array of ECM proteins, or cell adhesion molecules (CAMs), that act as 'counterreceptors'.

Integrins are more than CAMs: they can transmit bidirectional signals across the plasma membrane. On the cell surface, integrins are normally in the low affinity state, and they can be activated through an 'inside-out' signalling pathway. During this process, two cellular activators, talin and kindlin, play an essential role, and the binding of talin to the β integrin cytoplasmic tail is proposed to be the final step in integrin activation [35]. Conversely, the binding of the integrin extracellular ligands transmits signals inward, a process called 'outside-in' signalling. Outside-in signalling of integrin exerts significant influences on cell mobility, proliferation, differentiation, etc. [36]. Because the list of papers describing the many facets of integrin-mediated signalling pathways is immense, we refer to those [33,37,38], and only limit to summarize that integrins seem to be linked to almost all of the known signalling pathways, including induction of cytosolic kinases, stimulation of the phosphoinositide metabolism, activation of Ras/MAPK and protein kinase C (PKC) pathways and regulation of small GTPases. Moreover, integrin signalling often overlaps with that triggered by growth factor or cytokine receptors [38]. The overlap and proper integration of differently arising signals, makes physiological sense, because cells must integrate multiple stimuli from the ECM, growth factors, hormones and mechanical stress, to organize appropriate responses. The same integration occurs and determines the fate of even more in tumour cells inside the cancer tissue.

(b) Integrin relationships with ion channels

The relationships between integrins and ion channels in the cell-to-cell and cell-to-matrix interactions have been long described [39]. The earliest indications came from studies on neuronal and leukaemic cells, in which many cellular processes elicited by the engagement of adhesive proteins, such as differentiation, migration and neurite extension, turned out to depend on ion channel activation [40–43]. When associated with integrins, ion channel function becomes bidirectional itself: it is regulated by extracellular signals (through integrins) and in turn controls integrin activation and/or expression [39]. Interestingly, the same kind of complex bidirectional signalling has been observed for some ion transporters, in particular those mediating proton fluxes [44–46], which are so relevant in the establishment of a reversed H^+ gradient which characterizes neoplastic malignancy [47].

The bidirectional crosstalk between integrins and ion channels occurs through different mechanisms: it may rely on cytoplasmic messengers, such as Ca^{2+} or protein kinases, commuting between the two proteins (reviewed in [39]). For example, T lymphocyte activation in which β1 integrins are involved is underlied by a coordinated influx of Ca^{2+}, which is controlled by and, conversely, regulates K^+ channels [16]. The transmission of mechanical forces at focal adhesion sites is triggered by integrins and mediated by calcium [48], but also involves the activation of signalling molecules, such as FAK and c-Src [49].

Another aspect of integrin/ion channel interaction is the fact that integrins and ion channels can interact directly at the plasma membrane level. In other words, the two proteins co-assemble on the plasma membrane and give rise to supramolecular complexes, which constitute platforms for triggering and orchestrating downstream intracellular signals. The first evidence was obtained in immune cells by Levite et al. [50], who found that the β1 integrin subunit associated with Kv1.3 channels in T lymphocytes. Shortly afterwards, a physical link between Kv1.3 channels and β1 integrins was described in melanoma cells [51]. Our group found that the β1 integrin subunit associates with another K^+ channel, Kv 11.1 or hERG1, on the plasma membrane of tumour cells, either leukaemias or solid cancers [52–56]. This complex can also involve growth factor or chemokine receptors and, once assembled, recruits cytosolic signalling proteins, which in turn activate intracellular signalling in an integrin- and ion channel-dependent manner. This has a clear negative impact on the leukaemia disease [54], can trigger chemoresistance [55] or control angiogenesis and tumour progression [56].

Another mechanism involving the interaction between integrins and ion channels contributes to determine integrin recycling [57]. In particular, CLIC3 chloride channels colocalize with active α5β1 integrins in late endosomes/lysosomes, allowing the integrin to be retrogradely transported and recycled to the plasma membrane at the cell rear. This mechanism also involves Rab25 and has a clear impact on cancer behaviour. In fact, in PC, active integrins and CLIC3 are necessary for cancer cell invasion [57].

(c) Integrins and ion channels: role in cell migration

A most interesting aspect regards the comprehension of several mechanisms by which integrins and different channel types interact in controlling cell migration. Besides being a fundamental component of embryogenesis and tissue remodelling in the adult, these processes are relevant in tumour cell invasiveness and metastatic spread. As typical mediators of cell interaction with the environment, it is not surprising that integrins play major roles in eukaryotic cell migration.

Table 2. Examples of interactions between ion channels and integrin subunits in cells of TME.

cell type	ion channel type	integrin subunit type	evidence of ion channel expression
endothelial cells	Ca^{2+} channels (BKCa — increased cytosolic Ca^{2+})	$\beta1$, $\beta3$	[39,68]
endothelial cells	TRPV4	$\beta1$	[11]
vascular smooth muscle cells	L.type Cav1.2	$\alpha5\beta1$	[39]
neutrophils	Cl^-	$\beta2$	[45]
lymphocytes	Kv 1.3, K_{Ca}	$\beta1$	[16,50]

Moreover, we are nowadays aware that several types of Ca^{2+}-activated and voltage-dependent K^+ channels are also implicated in the cell migration machinery. This rapidly growing field has been reviewed recently [58,59] and will not be discussed in detail here. We limit our discussion to the fact that K^+ channels can form complexes, and thereby modulate several proteins involved in cell movement, such as FAK [60,61], cortactin [62,63] and integrins themselves. Interesting speculations can derive from studies on $\alpha9\beta1$ integrins, which can regulate cell movement by activating inward rectifier K^+ channels (IRK) [64]. IRK channels, along with the integrin, are physically linked to spermidine/xspermine N1-acetyltransferase, the key enzyme in the pathway that acetylates spermine and spermidine to putrescine, thus controlling the intracellular concentration of polyamines. Polyamines are critical regulators of neoplastic growth and also the main intracellular messengers controlling IRK activity. A functional network may hence be figured out, where an adequate intracellular concentration of polyamines converges to trigger a proper $\alpha9\beta1$-dependent cell movement, through the modulation of IRK channels [65].

(d) Integrins and ion channels in the cells of the tumour microenvironment

Integrins and ion channels also interact at the level of the TME. One example involves cells of the innate immune system: neutrophils release Cl^- to accomplish their antimicrobial activity; Cl^- release occurs through the activation of Cl^- channels which is, at least in part, dependent on β_2 integrin-mediated adherence to fibronectin [66]. Macrophages express K_{IR} channels, whose activity is modulated by VLA4 ($\alpha4\beta1$) integrin receptors and hence by cell adhesion, which in turn affects the Ca^{2+}-dependent macrophage activation [28]. Another example is represented by ECs and their Cl channels of the CLCA protein family. In ECs, CLCA2 behaves as a vascular addressin for metastatic, blood-borne, cancer cells, facilitating vascular arrest of cancer cells via adhesion to $\beta4$ integrins, and hence promoting metastatic spread. In addition, the $\beta4$-integrin–CLCA complex stimulates Src-dependent cell signalling through FAK and extracellular signal-regulated kinase (ERK), leading to increased proliferation of metastatic foci [67].

A list of ion channels physically or functionally linked to integrins in the cells of TME is reported in table 2.

4. The role of the tumour microenvironment in the progression of pancreatic cancer

PC, mainly represented by the histological form of pancreatic ductal adenocarcinoma, is one of the most lethal gastrointestinal malignancies, representing the second leading cause of death among them. The overall 5-year survival rate is less than 6%, in the most recent American Database (http://seer.cancer.gov/csr/1975_2006), and a median survival of 18 months from diagnosis for those operated-on patients with no evidence of residual disease. Similar disappointing figures are available from European surveys [69]. The malignant nature of PC is mainly due to its aggressive growth and rapid development of distant metastases, thus making treatment extremely difficult. Additionally, PC is locally invasive, surrounded by a dense desmoplastic reaction (see §1) which can involve adjacent vital structures, limiting the chance for complete resection. Indeed, less than 20% of patients are candidates for surgical resection at the time of diagnosis, while almost one half have metastatic disease. Moreover, of the few patients who undergo surgery with radical intent (R0), most will develop a recurrence within 15 months. Although surgery remains the cornerstone of cure, the addition of adjuvant treatments is required [70]. Chemotherapy (in Europe) or radiochemotherapy (in North America) has been used, either as adjuvant to surgery or as definitive treatment for unresectable disease, with conflicting results [71,72]. Failure of traditional therapeutic approaches for this devastating disease had led to many efforts towards the study of molecular biology and targeted therapies, in order to create a multimodal therapeutic strategy [73]. Among target therapy drugs, only erlotinib that targets the EGF-R, has been shown to improve survival when used in combination with gemcitabine, compared with gemcitabine alone. Nevertheless, the clinical response rate remains modest, mainly owing to the intrinsic chemoresistance of PC cells [74]. Investigating mechanisms mediating chemoresistance is therefore of clinical interest in drug development of new agents. As stated in §1 one of the major contributors to PC malignancy and therapy resistance is represented by the desmoplastic reaction. Further understanding of how the TME facilitates PC cell malignancy will identify unique targets that may finally improve the treatment of patients with PC [6]. Some insights are briefly reported below.

(a) Interaction between tumour and stromal cells in pancreatic cancer: the role of pancreatic stellate cells

Normal development of glandular structures requires interactions between stromal cells and the epithelial cells that will eventually line the surface of the gland. In the pancreas, mesenchymal cells stimulate adjacent pluripotent cells to form acini, while inducing other remote cells to complete the
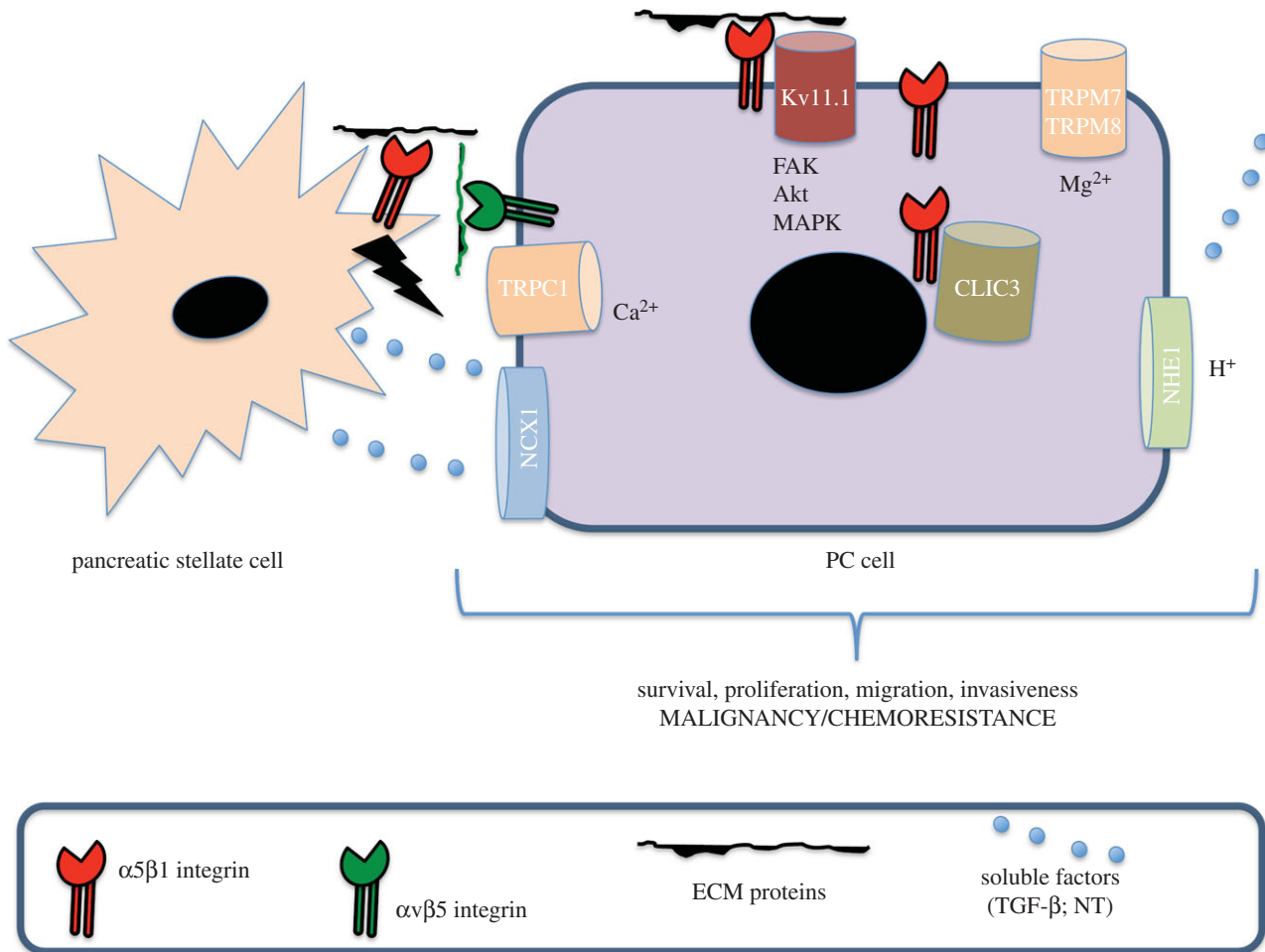
Figure 1. A model illustrating tumour–stroma interaction in PC as well as the involvement of integrins.

endocrine pathway. These interactions in normal development require specific proteins within the ECM, notably laminin. Crosstalk between mesenchymal and epithelial compartments occurs through soluble messengers acting on a paracrine (or autocrine) manner, cell-surface receptor activation through direct cell-to-cell contact, and specific ECM proteins secreted by mesenchymal cells [75,76]. Perturbations of the normal mesenchymal–epithelial interactions can lead to unregulated cell growth, as occurs in cancer.

PSCs are the predominant mesenchymal cells within the PC stroma and the main determinant of the desmoplastic reaction [77]. PSCs originate from bone-marrow-derived mesenchymal stem cells, and are similar to myofibroblasts found in other tumour stroma (breast, prostate cancer). PSCs have the ability to transdifferentiate from a 'quiescent', retinoid/lipid storing phenotype in the normal pancreas to an 'activated', α-smooth muscle actin producing myofibroblastic phenotype. Activators of PSCs *in vivo* include cytokines (IL1, IL6, IL8 and TNF-α) growth factors (PDGF and TGF-β) and reactive oxygen species released by damaged inflammatory cells recruited in response to injury to the pancreas. Activated PSCs, in turn, can produce autocrine factors, such as PDGF, TGF-β, cytokines and proinflammatory molecules which may potentiate an activated phenotype [77–79]. PSCs, besides being implicated in the genesis of chronic pancreatitis, are critical for the development of the desmoplastic reaction in PC, being the main source of ECM proteins [78]. Moreover, because PSCs are activated by PC cells *in vitro*, a synergistic relationship between the

two types of cells occurs, that favours the development and progression of PC.

(b) Integrins in pancreatic cancer

In PC, it is well established that ECM proteins, such as collagen, fibronectin and tenascin-C, interact with cell-surface integrins to provide intracellular signals to both PSCs and PC cells [80]. For example, in PSCs, β1 integrins are important not only to modulate cell adhesion to the basement membrane, but also to determine the proper formation and differentiation of acini during normal development [81]. PC cells express at least two major classes of integrins: β1-containing integrins (mainly α2β1 and α5β1), which mediate cell adhesion to fibronectin, laminin and collagen (type I and IV), and αvβ5, which is mainly involved in PC cell adhesion to vitronectin [82,83]. Integrin engagement has been reported to determine the malignant phenotype of PC cells by regulating cell proliferation [82], invasion [84] as well as by cytokine secretion [85]. For example, tenascin-C can affect PC cell growth and migration through the activation of β1 integrin intracellular signalling pathways [86]. Some recent evidence indicates that intergrin involvement in PC cell invasion can also occur through a complex interaction with EGF receptor (EGF-R) and the modulation of Src-centred intracellular signalling pathways [84].

On the whole, integrins, mainly those containing the β1 subunit, being expressed both in PC cells and in PSCs, and

regulating the production of ECM proteins by the latter, are pivotal in regulating tumour–stroma interaction in PC. β1 integrins can hence drive different aspects of tumour cell behaviour, including chemo- and radio-resistance (see below).

(c) Ion channels and transporters in pancreatic cancer

In the past few years, different ICT proteins have been characterized in PC cells: TRP cationic channels of the 'melastatin-related' type (TRPM) have been reported to be overexpressed in PC cells. In particular, both TRPM8 [87] and TRPM7 [88] are overexpressed in pancreatic ductal adenocarcinoma cells, where they regulate either cell proliferation or migration. TRPM7 activation triggers a Mg^{2+}-sensitive 'suppressor of cytokine signalling 3a' (Socs3a) pathway, which regulates exocrine pancreatic epithelial cell proliferation, both in development and cancer [89]. Moreover, Dong et al. [90] reported that TGF-β induces Ca^{2+} entry in PC cells via TRPC1 channels and the Na^+/Ca^{2+} exchanger NCX1, thus raising intracellular Ca^{2+} concentration. Ca^{2+} increase is in turn essential for PKCα activation and subsequent tumour cell invasion [90]. As described above [57], CLIC3 channels regulate integrin recycling and behave as independent prognostic indicators in PC. This highlights the importance of active integrin trafficking as well as Cl^- channels as potential drivers to cancer progression. Among voltage-gated K^+ channels, Kv 11.1 (hERG1) is expressed in PC and identifies a patient group with worse prognosis [91]. Because hERG1 physically and functionally interacts with β1 integrins in different tumour cells (see above) including PC [91], it is tempting to speculate that it could drive PC malignancy through the modulation of integrin-mediated signalling. Finally, the sodium hydrogen exchanger 1 (HNE1) is expressed in PC cells [92,93] and is activated by growth factors such as neurotensin (NT). The NT/HNE1 pathway may be implicated in the early progression of PC by localized acidification and induction of an aerobic glycolytic phenotype with higher metastatic potential.

A model illustrating tumour–stroma interaction in PC, as well as the involvement of integrins, is reported in figure 1. Those ICTs which interact with integrins to modulate the crosstalk between PC cells and the TME are also illustrated.

(d) The tumour microenvironment drives therapy resistance in pancreatic cancer

The altered crosstalk between stromal ECM proteins and integrins expressed on both tumour and TME cells is also implicated in mechanisms of acquired resistance to chemo- and radiotherapy in PC. An in vitro study using different PC cell lines cultured in the presence of collagen, fibronectin or laminin, showed different induction of chemosensitivity, depending on the type of substrate [94]. Indeed, inhibiting ECM-integrin function in combination with chemotherapy may be a potential therapeutic intervention that could specifically target the desmoplastic reaction. One example could be the monoclonal antibody targeting α5β1 or αvβ3 integrin (Vitaxin). Moreover, PSCs are known to promote radioprotection of PC cells: this effect is dependent on a signalling pathway triggered by β1 integrin and converging on FAK [95].

Treatment failure in PC may be due, at least in part, to our limited understanding of how the fibrotic tissue and the stromal cells present within this tumour can facilitate the rapid progression of this cancer type. Therefore, a better understanding of the mechanisms which regulate PC cell interactions with the TME, with particular attention to the crosstalk between integrins and ICTs, could open a new perspective to effectively treat PC.

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