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Immune evasion on the nanoscale: Small extracellular vesicles in pancreatic ductal adenocarcinoma immunity

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is a type of cancer alarmingly expanding in our modern societies that is still proving to be very challenging to counteract. This disease constitutes a quintessential example of the multiple interactions existing between the tumour and its surrounding microenvironment. In particular, PDAC is characterized by a very immunosuppressive environment that favours cancer growth and makes this cancer type very resistant to immunotherapy. The primary tumour releases many factors that influence both the microenvironment and the immune landscape. Extracellular vesicles (EVs), recently identified as indispensable entities ensuring cell-to-cell communication in both physiological and pathological processes, seem to play a pivotal function in ensuring the delivery of these factors to the tumour-surrounding tissues. In this review, we summarize the present understanding on the crosstalk among tumour cells and the cellular immune microenvironment emphasizing the pro-malignant role played by extracellular vesicles. We also discuss how a greater knowledge of the roles of EVs in tumour immune escape could be translated into clinical applications.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive silent malignancies [2]. In its early stages, PDAC lacks distinct symptoms and there are no reliable, quantifiable and non-invasive biomarkers facilitating early detection [3]. Subsequently,

Abbreviations: AA, Arachidonic acid; Arg-1, Arginase 1; ATP, adenosine triphosphate; CAF, Cancer-associated fibroblast; CAR, chimeric antigen receptor; CA19–9, Serum carbohydrate antigen 19–9; CCL2, Chemokine (C-C motif) ligand 2; COX2, Cyclooxygenase-2; CTLA-4, Cytotoxic T-lymphocyte associated-antigen-4; DAMP, Damage-associated molecular pattern; DC, Dendritic cell; EV, Extracellular Vesicle; FAK, focal adhesion kinase; FasL, Fas ligand; FFA, Free Fatty Acid; GPC1, glypican-1; HMGB1, high mobility group box 1; Hsp, Heat shock protein; IDO, indoleamine 2,3-dioxygenase; IIC, Infiltrating Immune cell; IFN-γ, interferon-gamma; IL, Interleukin; ISEV, International Society for Extracellular Vesicles; lncRNA, Long non-coding RNA; MDSC, Myeloid-derived suppressor cell; MHC I/II, Major histocompatibility complex proteins I and II; MIF, Macrophage migration inhibitory factor; M-MDSC, Monocytic MDSCs; MSCs, Mesenchymal stem cells; mTOR, Mammalian target of rapamycin; PAMP, Pathogen-associated molecular pattern; PD-1, Programmed cell death protein 1; PD-L1, Programmed death-ligand 1; PDAC, Pancreatic ductal adenocarcinoma; PGE_2, Prostaglandin E2; PI3K, phosphoinositide-3-kinase; PKB, Protein kinase B, also known as AKT; PMN, Pre-metastatic niche; PMN-MDSC, Polymorphonuclear MDSCs; PNI, Perineural invasion; PRKDC, DNA-dependent protein kinase catalytic subunit; PSC, Pancreatic stellate cell; PTEN, Phosphoinositide-3-kinase/phosphatase and tensin homolog; SBF2-AS1, SET-binding factor 2 antisense; *SMAD4*, SMAD family member 4; S100A4,, S100 calcium binding protein A4; S100A8/9, S100 calcium binding protein A8/9; STAT3, Signal transducer and activator of transcription 3; TAA, Tumour-associated antigen; TAM, Tumour-associated macrophage; TCR, T-cell receptor; TF, Tissue factor; TGF-β, Transforming growth factor-beta; TGFBR, Transforming growth factor-beta receptor; TLR, Toll-like receptor; TME, Tumour Microenvironment; TNM, Tumour node metastasis; Treg, Regulatory T cell; VEGF, Vascular endothelial growth factor.

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80% of cases exhibit local tumour invasion or distant metastases at the time of diagnosis [4,5], precluding the use of surgical resection [6]. Additionally, cytotoxic and immunological therapies (e.g., checkpoint inhibitors) offer only modest survival benefits and are often associated with significant side effects [6,7]. Interestingly, specific immunotherapies (e.g., tumour-antigen antibodies, tumour protein vaccines) have been associated with improved immune response and overall survival in PDAC patients [8]. This indicates immune-based therapy can stimulate an anti-tumour response, although this is limited by a low mutational burden and the immunosuppressive tumour microenvironment (TME) [9]. For example, myeloid-derived suppressor cells (MDSCs) and tumour associated macrophages (TAMs) contribute to tumorigenesis and immune invasion by suppressing T cell responses [10].

PDAC progression is facilitated by an intricate network of intercellular communication within the tumour microenvironment (TME). In recent years, extracellular vesicles (EVs), particularly small EVs (sEVs) have been highlighted for their role in mediating the crosstalk between tumour, stromal and immune cells within this TME [11]. SEVs are lipid-bound, anucleate particles released from all cells that contain proteins, lipids, nucleic acids, and other functional biomolecules derived from their cell of origin [12–14]. sEVs released from different cell types, or the same cell type in different conditions, exhibit variation in size and contents, contributing to these vesicles' unique and highly heterogeneous nature [13,15,16]. Moreover, this indicates that sEV biogenesis relies on active cargo recruitment and provides a 'real-time' indication of the intracellular environment of the parent cell [17].

In PDAC, sEVs from cancer cells influence neighbouring immune cells by transferring various biomolecules, including proteins, lipids, and nucleic acids, modifying signal transduction and altering cellular function. For instance, PDAC-derived sEVs can induce the pro-tumorigenic polarisation of macrophages and inhibit effector T-cell responses to promote immunosuppression and anti-tumour immunity [11]. Therefore, understanding the crosstalk between sEVs and immune cells may suggest new strategies to improve the efficacy of immunotherapy in PDAC. In this review, we will summarise the existing knowledge of sEVs in PDAC in the context of the immune response, immune evasion, and cancer progression. Furthermore, we will discuss the status of sEVs as biomarkers to screen and predict disease progression, and their potential as therapeutic devices to increase tumour response and patient survival.

2. Small extracellular vesicles: definition, characterisation and function

EVs were first described as a mechanism of cellular waste disposal [18], however significant research has demonstrated that these vesicles are critical mediators of intercellular communication between adjacent and distant cells, in both physiological and pathophysiological processes [12,19]. The term 'extracellular vesicle' describes membrane-bound, anucleate particles released by cells with a size range of 30–1000 nm [20]. EVs can be broadly classified into three groups based on their size, molecular composition and sub-cellular origins, with small EVs (sEVs) ranging $<$ 200 nm, while medium EVs (mEVs) and large EVs (lEVs) range above 200 nm and 1000 nm, respectively.

Although the term "exosome" is commonly used to indicate EVs within the 30–150 nm size range, there are currently no specific markers to clearly differentiate exosomes (endosome-origin) and ectosomes (plasma membrane-derived). Therefore, The International Society for Extracellular Vesicles (ISEV) endorses the term 'small extracellular vesicles' to describe EVs within this size range [20]. Additionally, unless the particles' subcellular origins can be confirmed (via live cell imaging), it is recommended that the physical characteristics (size range or density), biochemical composition and conditions of cellular origins are described during reporting [20]. In this review, 'sEVs' will be used per the ISEV nomenclature.

sEVs have received increasing attention within the field due to their nano-scale size, extensive molecular contents, and highly heterogeneous nature [12–14]. Several studies have also demonstrated that sEVs released from different (or the same) cell types can vary in size and contents, contributing to the unique and highly heterogeneous nature of these vesicles [13,15,16]. The bi-lipid structure and lipid arrangement of sEVs provide them with a significant stability and bioavailability in bodily fluids, including blood, urine, saliva, ascites, and breast milk. Recently, sEVs have been investigated as key players in cancer progression and as a mechanism for the targeted delivery of drugs to the tumour core. In addition, the extensive and specialised molecular composition of sEVs makes them an attractive source for novel non-invasive diagnostic or prognostic biomarkers, particularly in diseases such as PDAC.

3. sEV crosstalk between PDAC and immune cells

PDAC is characterised by a myeloid-inflamed stroma with an immunosuppressive local environment. During tumour formation, cancer-derived sEVs restrain immunosurveillance and promote immune tolerance to provide a landscape favourable for tumour growth and survival [21,22]. For example, PDAC-derived sEVs are enriched with immunoinhibitory molecules (e.g., PD-1, PDL1, CTLA4), tumour-associated antigens (TAAs), major histocompatibility complex (MHC) class I and II proteins, co-stimulatory molecules and cytokines, which can either activate or inhibit immune cells within the TME [23,24].

As the disease progresses, sEVs from PDAC cells re-program immune cell infiltrates by stimulating pro-tumorigenic and anti-inflammatory phenotypes whilst suppressing cytotoxic immune activity [25]. Specifically, tumour-derived sEVs can stimulate MDSC accumulation and inhibit dendritic cell (DC) maturation, induce pro-tumorigenic macrophage polarisation and neutralise natural killer (NK) cytotoxicity [23]. *In vitro* studies have also shown that PDAC-derived sEVs promote the expansion, suppress activation and decrease apoptosis of tumour-associated regulatory T cells (Tregs) [26], with further evidence indicating a role for sEVs in T-cell exhaustion and B-cell inactivity [27,28] (Fig. 1).

Whilst the tumour-promoting effects of sEVs have been well-documented in several cancers, including ovarian, breast, melanoma, and lung [29–32], the underlying molecular mechanisms by which sEVs facilitate anti-tumour immunity in PDAC remain elusive. The following subsections will discuss current knowledge of the role of sEVs in the modulation of the PDAC immune landscape, focusing on several critical immune cell populations.

3.1. Macrophages

Monocyte-derived macrophages infiltrate the TME early in tumour formation in response to chemokines (e.g., chemokine [C-C motif] ligand 2, CCL2), cytokines (e.g., interleukin 4, IL-4), growth factors (e.g., vascular endothelial growth factor, VEGF), and changes in tissue homeostasis [33]. In addition, tissue-resident macrophages also contribute to the TAM population, and these may have functions independent of bone-marrow derived macrophages [33]. TAMs are intricately involved in tumorigenesis due to their intrinsic plasticity and ability to polarise between pro- and anti-tumorigenic phenotypes [33–35]. Whilst anti-tumorigenic macrophages suppress tumour cell growth, pro-tumorigenic TAMs facilitate stromal regeneration, inhibit cytotoxic behaviours and support tumour cell survival [36,37]. Pro-tumorigenic TAMs are the most abundant immune cell within the TME and drive immunosuppression through the sustained release of anti-inflammatory factors such as IL-6, IL-8, IL-10, transforming growth factor-beta (TGF-β) and prostaglandin E2 (PGE_2) [35,37,38]. Moreover, high numbers of pro-tumorigenic TAMs in the TME are accompanied by increased resistance to chemotherapy and poor prognosis.

TAMs can adopt a lipid-dependent metabolic state, an advantage in

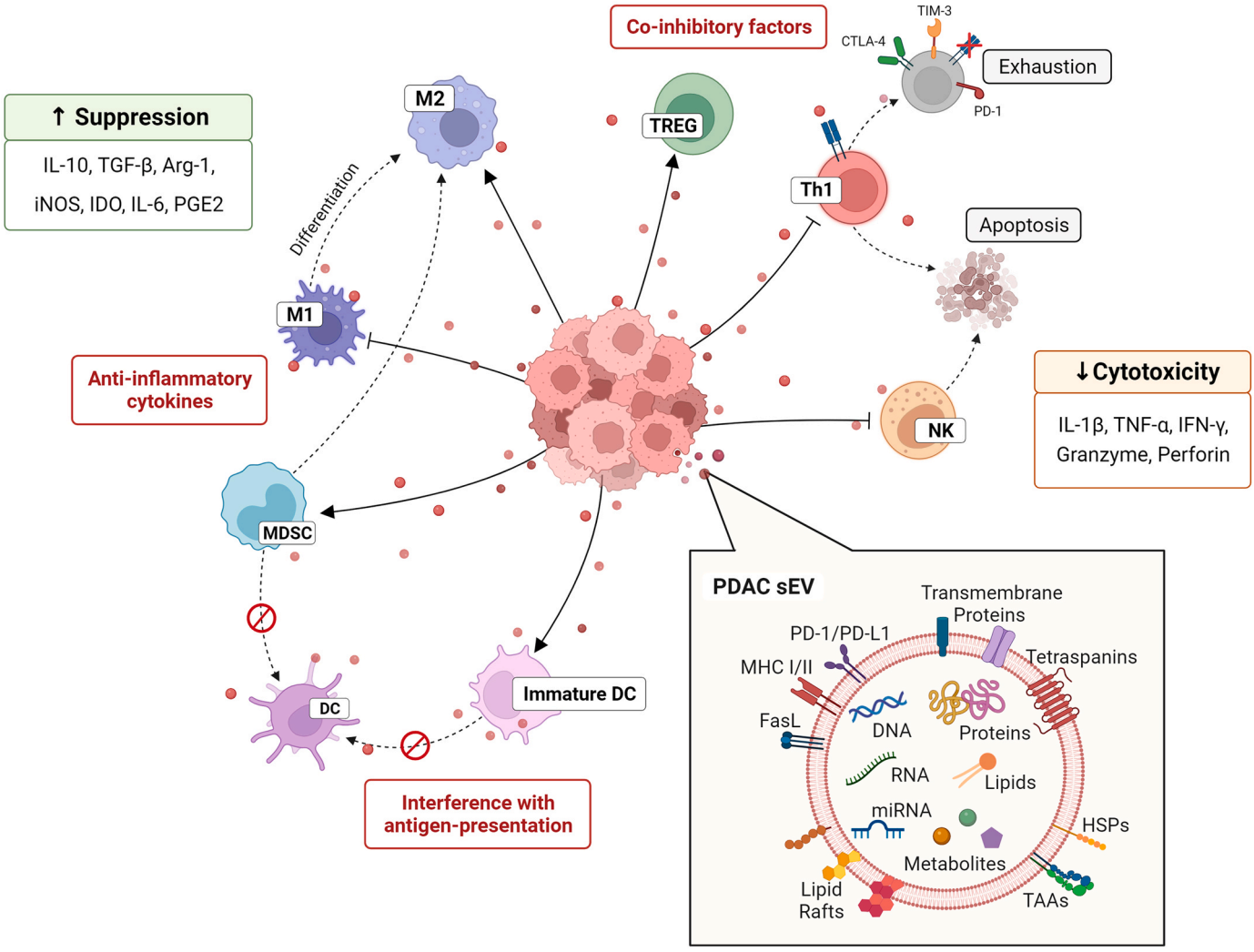

Fig. 1. Immune microenvironment of pancreatic ductal adenocarcinoma (PDAC). Pancreatic cancer cells and immune cells both release small extracellular vesicles (sEVs), which facilitate cross talk and contribute to an immunosuppressive, pro-tumorigenic microenvironment. sEVs released by cancer cells promote T cell exhaustion, apoptosis of natural killer (NK) cells, a pro-tumorigenic macrophage phenotype, and inhibition of dendritic cells (DC) function. Arg-1, arginase-1; CTLA-4, cytotoxic lymphocyte associated antigen-4; FasL, Fas ligand; HSPs, heat shock proteins; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; iNOS, inducible nitric oxide synthase; IFN-γ, interferon-gamma; M1, M1 macrophage; M2, M2 macrophage; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility class I/II; PD-1, Programmed cell death protein 1; PD-L1, programmed death-ligand 1; PGE2, prostaglandin E2; TAAs, tumour-associated antigens; TGFβ, transforming growth factor-beta; Th1, T helper 1 cells; TIM-3, T cell immunoglobulin and mucin domain 3; TREG, regulatory T cell; TNF-α, tumour necrosis factor-alpha.

the nutrient-deficient environment within the TME [37,39]. Following uptake by TAMs, PDAC-derived sEVs undergo degradation, leading to the deposition of lipids such as cholesterol, gangliosides, sphingolipids and free fatty acids (FFAs) within the endosomal compartment. This disrupts lipid homeostasis, leading to alterations to macrophage metabolism, activation and function [38,40]. Further, tumour-derived sEVs are enriched with several key lipid species, which coordinate the balance between pro- and anti-inflammatory phenotypes [20]. PDAC-derived sEVs also contain functional enzymes and lipid precursors [41] such as arachidonic acid (AA), a prerequisite for the biosynthesis of prostaglandins, thromboxanes, and leukotrienes. AA constitutes approximately 30% of the polyunsaturated FFA content of sEVs and is critical in determining the efficacy of sEV uptake by immature macrophages [41].

PDAC-derived sEVs may further increase the synthesis of inflammatory prostaglandins in macrophages via the transfer of cyclooxygenase-1/2 (COX1/2). The AA/Cyclooxygenase pathway upregulates PGE_2 production and promotes the secretion of IL-10, TGF-β, indoleamine 2,3-dioxygenase (IDO) and inducible nitric oxide synthase. This stimulates MDSC accumulation whilst inhibiting DC and pro-tumorigenic macrophage differentiation [34,42,43]. A previous study from our research group revealed that prostaglandin E2 synthase (*PTGES3*) – which converts prostaglandin endoperoxide H2 (PGH_2) to PGE_2 – is distinctly enriched in PDAC-derived sEVs. However, the functional implications in macrophages are yet to be explored [44]. Nevertheless, intracellular PGE_2 positively regulates the expression of COX2 and prostaglandin E2 receptor 4 in macrophages and stimulates Treg recruitment and polarisation of pro-tumorigenic macrophages, amplifying the immunosuppressive environment of the TME. Notably, PGE_2 is enriched in the blood and urine of PDAC patients and is negatively correlated with patient survival [43,45].

In addition, various microRNAs (miRNA) have been implicated in sEV-induced macrophage function within the TME. For instance, miR-301a-3p in sEVs can promote the polarisation and infiltration of pro-tumorigenic TAMs into the TME via modulation of the phosphoinositide-3-kinase (PI3K)/phosphatase and tensin homolog (PTEN) pathway [22]. By inhibiting PTEN phosphorylation, miR-301a-3p enhances phosphorylation of PI3K, protein kinase B (PKB) and the mammalian target of rapamycin (mTOR) in macrophages, stimulating metabolic remodelling, cell survival and anti-inflammatory

cytokine production [22]. Additionally, miR-301a-3p upregulates PD-L1 expression on TAMs, thereby promoting T-cell exhaustion and Treg recruitment and inhibiting cytotoxicity within the TME [46]. Notably, miR-301a-3p levels in circulating sEVs directly correlate with late tumour-node-metastasis (TNM) stage, depth of tumour invasion, and lymph node metastasis and are negatively associated with overall survival in PDAC patients (Fig. 2) [22,47].

Some sEVs are also reported to carry critical immunosuppressive cytokines. For example, TGF-β-bearing tumour sEVs further enhance the inhibitory activity of macrophages via activation of the canonical TGF-β pathway to promote the secretion of IL-10 production. Lima et al. [38] demonstrated that sEVs could bind to cytokines present in the TME via interaction with exposed glycosaminoglycan side chains. These conjugates then accumulate in an organ-specific manner to promote disease progression as well as pre-metastatic niche formation.

Beyond the TME, PDAC-associated sEVs enhance metastasis by priming the pre-metastatic niche (PMN) at distant sites. The best-described example is within the liver, where the uptake of macrophage migration inhibitory factor (MIF)-containing sEVs by resident Kupffer cells stimulates the secretion of TGF-β [48]. The local increase in TGF-β induces fibronectin production by hepatic stellate cells (HSCs), thereby promoting fibrosis and PMN formation [48]. Our previous proteomic data have revealed that MIF is exclusively included in the cargo of malignant pancreatic cell-derived sEVs, whilst non-malignant

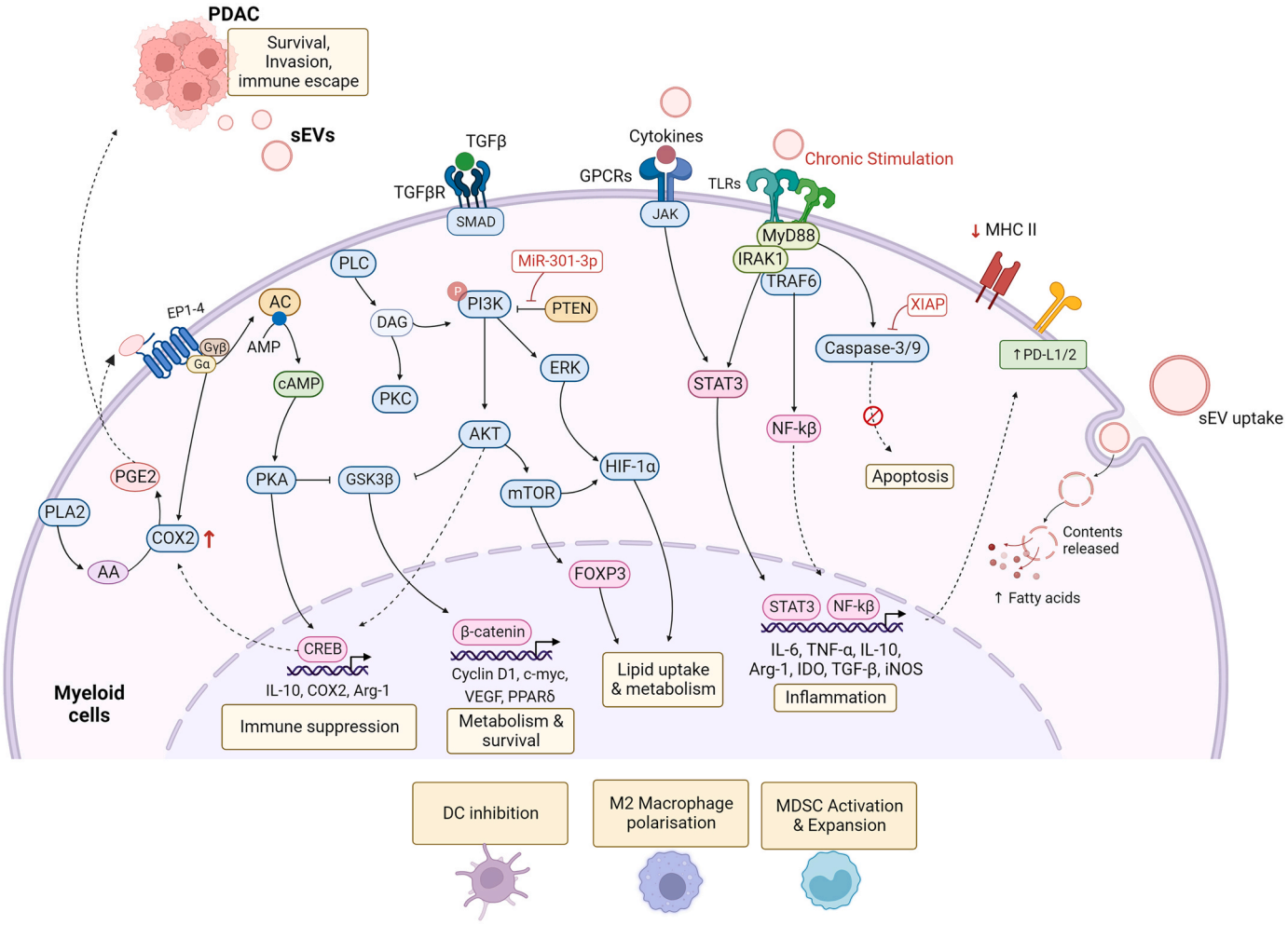

Fig. 2. PDAC-derived sEVs promote the suppressive activation of myeloid cell populations. Small extracellular vesicles (sEVs) released from cancer cells induce metabolic changes in myeloid cell populations by enhancing lipid uptake and metabolism pathways via the phosphatase and tensin homologue phosphoinositide-3-kinase (PTEN/PI3K) signal transduction pathways. Chronic stimulation of toll-like receptors (TLRs) by antigens on sEVs shifts immune responses towards signal transducer and activator of transcription-3 (STAT3) signal transduction to upregulate anti-inflammatory cytokine production and suppressive activation. Myeloid differentiation primary response 88 (MyD88) is critical for the suppressive function of myeloid cells by inhibiting caspase-dependant apoptosis to induce the expansion of immature myeloid cells. Lipid accumulation in macrophages promotes pro-tumorigenic signalling and increases activation of phospholipase A2 (PLA2), which cleaves arachidonic acid (AA) from the cell membrane to upregulate prostaglandin E2 (PGE2) secretion. PGE2 both increases tumour cell survival and proliferation and positively regulates cyclooxygenase 2 (COX2) expression, thereby amplifying the suppressive capacity of macrophages. AA, arachidonic acid; AC, adenylyl cyclase; AMP, Adenosine monophosphate; Akt, protein kinase B/Akt; Arg-1, arginase-1; cAMP, cyclic AMP; CDK, cyclin-dependent kinase; COX2, cyclooxygenase-2; CREB, cAMP response element-binding protein; DAG, diacylglycerol; DC, dendritic cell; EP1–4, prostaglandin receptors EP1–4; ERK, extracellular signal-regulated pathway; FOXP3, forkhead box protein 3; GPCR, G-protein coupled receptor; GSK3B, glycogen synthase kinase 3 Beta; HIF-1α, hypoxia-inducible factor-1 alpha; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; iNOS, inducible nitric oxide synthase; IRAK1, interleukin-1 receptor-associated kinase 1; JAK, Janus Kinase; MDSC, myeloid-derived suppressor cells; MHC, major histocompatibility class I/II; MEK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NF-kβ, nuclear factor; PGE2, prostaglandin E2; PD-1/L1, programmed cell death protein-1/ligand-1/2; PDAC, pancreatic ductal adenocarcinoma; PI3K, phosphoinositide-3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PKA, protein kinase A; PLA2, phospholipase-A2; PLC, phospholipase C; PPARδ, Peroxisome proliferator-activated receptor delta; PTEN, phosphatase and tensin homologue; SMAD, small mothers against decapentaplegic homolog; STAT, signal transducer and activator of transcription; TCR, T-cell receptor; TGF-β, Transforming growth factor-beta; TGFβR, transforming growth factor receptor; TLR, toll-like receptor; TNF-α, tumour necrosis factor-alpha; TRAF6, TNF receptor associated factor 6; VEGF, vascular endothelial growth factor; XIAP, X-linked inhibitor of apoptosis protein.

pancreatic-derived sEVs lack MIF expression [44].

sEV-mediated communication in the TME is bi-directional, and it is well accepted that TAM-derived sEVs influence the TME and cancer cells (Fig. 2). For instance, TAM-derived sEVs modify TGF-β-mediated signalling via the transfer of miR-501–3p, which blocks the transcription of TGF-β receptor 3 (TGFBR3) - a putative tumour suppressor and negative regulator of TGF-β transduction [49]. Notably, TGFBR3 inhibition in PDAC is associated with increased TGF-β-mediated tumour growth, migration and metastatic potential [50]. Concomitantly, miR-365 in TAM sEVs selectively inhibits the B-cell translocation gene 2 (*BTG2*) in PDAC cells, leading to activation of focal adhesion kinase (FAK) and PKB, mTOR complex-1 mediated transcription, and enhanced proliferation, migration, and invasion of PDAC cells [51]. Additionally, the transfer of miR-365 promotes gemcitabine resistance in PDAC by modulating pyrimidine metabolism and stimulating cytidine deaminase activity, which inhibits gemcitabine activity [42].

In addition to miRNA, Yin et al. [52] recently reported that TAM-derived sEVs contain high levels of the long non-coding RNA (lncRNA), SET-binding factor 2 antisense (SBF2-AS1). When transferred to PDAC cells, this upregulates the expression of X-linked inhibitor of apoptosis protein to increase cell survival and enhance their invasive capacity. Notably, the overexpression of SBF2-AS1 in PDAC tissues has been shown to positively correlate with TNM stage and degree of drug resistance. However, the prognostic potential of this lncRNA in EVs is yet to be explored [53].

3.2. Immature myeloid cells (MDSCs and DCs)

MDSCs are a heterogeneous population of immature myeloid cells which are critical to the regulation of innate and adaptive immune responses [54]. Under homeostatic conditions, the entry of immature myeloid cells into peripheral organs stimulates their differentiation into functional immune cells [54]. In cancer, these cells fail to respond to maturation cues and instead expand and proliferate as MDSCs. MDSCs can be classified into two subtypes; polymorphonuclear MDSCs (PMN-MDSC) and monocytic MDSCs (M-MDSC) [55]. PMN-MDSCs suppress T-cell responses by producing reactive oxygen species (ROS) and interfering with antigen-mediated T-cell activation [54]. Alternatively, M-MDSCs – which constitute the majority of MDSCs – secrete potent immunosuppressive cytokines and factors, including IL-10, Arginase-1 (Arg-1) and nitric oxide (Fig. 2). The increased secretion of Arg-1 within the TME depletes the local arginase pool and inhibits the correct assembly of the T-cell receptor (TCR) complex in T-cells, resulting in their decreased proliferation and increased tolerance [56]. Notably, in PDAC patients, the frequency of MDSCs in the periphery has been associated with advanced metastatic disease and poor clinical outcomes [57].

The differentiation, survival, and activation of MDSCs are primarily modulated by pattern-recognition receptors (PRRs), particularly toll-like receptors (TLRs), present on the surface of immune cells. In response to different pathogen-associated molecular patterns (PAMPs) or damage/danger-associated molecular patterns (DAMPs), these transmembrane receptors can induce specific intracellular signalling pathways, stimulating cellular activation and immune responses [58].

Shen et al. showed that heat shock proteins (Hsp) expressed by sEVs, particularly Hsp105 and Hsp72, interact with TLR2/4 on immature DCs to upregulate the production of IL-6 via activation of the signal transducer and activator of transcription 3 (STAT3) signalling pathway [59]. Likewise, Hsp72 expression on tumour sEVs can enhance the expansion and accumulation of MDSCs, which is accompanied by an upregulated secretion of IL-1, tumour necrosis factor (TNF), VEGF and CCL2 [60]. Basso et al. also showed that PDAC-derived sEVs could disrupt DC maturation and promote MDSC activation via the transfer of miRNAs, including miR-494–3p and miR-1260a. Interestingly, this study showed that these miRNAs are upregulated in sEVs derived from PDAC cells with aberrant SMAD family member 4 (*SMAD4*) expression – a tumour suppressor gene commonly associated with PDAC – indicating a potential role of *SMAD4* expression with cargo sorting in assortment of tumour-derived sEVs [61].

Rashid et al. recently demonstrated that tumour-associated MDSCs could secrete sEVs expressing Fas ligand (FasL) and TNF-related apoptosis-inducing ligand, which trigger apoptotic pathways in recipient CD8 + T-cells [62]. MDSC-derived sEVs have also been shown to inhibit the production of IL-2 and induce a pro-tumorigenic phenotype in macrophages [63]. Additionally, these sEVs have been shown to contain several pro-inflammatory molecules, such as S100 calcium binding proteins A8/9 (S100A8/9), which facilitate infiltration and accumulation of suppressive immune cells into the TME [64]. In addition, MDSCs have receptors for S100A8/A9, and this initiates an autocrine loop leading to increased secretion of this protein either directly or via exosome release [65]. Recent proteomic data has also revealed the enrichment of members of the S100 calcium-binding family of proteins (e.g., S100A4) in PDAC-derived sEVs [44]. Thus, both tumour and immune cell derived sEVs contain these calcium binding proteins, and it is likely both contribute to an immunosuppressive local environment and the development of metastasis via priming of the PMN [64].

Mature dendritic cells (DCs) are essential to initiating immune responses, acting as primary antigen-presenting cells, which facilitate cytotoxic T-cell activation in an antigen-dependent manner. However, immature DCs lack the capacity for proper antigen presentation and instead promote T-cell immune tolerance and suppression by expressing PD-L1 and cytotoxic T-lymphocyte associated protein-4 (CTLA-4) [66]. Additionally, the increased secretion of IL-6 and PGE_2 by immature DCs has been shown to induce STAT3-dependant matrix metalloproteinase 9 transcriptional activity in tumour cells to promote invasive tumour phenotypes. Thus, immature DCs are a potent anti-inflammatory, pro--tumorigenic immune subtype.

PDAC cells and their sEV derivatives have been shown to participate in DC activation and function [67]. Zhou et al. demonstrated that miR-203 in PDAC-derived sEVs can inhibit immune-activating toll-like receptor 4 (TLR4) expression in DCs. MiR-203 has also been shown to modulate DC cytotoxicity by inhibiting the secretion of IL-12 and TNF, thereby decreasing their capacity to neutralise surrounding cancer cells [68]. Likewise, Ding et al. [69] demonstrated that miR-212–3p in PDAC-derived sEVs targets and suppresses regulatory factor X-associated protein in DCs, resulting in the downregulation of MHC II. The collective downregulation of TLR4 and MHC II impedes the activation and processing of antigen presenting DCs.

3.3. Natural killer cells

Natural killer (NK) cells – lymphoid-derived immune cells – are an additional innate cell involved in the first-line protection against pathogenic processes, such as viral infection and cancer [70]. These cells detect and directly abrogate infected cells through the release of cytotoxic factors, granzyme and perforin, as well as cytokines such as interferon-gamma (IFN-γ) and TNF [70,71]. In PDAC, the role of NK cells is poorly understood, although it is known they exhibit poor tumour localisation and infiltration and constitute less than 0.5% of the immune population [72]. In addition, the enrichment of immuno-inhibitory elements such as TGF-β, IDO, PGE_2 and IL-10 within the TME impairs the lytic activity of NK cells in PDAC and increases their exhaustion [73].

Zhao et al. showed that NK cells treated with metastatic pancreatic cancer or PDAC-derived sEVs demonstrated a significant reduction in the expression of natural killer group 2 member D and CD107, which are crucial markers of cellular activation and function, respectively. Furthermore, these sEV-treated cells had a decreased production of IFN-γ and TNF, along with impaired glucose uptake [73]. Evidently, PDAC-sEVs potentiate NK neutralisation and inhibition, which may contribute to this population's low tumoral and peripheral frequency. On the other hand, some NK-derived sEVs have been found to possess

anti-tumorigenic cargoes, including miR-607–3p, which can inhibit the malignant transformation of PDAC cells, likely via the direct targeting of IL-26 [74].

3.4. T- and B-lymphocytes

T-cells typically engage in intercellular communication via receptor-ligand interactions, leaving them susceptible to sEV-mediated changes. Notably, activated Tregs are the most responsive to sEV interaction, whilst inactivated or resting Tregs are the least responsive [28]. T-cell activity is regulated by the interaction between programmed cell death protein-1 (PD-1) and its ligand, PD-L1. Whilst a moderate level of PD-1/PD-L1 is required to suppress T-cell cytotoxicity and IFN-γ production, minimal levels can inhibit TNF, IL-2 and T-cell expansion. The overexpression of PD-L1 in PDAC-derived sEVs facilitates self-tolerance by inhibiting T-cell-mediated cytotoxicity and promoting Treg expansion. Moreover, tumour derived sEVs enhance the gene transcription of inhibitory proteins such as TGF-β, COX2, CTLA-4 and Fas/FasL in CD4 + T-cells, leading to reduced activation as shown by decreased CD69 expression (Fig. 3) [27,28].

Tumour sEVs have been shown to cause DNA damage, disrupt metabolism and promote the death of activated T-cells in the periphery by inhibiting tumour infiltration of cytotoxic subsets. Shen et al. demonstrated in vitro that PDAC-derived sEVs can induce p38-induced endoplasmic reticulum stress and apoptosis by amplifying the expression of pro-apoptotic transcription factors, Jun, and DNA damage-inducible transcript 3 protein in T lymphocytes. Additionally, it has been shown that serine-protein kinase ATM, adenosine monophosphate-activated protein kinase, and surtuins 1, 2 and 6 are sequentially upregulated upon interaction with tumour sEVs, leading to increased expression of the transcriptional regulators forkhead box proteins -O1A, -O3A and -P3, and subsequent Treg expansion [75].

Tumour-derived sEVs have also been shown to induce sustained Ca^{2+} influx to promote activated T-cell apoptosis and Treg behaviour [28]. In naïve T-cells, Ca^{2+} accumulation stimulates the conversion of adenosine triphosphate (ATP) to inosine to increase the production of adenosine,

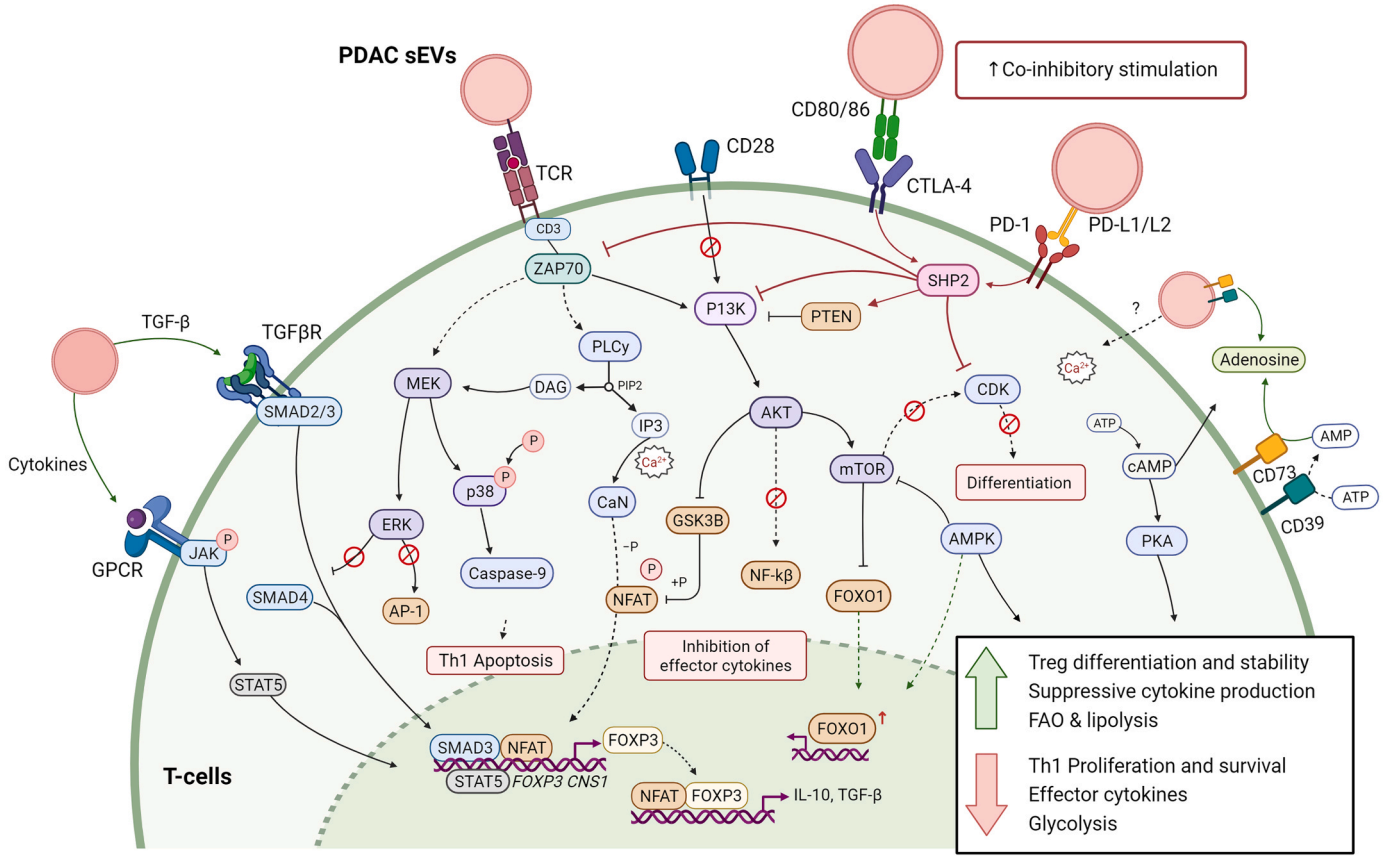

Fig. 3. Mechanisms of tumour-derived sEV-induced T-cell suppression. Tumour-derived sEVs are enriched in co-inhibitory ligands, including CD80/CD86 and programmed death ligand 1/2 (PD-L1/2), which bind to cytotoxic lymphocyte associated antigen-4 (CTLA-4) and programmed cell death protein-1 (PD-1) on the surface of T-lymphocytes. Stimulation of T-cell receptor (TCR) along with these co-inhibitory receptors operate through SH2 containing protein tyrosine phosphatase-2 (SHP2) to inhibit effector T-cell activation and cytokine production and promote regulatory T-cell (Treg) differentiation. Upregulation of phosphatase and tensin homologue (PTEN) suppresses phosphoinositide-3-kinase/protein kinase-B/mammalian target of rapamycin (PI3K/AKT/mTOR) signalling, resulting in the down-regulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kβ) pro-inflammatory cytokine production. Transforming growth factor-beta (TGF-β) upregulates mothers against decapentaplegic homolog-3 (SMAD3) which, when bound to nuclear factor activator of T-cells (NFAT) stabilises the expression of forkhead box P3 (FOXP3) to enhance Treg transcriptional programming. AKT, protein kinase B; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; AP-1, activator protein-1; ATP, adenosine triphosphate; cAMP, cyclic AMP; Ca^{+2}, Calcium ions; CaN, calcineurin; CDK, cyclin-dependent kinase; CTLA-4, cytotoxic T-lymphocyte associated antigen-4; DAG, diacylglycerol; ERK, extracellular signal-regulated pathway; FAO, fatty acid oxidation; FOXO1, forkhead box protein-1; FOXP3, forkhead box protein 3; GPCR, G-protein coupled receptor; GSK3B, glycogen synthase kinase 3 Beta; IP3, inositol 1,4,5-trisphosphate; JAK, Janus Kinase; MEK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NF-kβ, nuclear factor; NFAT, nuclear factor activator of T-cells; p38, p38 mitogen-activated protein kinases; PI3K, phosphoinositide-3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PKA, protein kinase A; PLCγ, phospholipase-C gamma; PTEN, phosphatase and tensin homologue; SHP2, SH2 containing protein tyrosine phosphatase-2; SMAD, small mothers against decapentaplegic homolog; STAT, signal transducer and activator of transcription; TCR, T-cell receptor; TGF-β, transforming growth factor-beta; TGFβR, transforming growth factor receptor; ZAP70, zeta-chain associated protein kinase 70.

which is an essential factor involved in the suppressive activity of Tregs [28]. Similarly, tumour-derived sEVs are reported to overexpress CD39 and CD73, which convert extracellular ATP to 5'-adenosine monophosphate, and then to adenosine, respectively [76]. Further, Smyth et al. [77] demonstrated that TCR activation of Tregs ($CD25^{+}FoxP3^{+}$) stimulated the secretion of CD73-bearing sEVs, thereby providing an additional mechanism to produce adenosine within the TME (Fig. 3).

Small EVs bearing TGF-β stimulate Treg expansion and inhibit NK-cell cytotoxicity in a mechanism similar to macrophages [78]. Further, our studies have shown that PDAC-derived sEVs are selectively enriched with immunomodulatory proteins, including high mobility group box 1 (HMGB1) and DNA-dependent protein kinase catalytic subunit (PRKDC), which facilitate cancer-associated inflammation, immune tolerance and DNA damage [44]. In several cancer models, HMGB1 has been shown to promote MDSC survival [79], and stimulate the expansion of PD-$L1^{+}$ TAMs and neutrophils within the TME, thereby mitigating T-cell activity and promoting immunosuppression [80,81]. Additionally, PRKDC has been associated with poor clinical outcomes in several cancers, including PDAC [82], and is under investigation as a therapeutic target for the treatment of multiple myeloma, non-Hodgkins lymphoma, glioblastoma and a range of advanced solid tumours [83].

Under homeostatic conditions, B-cells produce antibodies necessary for the neutralisation or opsonisation of pathogens and the activation of antibody-dependant T-cell cytotoxicity [84]. However, B-cells may also contribute to a tolerogenic and immunosuppressive TME. Despite the low immunogenicity of PDAC, PDAC-derived sEVs contain a large repertoire of TAAs, including pyruvate kinase PKM2, annexin A1, HspA8 and H2A histone family member Z, which are targets of circulating autoantibodies produced as a result of tumorigenesis [85]. These circulating autoantibodies in PDAC patient plasma were found to be more reactive to sEVs than tumour cells, suggesting that sEVs can act as a decoy that decreases complement-dependent cytotoxic activity towards tumour cells [85].

3.5. Neutrophils

Neutrophils are indispensable for defence against microbial infections and play key roles in innate and adaptive immunity [86]. Notably, high neutrophil infiltration within the TME is negatively associated with survival outcomes in PDAC (Piciucchi, 2017 #859). Like macrophages, neutrophils display significant plasticity and can polarise between both pro- and anti-tumorigenic phenotypes within the TME [87–89]. In PDAC, one study in mice showed pancreatic cancer-derived sEVs promoted the recruitment of bone marrow-derived cells (including neutrophils), which provided a favourable niche for liver metastasis [48]. Given this, neutrophil-derived EVs have been implicated as anti-inflammatory, pro-inflammatory and antibacterial mediators, which likely reflects the active state of the parent cell [90,91]. Neutrophil-derived EVs have been shown to contain factors that promote tumour progression, such as neutrophil elastase (NE) and lipocalin-2 [92], to stimulate oncogenic signalling and proliferation [1], and modulate pro-inflammatory cytokine production by PSCs within the pancreatic TME [93]. While studies have hypothesised the roles neutrophil derived-EVs in inflammation and tumour progression, further investigations are needed to understand the role of sEVs in the cancer-neutrophil communication within the PDAC landscape.

3.6. Platelets

Platelets are primarily known for their contribution to haemostasis and thrombosis and have a well-documented role in promoting PDAC progression and thrombotic complications [99,100]. Considered part of innate immune responses, evidence suggests platelets are involved in regulating innate immune cells during pathological processes such as infection, inflammation, and cancer via the secretion of various factors and surface receptor-ligand interactions [94,95]. In addition, DAMPs and PAMPs derived from host tissues and microbes can trigger TLRs on the platelet surface, promoting inflammation and thrombosis during infection and cancer [96–98]. Whilst the influence of platelet-derived microvesicles on cancer biology has been somewhat enlightened [99], the effects of cancer-derived sEVs on platelet function have so far commanded much less attention. Tissue factor (TF) - the main agonist of cancer-associated blood clotting - is abundant in platelet microparticles and is aberrantly expressed in cancer cells and cancer-derived sEVs [100]. The presence of TF in sEVs may be an indicator of thrombotic complications as it is not normally present in cells exposed to circulating blood. However, these TF-negative EVs have also been reported to elicit platelet activation [101]. Additionally, the enrichment of DAMPs, including histones, HMGB1 and S100A4 in PDAC sEVs are known to modulate platelet reactivity [44] [102]. Given the above, the effect of cancer-derived sEVs on platelets and the repercussions on the tumour-immune response requires further investigation.

4. Immune-stroma-tumour communication

The stroma within the TME encompasses a heterogeneous population of cells derived from adjacent tissues or myeloid tissue which, when exposed to chronic inflammation, undergoes phenotypic and functional transformation to support tumour growth [103]. One hallmark of the PDAC TME is the presence of large amounts of extracellular matrix proteins such as collagen, fibronectin and glycoproteins, which restricts access of drugs to tumour cells, and is linked to treatment resistance and poor patient outcome [22,104].

Angiogenic vascular endothelial cells, infiltrating immune cells (IICs), and cancer-associated fibroblastic cells (CAFs) represent the major stromal subpopulations within the TME and collectively enhance immune evasion to promote cancer cell proliferation, invasion, metastasis and angiogenesis (CAFs and IICs), in addition to impairing cellular respiration (CAFs) [105]. It is possible the interaction among immune and stromal cells within the TME can occur through sEV exchange, although few studies have focused on this in PDAC. In one study, it was revealed that CAF-derived sEVs can promote pro-tumorigenic TAM polarisation through the transfer of miRNA-320a [106]. In melanoma, CAFs were found to release sEVs that induce modification of the stroma by lowering the intratumoral pH, to favour tumorigenesis [107]. Additionally, various RNA cargoes in CAF-derived sEVs promote resistance to chemo and radiotherapy, not only in pancreatic cancer but also in lung, oesophageal, prostate, gynaecologic, head and neck, hepatocellular, bladder, gastric, breast and colorectal cancers [108]. Research has shown that fibrotic pancreatic stellate cells (PSCs) can facilitate MDSC differentiation and accumulation by releasing IL-6 and activating STAT3 signalling [109].

Interestingly, CAF-derived sEVs have also been implicated in metabolic re-programming of PDAC cells. sEVs from CAFs were able to support proliferation of PDAC cells cultured without glutamine, leucine, lysine, phenylalanine, and pyruvate, suggesting PDAC cells internalise metabolites provided by these sEVs [110]. Furthermore, it appeared that CAF-derived sEVs disabled normal oxidative respiration in PDAC cells and increased glycolysis [110].

Recently, sEV-mediated cross talk between cancer cells, endothelial cells and TAMs has been described [111]. miRNAs in cancer cell-derived sEVs were shown to stimulate a pro-tumorigenic TAM phenotype. These TAMs then released sEVs carrying miR-155–5p and miR-221–5p into TME, which were taken up by endothelial cells, leading to decreased E2F Transcription Factor 2 expression and enhanced angiogenesis of endothelial cells [111,112]. This enhanced peritumoral angiogenesis, thereby supporting tumour growth and metastatic dissemination [111]. In turn, EC-derived sEVs have been implicated in tumour progression in hepatocellular carcinoma and lung cancer, but in contrast, are linked with tumour suppression in breast cancer [113].

Although most data to date describe pro-tumorigenic relationships between cancer, stromal and immune cell-derived sEVs in PDAC, some

studies have described anti-tumoral interactions [114], illustrating the dual nature of tumour-stroma communications. For example, sEVs from activated CD8 + T cells were found to deplete stromal cells including mesenchymal stem cells (MSCs) and CAFs, mediated in part by miR-298–5p [78]. This loss of stromal cells was then linked with reduced tumour growth and invasion, although the mechanism driving this anti-tumour activity remains unclear [78].

5. Perineural invasion and immune crosstalk

The term perineural invasion (PNI) refers to the interaction between cancer cells and nerves and can involve cancer cells invading any of the three layers of the nerve, or cancer cells closely contacting the nerve and surrounding at least one third of the nerve periphery [115,116]. PNI can be found in all PDAC stages, with changes in innervation visible prior to the early tumour development in animal models [117]. Additionally, numerous studies have confirmed that PNI is a negative prognostic factor in the survival of PDAC patients, with the depth of infiltration shown to drastically affect metastasis formation and survival [118]. Further, most PDAC patients suffer from severe pain, and PNI has been highlighted as a critical factor affecting their quality of life[118].

Several molecular mediators expressed by both neural and PDAC cells are involved in stimulating nerve growth and modulating PNI, including nerve growth factor, neurotrophins 3 and 4, brain-derived neurotrophic factor, glial cell-derived neurotrophic factor, midkine growth factor, pleiotrophin, chemokines, neurotransmitters like catecholamines, as well as axon guidance gene family members such as semaphorin 3D [118]. Interactions between PSCs, CAFs, TAMs, MDSCs, T cells and Schwann cells have also been implicated in PNI regulation [118,119]. Notably, increasing evidence supports a key role of EVs in both cancer-nerve and immune cell-nerve crosstalk [119,120]. Macrophages, which are central to the peripheral nerve microenvironment, can boost cancer-nerve-crosstalk through the release of EVs [120]. Additionally, nerve fibres co-localising with lymphoid tissues, such as the thymus, are critical to the stimulation and maturation of peripheral immune cells and play a significant role in facilitating tissue protective behaviours and immunoregulation [121]. However, immune cell-nerve sEV crosstalk has yet to be fully explored but may prove significant in PDAC progression and treatment.

6. sEVs as PDAC biomarkers

Currently, serum carbohydrate antigen 19–9 (CA19–9) is the best serum biomarker available for the detection of PDAC. However, with a sensitivity of 72.4% and specificity of 84.2% [122,123], ~25% of PDAC patients will not be identified via CA19–9 screening, indicating additional biomarkers are required for diagnosis and disease staging of PDAC. Multimarker panels involving up to eight serum markers have recently been described that may better identify early PDAC patients that using a single biomarker alone [124].

However, in a recent systematic review, various methods of liquid biopsy were compared to determine their relative value as diagnostic markers for pancreatic cancer. This study revealed that compared to circulating tumour cells and circulating tumour DNA, sEVs showed the most sensitivity and specificity [125]. Table 1 shows a non-exhaustive list of potential sEV biomarker molecules.

Several studies have suggested that the enrichment of glypican-1 (GPC1) in PDAC-derived sEVs may serve as an indicator of disease progression and metastatic potential due to its involvement in cell division, morphogenesis, and differentiation [126,127]. As a biomarker for PDAC, GPC-1 is contentious due to its inconsistent presence in sEVs across studies, with several reporting either an insignificant difference between malignant and non-malignant sEVs [44] or an absence of this marker. Importantly, the credibility of the original study by Melo et al. [133] has been questioned by many and insinuations have been made about data manipulation and academic integrity. At the centre of this controversy is the polyclonal GPC-1 antibody used in the study, which has since been proven to be producing staining artefacts and the fact that the data reported in the 2015 paper had been manipulated. Additionally, in a re-release of the article, the authors stated they had adjusted the flow cytometry parameters for each sample to detect GPC-1 in patient-derived exosomes. While other studies have backed the original claims of GPC-1 as a putative biomarker for PDAC [127,128], unfortunately, the controversies surrounding the initial findings may have prematurely damaged its reliability.

Other exosomal surface proteins identified as potential biomarkers include a panel comprising claudin 4, epithelial cellular adhesion molecule, CD151, galectin-3 binding protein, histone H2B type 2-E and histone H2B type 2-F [129].

Table 1
Putative biomarkers contained within PDAC-associated sEVs.

Origins	Marker	Expression	Function	Source
Pancreatic cancer-derived sEVs				
Plasma sEVs	GPC1	↑	Stratifies PDAC from chronic pancreatitis	[128, 134]
PAN02 cells	MIF	↑	Targets Kupffer cells to promote liver metastasis	[47]
Panc1, BxPC-3 cells	miR-301a-3p	↑	Promotes M2 TAMs via PTEN/PI3K-γ activation and increases metastatic properties of PDAC. Positively correlates with tumour invasion and depth.	[21]
Panc1 cells	miR-203	↑	Inhibits TLR4 expression on DCs and promotes	[68]
PDAC tissue samples	miR-155, − 203, − 210, − 222	↑	Expression negatively correlates with patient survival	[67]
Patient serum, PDAC cell lines	c-MET, PD-L1	↑	High sensitivity and specificity for detecting PDAC	[123]
Panc1, BxPC-3, SW1990	miR-212–3p	↑	Induced suppression of RFXAP in dendritic cells to inhibit MHC II expression	[69]
Plasma sEVs	Tumour associated antigens	↑	High expression of TAAs deters tumour cell-targeted cytotoxic activity	[85]
Malignant pancreatic cell lines	S100 calcium binding protein family	↑	Promotes pre-metastatic niche formation	[44]
Immune cell-derived sEVs				
M2-TAM	miR-365	↑	Inhibits BTG2 expression to promote FAK/PKB activity. Promotes chemoresistance in PDAC cells	[42, 51]
M2-TAM	miR-501–3p	↑	Inhibits anti-tumorigenic TGF-β signalling in PDAC cells	[49]

Abbreviation: BTG2, B-cell translocation gene 2; c-MET, mesenchymal-epithelial transition factor; DCs, dendritic cells; FAK, focal adhesion kinase; GPC1, glypican-1; MHC, major histocompatibility complex; MIF, migration inhibitory factor; PDAC, pancreatic ductal adenocarcinoma; PD-L1, programmed death-ligand 1; PI3K, Phosphoinositide-3-kinase; PKB, protein kinase B/Akt; PTEN, phosphatase and tensin homolog; RFXAP, regulatory factor-x associated protein; sEVs, small extracellular vesicles; TAAs, tumour-associated antigens; TAMs, tumour-associated macrophages; TGFβ, transforming growth factor-beta; TLR4; toll-like receptor 4.

RNA transcripts within sEVs also show potential as biomarkers. Specifically, miR-181a, − 106b, − 30c, − 21, − 20a and − 10b were all enriched in PDAC-derived (but not healthy pancreatic) sEVs, whilst let7a, miR-181a, − 106b, − 30c, − 21, − 20a and − 10b were all under-expressed [130]. Notably, the same study showed that the level of these miRNAs normalised within 24 h of surgical tumour resection. Whether this occurs in patients undergoing chemotherapy is yet to be determined, but it may provide a method to track treatment response, predict re-occurrence and screen for early disease (Table 1). In addition to miRNA, mRNA and small nucleolar RNAs cargo may also have potential as biomarkers for PDAC. A panel of two small nucleolar RNAs (H/ACA Box 74A and H/ACA Box 25) and two mRNA species (WASP Family Member 2 and ADP Ribosylation Factor 6) was recently described and may be superior to CA19–9 in distinguishing patients with early PDAC from people without the disease [131].

7. The potential of sEVs as a delivery system

sEVs are an attractive delivery system, as their cell-like lipid structure provides protection for the targeted delivery of biological or pharmacological modulators and can facilitate the trans-membrane transport of otherwise insoluble cargoes into cells. For example, Zhou et al. loaded sEVs with the chemotherapeutic oxaliplatin (OXA), and galactin-9 siRNA and explored the ability of these sEVs to affect pancreatic cancer cell growth in vitro and in vivo. Oxaliplatin caused cell death by inhibiting DNA synthesis and repair but also triggered immunogenic cell death which stimulated DC maturation and subsequent infiltration of anti-tumour cytotoxic T lymphocytes. In addition, downregulation of tumour cell galectin-3 modulated tumour-macrophage interactions, to reduce the immunosuppressive effect of TAMs in the TME [132]. This study also showed that sEVs specifically targeted the tumour to promote local drug accumulation and limit systemic distribution, thereby reducing the associated toxicity [132].

MSC-derived sEVs modified to contain a miRNA-1231 mimic have also been used to target pancreatic cancer cells [133]. These experiments revealed pancreatic cancer cells took up the MSC-derived sEVs containing the miRNA-1231 mimic, and this affected expression of known target mRNA (e.g., epidermal growth factor receptor, cyclin E), inhibited cell proliferation, migration, and invasion of PDAC cells in vitro. These modified exosomes also inhibited pancreatic cancer tumour growth in a mouse model [133]. MSC-derived sEVs have also been loaded with gemcitabine and paclitaxel and tested on PDAC cells in tissue culture and in tumour spheroids. The exosomes were taken up by PDAC cells, penetrated tumour spheroids and inhibited cell proliferation [134]. *In vivo* studies also showed reduced tumour growth and increased survival when mice were treated with sEVs containing gemcitabine and paclitaxel, compared to standard chemotherapy, suggesting the sEVs facilitate delivery of the chemotherapy drugs deeper into the tumour [134].

Currently, there is also a phase I clinical trial involving MSC-derived sEVs in PDAC, testing the dose and side effects of MSCs loaded with a siRNA targeting a common mutation in the Kirsten rat sarcoma viral oncogene homologue (KRAS; G12D). This trial is based on successful pre-clinical data showing that the modified sEVs suppressed cancer and augmented overall survival in various mouse models of PDAC [135].

Presently, most therapeutic strategies aimed at inhibiting tumour-induced immunosuppression target immune checkpoints, including CTLA-4, PD-1 or PD-L1. The use of immune checkpoint inhibitors (ICI), sometimes in combination with chemotherapy, has revolutionised the treatment of a range of cancers including melanoma, renal cell carcinoma and lung cancer [136]. However, checkpoint inhibitors have not been successful in PDAC therapy, as the low immunogenicity of PDAC tumours impacts the capacity for cytotoxic activation and infiltration [137,138].

Additionally, an important consideration when targeting the immune landscape in PDAC is the presence of compensatory immune responses. For example, PDAC mouse models have shown that depleting Tregs within the TME induces myeloid cell infiltration, promoting pro-tumour macrophage polarisation (and vice versa) [139]. One approach explored to restrict this compensation is to target all myeloid cells via integrin CD11b. Early trials using a CD11b antagonist were unsuccessful, as they were not well tolerated by patients due to toxicity [139]. However, CD11b agonists have shown some promise in that they reduce myeloid infiltration, increase T-cell infiltration, and sensitize tumours to chemotherapy and immunotherapy.

Additionally, Hussain et al. revealed that PDAC cells upregulate the expression of PD-L1 when treated with gemcitabine or galunisertib, either alone or in combination. Given that gemcitabine is a first-line chemotherapy protocol, this information advocates for the use of a combination of immune- and chemotherapies to target both the stromal and immune components in the treatment of PDAC [50].

The emergence of engineered T-cell treatments, such as chimeric antigen receptor (CAR)-T cell therapy, has provided significant advances in the treatment of some B cell leukaemia and lymphomas [140]. However, this strategy remains ineffective in PDAC given that it relies on the capacity of cytotoxic T-cells to infiltrate the TME. Furthermore, it has been shown that CAR-T cells can be inactivated within the TME via the PD-1/PD-L1 pathway, resulting in reduced cytotoxicity and proliferation [141]. However, it has been demonstrated that exosomes derived from CAR-T cells express CAR on their surface, but do not express PD-1 and are thus unresponsive to PD-L1 [141]. These CAR-exosomes also exhibited potent anti-tumour properties whilst lacking the toxicity associated with CAR-T treatment. These data suggest that (CAR)-T-derived sEVs could be used as cancer-targeting agents and may improve therapeutic efficacy [141].

Lastly, exosomes are also being investigated for their potential to deliver cancer vaccines. DC-derived exosomes are currently in Phase 2 clinical study as vehicles for the delivery of tumour-antigen vaccinations in non-small cell lung cancer (NCT01159288).

8. Conclusion and perspectives

Given its high fatality and intractability, an extensive body of research has focussed on understanding the oncogenic behaviours driving PDAC and increasing its response to treatment. Unfortunately, despite significant advances in the field, PDAC remains ill-defined, lacking distinct, non-invasive diagnostic or prognostic markers and minimal improvements in therapeutic intervention. sEVs released by tumour-associated cells – such as stromal and immune cells – are unique in their molecular profile as dictated by the TME. This information provides unprecedented access into the tumour core, proving to be critical to understanding the oncogenic behaviours of cells within the microenvironment. Therefore, molecular profiles of sEVs could serve as markers of disease progression, possible complications and prognosis [142]. Given the nature of oncogenesis, PDAC-specific biomarkers are yet to be established, with those currently identified either failing to demonstrate consistency or being also observed in cancers of different origins. Thus, establishing a multi-omics approach of sEVs for the identification and stratification of PDAC tumours may provide increased sensitivity in detection and significant predictive value in treatment outcomes.

Our knowledge of the EV-mediated crosstalk among the primary tumour and immune cells poses many opportunities for better diagnosis or treatment of PDAC. For instance, the inhibition of cancer-derived EVs, either by affecting their release, or uptake, or by blocking specific mediators carried by EVs, could avert the effect of cancer-derived EVs on immune cells or potentially potentiate the effect of immunotherapy. This implies the imperative to gather more information on specific targets of the cargo carried by EVs as well as to improve our knowledge of the mechanisms of EVs' release and uptake by recipient cells. As regards the potential application of EVs as biomarkers, there are still many issues that need to be addressed in order to gather reproducible

information based on blood collection. The presence of contaminants, the scarce concentration in the collected samples and the lack of specificity due to the presence of different EV subpopulations are still representing a barrier to the mass clinical use of EVs as biomarkers. Nonetheless, EVs are presently one of the few novel viable options to explore for developing efficacious tools for the diagnosis and treatment of challenging cancers such as PDAC.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Data availability

No data was used for the research described in the article.

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References

[1] I. Lerman, et al., Infiltrating myeloid cells exert protumorigenic actions via neutrophil elastase, Mol. Cancer Res 15 (9) (2017) 1138–1152.
[2] M. Orth, et al., Pancreatic ductal adenocarcinoma: Biological hallmarks, current status, and future perspectives of combined modality treatment approaches, Radiat. Oncol. 14 (1) (2019).
[3] N. Sturm, T.J. Ettrich, L. Perkhofer, The impact of biomarkers in pancreatic ductal adenocarcinoma on diagnosis, surveillance and therapy, Cancers (Basel) 14 (1) (2022).
[4] N. Martinez-Bosch, J. Vinaixa, P. Navarro, Immune evasion in pancreatic cancer: From mechanisms to therapy, Cancers 10 (1) (2018) 6.
[5] A. Adamska, A. Domenichini, M. Falasca, Pancreatic ductal adenocarcinoma: Current and evolving therapies, Int. J. Mol. Sci. 18 (7) (2017) 1338.
[6] D.R. Principe, et al., The current treatment paradigm for pancreatic ductal adenocarcinoma and barriers to therapeutic efficacy, Front Oncol. 11 (2021), 688377.
[7] F.E.F. Timmer, et al., Pancreatic cancer and immunotherapy: A clinical overview, Cancers (Basel) 13 (16) (2021).
[8] J. Chen, G. Xiao-Zhong, X.S. Qi, Clinical outcomes of specific immunotherapy in advanced pancreatic cancer: A systematic review and meta-analysis, J. Immunol. Res 2017 (2017) 8282391.
[9] M. Huber, et al., The immune microenvironment in pancreatic cancer, Int J. Mol. Sci. 21 (19) (2020).
[10] A.S. Bear, R.H. Vonderheide, M.H. O'Hara, Challenges and opportunities for pancreatic cancer immunotherapy, Cancer Cell 38 (6) (2020) 788–802.
[11] I.A. Batista, S.A. Melo, Exosomes and the future of immunotherapy in pancreatic cancer, Int J. Mol. Sci. 20 (3) (2019).
[12] M. Colombo, G. Raposo, C. Théry, Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles, Annu. Rev. Cell Dev. Biol. 30 (1) (2014) 255–289.
[13] R. Kalluri, V.S. Lebleu, The biology, function, and biomedical applications of exosomes, Science 367 (6478) (2020) p. eaau6977.
[14] A. Lo Cicero, P.D. Stahl, G. Raposo, Extracellular vesicles shuffling intercellular messages: For good or for bad, Curr. Opin. Cell Biol. 35 (2015) 69–77.
[15] Y. Zeng, et al., Biological features of extracellular vesicles and challenges, Front. Cell Dev. Biol. 10 (2022).
[16] R.A. Haraszti, et al., High-resolution proteomic and lipidomic analysis of exosomes and microvesicles from different cell sources, J. Extracell. Vesicles 5 (1) (2016), 32570.
[17] D.K. Jeppesen, et al., Reassessment of exosome composition, Cell 177 (2) (2019) 428–445, e18.
[18] R.M. Johnstone, et al., Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes), J. Biol. Chem. 262 (19) (1987) 9412–9420.
[19] E.R. Abels, X.O. Breakefield, Introduction to extracellular vesicles: biogenesis, RNA cargo selection, content, release, and uptake, Cell. Mol. Neurobiol. 36 (3) (2016) 301–312.
[20] C. Théry, et al., Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines, J. Extracell. Vesicles 7 (1) (2018) 1535750.
[21] L. De Lellis, et al., Exosomes as pleiotropic players in pancreatic cancer, Biomedicines 9 (3) (2021) 275.
[22] X. Han, et al., Reversal of pancreatic desmoplasia by re-educating stellate cells with a tumour microenvironment-activated nanosystem, Nat. Commun. 9 (1) (2018), 3390.
[23] W. Chen, et al., Tumor-related exosomes contribute to tumor-promoting microenvironment: an immunological perspective, J. Immunol. Res. 2017 (2017) 1–10.
[24] T. Whiteside, Tumor-derived exosomes and their role in tumor-induced immune suppression, Vaccines 4 (4) (2016) 35.
[25] R. Xu, et al., Extracellular vesicles in cancer — implications for future improvements in cancer care, Nat. Rev. Clin. Oncol. 15 (10) (2018) 617–638.
[26] M. Szajnik, et al., Tumor-derived microvesicles induce, expand and up-regulate biological activities of human regulatory T cells (Treg), PLoS ONE 5 (7) (2010), e11469.
[27] J. Liu, et al., Immune suppressed tumor microenvironment by exosomes derived from gastric cancer cells via modulating immune functions, Sci. Rep. 10 (1) (2020).
[28] L. Muller, et al., Tumor-derived exosomes regulate expression of immune function-related genes in human T cell subsets, Sci. Rep. 6 (1) (2016), 20254.
[29] A. Benito-Martin, M.G. Jasiulionis, S. Garcia-Silva, Extracellular vesicles and melanoma: New perspectives on tumor microenvironment and metastasis, Front Cell Dev. Biol. 10 (2022) 1061982.
[30] J. Crow, et al., Exosomes as mediators of platinum resistance in ovarian cancer, Oncotarget 8 (7) (2017) 11917–11936.
[31] B.T. Kreger, et al., The enrichment of survivin in exosomes from breast cancer cells treated with paclitaxel promotes cell survival and chemoresistance, Cancers (Basel) 8 (12) (2016).
[32] M. Tine, et al., Extracellular vesicles in lung cancer: Bystanders or main characters? Biol. (Basel) 12 (2) (2023).
[33] L.R. Sanchez, et al., The emerging roles of macrophages in cancer metastasis and response to chemotherapy, J. Leukoc. Biol. 106 (2) (2019) 259–274.
[34] Y. Liu, X. Cao, Immunosuppressive cells in tumor immune escape and metastasis, J. Mol. Med. 94 (5) (2016) 509–522.
[35] X. Liu, et al., The reciprocal regulation between host tissue and immune cells in pancreatic ductal adenocarcinoma: New insights and therapeutic implications, Mol. Cancer 18 (1) (2019).
[36] S. Yang, Q. Liu, Q. Liao, Tumor-associated macrophages in pancreatic ductal adenocarcinoma: Origin, polarization, function, and reprogramming, Front. Cell Dev. Biol. 8 (2021).
[37] A. Puthenveetil, S. Dubey, Metabolic reprograming of tumor-associated macrophages, Ann. Transl. Med. 8 (16) (2020), 1030-1030.
[38] J. Zhou, et al., Tumor-associated macrophages: Recent insights and therapies, Front. Oncol. 10 (2020).
[39] M. Wan, et al., Metabolic manipulation of the tumour immune microenvironment, Immunology 165 (3) (2022) 290–300.
[40] K. Sagini, et al., Extracellular vesicles as conveyors of membrane-derived bioactive lipids in immune system, Int. J. Mol. Sci. 19 (4) (2018) 1227.
[41] C. Subra, et al., Exosomes account for vesicle-mediated transcellular transport of activatable phospholipases and prostaglandins, J. Lipid Res. 51 (8) (2010) 2105–2120.
[42] Y. Binenbaum, et al., Transfer of miRNA in macrophage-derived exosomes induces drug resistance in pancreatic adenocarcinoma, Cancer Res. 78 (18) (2018) 5287–5299.
[43] F. Finetti, et al., Prostaglandin E2 and cancer: Insight into tumor progression and immunity, Biology 9 (12) (2020) 434.
[44] A. Emmanouilidi, et al., Oncogenic and non-malignant pancreatic exosome cargo reveal distinct expression of oncogenic and prognostic factors involved in tumor invasion and metastasis, PROTEOMICS 19 (8) (2019), 1800158.
[45] M. Nakanishi, D.W. Rosenberg, Multifaceted roles of PGE2 in inflammation and cancer, Semin. Immunopathol. 35 (2) (2013) 123–137.
[46] L.-W. Hsu, et al., MicroRNA-301a inhibition enhances the immunomodulatory functions of adipose-derived mesenchymal stem cells by induction of macrophage M2 polarization, Int. J. Immunopathol. Pharmacol. 34 (2020), 2058738420966092.
[47] X. Xia, et al., MicroRNA-301a-3p promotes pancreatic cancer progression via negative regulation ofSMAD4, Oncotarget 6 (25) (2015) 21046–21063.
[48] B. Costa-Silva, et al., Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver, Nat. Cell Biol. 17 (6) (2015) 816–826.
[49] Z. Yin, et al., Macrophage-derived exosomal microRNA-501-3p promotes progression of pancreatic ductal adenocarcinoma through the TGFBR3-mediated TGF-β signaling pathway. J. Exp. Clin. Cancer Res. 38 (1) (2019).
[50] S.M. Hussain, et al., Role of TGF-β in pancreatic ductal adenocarcinoma progression and PD-L1 expression, Cell. Oncol. 44 (3) (2021) 673–687.
[51] X. Li, et al., miR-365 secreted from M2 Macrophage-derived extracellular vesicles promotes pancreatic ductal adenocarcinoma progression through the BTG2/FAK/AKT axis, J. Cell. Mol. Med. 25 (10) (2021) 4671–4683.
[52] Z. Yin, et al., Down-regulated lncRNA SBF2–AS1 in M2 macrophage-derived exosomes elevates miR-122–5p to restrict XIAP, thereby limiting pancreatic cancer development, J. Cell. Mol. Med. 24 (9) (2020) 5028–5038.

[53] Y.-Q. Hua, et al., Long non-coding SBF2-AS1 acting as a competing endogenous RNA to sponge microRNA-142-3p to participate in gemcitabine resistance in pancreatic cancer via upregulating TWF1, Aging 11 (20) (2019) 8860–8878.
[54] Z. Chen, et al., Roles of the exosomes derived from myeloid-derived suppressor cells in tumor immunity and cancer progression, Front. Immunol. 13 (2022), 817942-817942.
[55] V. Bronte, et al., Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards, Nat. Commun. 7 (1) (2016), 12150.
[56] Q. Liu, Q. Liao, Y. Zhao, Chemotherapy and tumor microenvironment of pancreatic cancer, Cancer Cell Int. 17 (1) (2017), 68.
[57] Thyagarajan, A., et al., *Myeloid-Derived Suppressor Cells and Pancreatic Cancer: Implications in Novel Therapeutic Approaches. LID - 10.3390/cancers11111627 [doi] LID - 1627.* (2072–6694 (Print)).
[58] F. Veglia, E. Sanseviero, D.I. Gabrilovich, Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity, Nat. Rev. Immunol. 21 (8) (2021) 485–498.
[59] Y. Shen, et al., Tumor-derived exosomes educate dendritic cells to promote tumor metastasis via HSP72/HSP105-TLR2/TLR4 pathway, OncoImmunology 6 (12) (2017), e1362527.
[60] F. Chalmin, et al., Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells, J. Clin. Investig. 120 (2) (2010) 457–471.
[61] D. Basso, et al., PDAC-derived exosomes enrich the microenvironment in MDSCs in a SMAD4-dependent manner through a new calcium related axis, Oncotarget 8 (49) (2017) 84928–84944.
[62] M. Rashid, et al., Critical immunosuppressive effect of MDSC-derived exosomes in the tumor microenvironment, Oncol. Rep. 45 (3) (2021) 1171–1181.
[63] N. Othman, R. Jamal, N. Abu, Cancer-derived exosomes as effectors of key inflammation-related players, Front. Immunol. 10 (2019).
[64] M. Eisenblaetter, et al., Visualization of tumor-immune interaction - target-specific imaging of S100A8/A9 reveals pre-metastatic niche establishment, Theranostics 7 (9) (2017) 2392–2401.
[65] Sinha, P., et al., *Proinflammatory S100 proteins regulate the accumulation of myeloid-derived suppressor cells.* (1550–6606 (Electronic)).
[66] A. Labani-Motlagh, M. Ashja-Mahdavi, A. Loskog, The tumor microenvironment: A milieu hindering and obstructing antitumor immune responses, Front. Immunol. 11 (2020).
[67] T. Greither, et al., Elevated expression of microRNAs 155, 203, 210 and 222 in pancreatic tumors is associated with poorer survival, Int. J. Cancer 126 (1) (2010) 73–80.
[68] M. Zhou, et al., Pancreatic cancer derived exosomes regulate the expression of TLR4 in dendritic cells via miR-203, Cell. Immunol. 292 (1) (2014) 65–69.
[69] G. Ding, et al., Pancreatic cancer-derived exosomes transfer miRNAs to dendritic cells and inhibit RFXAP expression via miR-212-3p, Oncotarget 6 (30) (2015) 29877–29888.
[70] A.M. Abel, et al., Natural killer cells: Development, maturation, and clinical utilization, Front Immunol. 9 (2018) 1869.
[71] X. Peng, et al., Application of natural killer cells in pancreatic cancer (Review), Oncol. Lett. 22 (3) (2021), 647.
[72] S.A. Lim, et al., Defective localization with impaired tumor cytotoxicity contributes to the immune escape of NK cells in pancreatic cancer patients, Front. Immunol. 10 (2019).
[73] J. Zhao, et al., Tumor-derived extracellular vesicles inhibit natural killer cell function in pancreatic cancer, Cancers 11 (6) (2019) 874.
[74] H. Sun, et al., Natural killer cell-derived exosomal miR-3607-3p inhibits pancreatic cancer progression by targeting IL-26, Front. Immunol. 10 (2019).
[75] T. Shen, et al., BxPC-3-derived small extracellular vesicles induce FOXP3+ Treg through ATM-AMPK-sirtuins-mediated FOXOs nuclear translocations, iScience 23 (8) (2020), 101431.
[76] A. Clayton, et al., Cancer exosomes express CD39 and CD73, which suppress T cells through adenosine production, J. Immunol. 187 (2) (2011) 676–683.
[77] L.A. Smyth, et al., CD73 expression on extracellular vesicles derived from CD4+ CD25+Foxp3+T cells contributes to their regulatory function. Eur. J. Immunol. 43 (9) (2013) 2430–2440.
[78] N. Seo, et al., Activated CD8+ T cell extracellular vesicles prevent tumour progression by targeting of lesional mesenchymal cells, Nat. Commun. 9 (1) (2018).
[79] S. Jin, et al., Roles of HMGB1 in regulating myeloid-derived suppressor cells in the tumor microenvironment, Biomark. Res. 8 (1) (2020), 21.
[80] B. Li, et al., Tumor-derived exosomal HMGB1 promotes esophageal squamous cell carcinoma progression through inducing PD1+ TAM expansion, Oncogenesis 8 (3) (2019), 17.
[81] Y. Shi, et al., Extracellular vesicles from gastric cancer cells induce PD-L1 expression on neutrophils to suppress T-cell immunity, Front. Oncol. 10 (2020).
[82] T. Nishimura, et al., Disease-related protein co-expression networks are associated with the prognosis of resectable node-positive pancreatic ductal adenocarcinoma, Sci. Rep. 12 (1) (2022).
[83] I.S. Mohiuddin, M.H. Kang, DNA-PK as an emerging therapeutic target in cancer, Front. Oncol. 9 (2019).
[84] J. Shang, H. Zha, Y. Sun, Phenotypes, functions, and clinical relevance of regulatory B cells in cancer, Front. Immunol. 11 (2020).
[85] M. Capello, et al., Exosomes harbor B cell targets in pancreatic adenocarcinoma and exert decoy function against complement-mediated cytotoxicity, Nat. Commun. 10 (1) (2019).
[86] P.X. Liew, P. Kubes, The neutrophil's role during health and disease, Physiol. Rev. 99 (2) (2019) 1223–1248.
[87] M.A. Giese, L.E. Hind, A. Huttenlocher, Neutrophil plasticity in the tumor microenvironment, Blood 133 (20) (2019) 2159–2167.
[88] S.B. Coffelt, M.D. Wellenstein, K.E. de Visser, Neutrophils in cancer: Neutral no more, Nat. Rev. Cancer 16 (7) (2016) 431–446.
[89] Z.G. Fridlender, et al., Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN, Cancer Cell 16 (3) (2009) 183–194.
[90] F. Kolonics, et al., The functional heterogeneity of neutrophil-derived extracellular vesicles reflects the status of the parent cell, Cells 9 (12) (2020).
[91] J. Dalli, et al., Heterogeneity in neutrophil microparticles reveals distinct proteome and functional properties, Mol. Cell Proteom. 12 (8) (2013) 2205–2219.
[92] S. Bekeschus, et al., A neutrophil proteomic signature in surgical trauma wounds, Int J. Mol. Sci. 19 (3) (2018).
[93] S.B. Gomez-Chou, et al., Lipocalin-2 promotes pancreatic ductal adenocarcinoma by regulating inflammation in the tumor microenvironment, Cancer Res 77 (10) (2017) 2647–2660.
[94] J. Rayes, et al., The dual role of platelet-innate immune cell interactions in thrombo-inflammation, Res. Pract. Thromb. Haemost. 4 (1) (2020) 23–35.
[95] A. Zarbock, R.K. Polanowska-Grabowska, K. Ley, Platelet-neutrophil-interactions: Linking hemostasis and inflammation, Blood Rev. 21 (2) (2007) 99–111.
[96] P. Damien, et al., LPS stimulation of purified human platelets is partly dependent on plasma soluble CD14 to secrete their main secreted product, soluble-CD40-Ligand, BMC Immunol. 16 (1) (2015), 3.
[97] F. Semeraro, et al., Extracellular histones promote thrombin generation through platelet-dependent mechanisms: Involvement of platelet TLR2 and TLR4, Blood 118 (7) (2011) 1952–1961.
[98] L.X. Yu, et al., Platelets promote tumour metastasis via interaction between TLR4 and tumour cell-released high-mobility group box1 protein, Nat. Commun. 5 (2014) 5256.
[99] S. Lazar, L.E. Goldfinger, Platelets and extracellular vesicles and their cross talk with cancer, Blood 137 (23) (2021) 3192–3200.
[100] T. Sasano, et al., Role of tissue-factor bearing extracellular vesicles released from ovarian cancer cells in platelet aggregation in vitro and venous thrombosis in mice, Thromb. Update 2 (2021), 100020.
[101] F.G. Gomes, et al., Breast-cancer extracellular vesicles induce platelet activation and aggregation by tissue factor-independent and -dependent mechanisms, Thromb. Res 159 (2017) 24–32.
[102] O. Elaskalani, N.B. Abdol Razak, P. Metharom, Neutrophil extracellular traps induce aggregation of washed human platelets independently of extracellular DNA and histones, Cell Commun. Signal 16 (1) (2018), 24.
[103] J.M. Pitt, et al., Targeting the tumor microenvironment: Removing obstruction to anticancer immune responses and immunotherapy, Ann. Oncol. 27 (8) (2016) 1482–1492.
[104] C.J. Herting, I. Karpovsky, G.B. Lesinski, The tumor microenvironment in pancreatic ductal adenocarcinoma: Current perspectives and future directions, Cancer Metastas-.-. Rev. 40 (3) (2021) 675–689.
[105] D. Hanahan, Lisa, Accessories to the crime: Functions of cells recruited to the tumor microenvironment, Cancer Cell 21 (3) (2012) 309–322.
[106] M. Zhao, A. Zhuang, Y. Fang, Cancer-associated fibroblast-derived exosomal miRNA-320a promotes macrophage M2 polarization in vitro by regulating PTEN/PI3Kγ signaling in pancreatic cancer, J. Oncol. 2022 (2022) 1–11.
[107] M. Shelton, et al., The role of CAF derived exosomal microRNAs in the tumour microenvironment of melanoma, Biochim. Et. Biophys. Acta (BBA) - Rev. Cancer 1875 (1) (2021), 188456.
[108] N. Masoudi-Khoram, et al., Exosomes derived from cancer-associated fibroblasts mediate response to cancer therapy, Crit. Rev. Oncol. /Hematol. 185 (2023), 103967.
[109] T.A. Mace, et al., Pancreatic cancer-associated stellate cells promote differentiation of myeloid-derived suppressor cells in a STAT3-dependent manner, Cancer Res. 73 (10) (2013) 3007–3018.
[110] H. Zhao, et al., Tumor microenvironment derived exosomes pleiotropically modulate cancer cell metabolism, eLife 5 (2016), e10250.
[111] Y. Yang, et al., M2 macrophage-derived exosomes promote angiogenesis and growth of pancreatic ductal adenocarcinoma by targeting E2F2, Mol. Ther. 29 (3) (2021) 1226–1238.
[112] C. Huang, et al., Regulation of miR-155 affects pancreatic cancer cell invasiveness and migration by modulating the STAT3 signaling pathway through SOCS1, Oncol. Rep. 30 (3) (2013) 1223–1230.
[113] J. Gao, et al., Tumor endothelial cell-derived extracellular vesicles contribute to tumor microenvironment remodeling, Cell Commun. Signal. 20 (1) (2022), 97.
[114] J.Y. Mun, et al., Dual relationship between stromal cells and immune cells in the tumor microenvironment, Front Immunol. 13 (2022), 864739.
[115] C. Liebig, et al., Perineural invasion in cancer: A review of the literature, Cancer 115 (15) (2009) 3379–3391.
[116] F. Selvaggi, et al., Perineural invasion in pancreatic ductal adenocarcinoma: From molecules towards drugs of clinical relevance, Cancers (Basel) 14 (23) (2022).
[117] R.E. Stopczynski, et al., Neuroplastic changes occur early in the development of pancreatic ductal adenocarcinoma, Cancer Res 74 (6) (2014) 1718–1727.
[118] J. Li, R. Kang, D. Tang, Cellular and molecular mechanisms of perineural invasion of pancreatic ductal adenocarcinoma, Cancer Commun. (Lond.) 41 (8) (2021) 642–660.
[119] R.D. Cervantes-Villagrana, et al., Tumor-induced neurogenesis and immune evasion as targets of innovative anti-cancer therapies, Signal Transduct. Target Ther. 5 (1) (2020), 99.

[120] Y. Guo, Z. Gil, The role of extracellular vesicles in cancer–nerve crosstalk of the peripheral nervous system, Cells 11 (8) (2022) 1294.
[121] X. Tan, et al., Nerve fibers in the tumor microenvironment in neurotropic cancer—pancreatic cancer and cholangiocarcinoma, Oncogene 40 (5) (2021) 899–908.
[122] Q. Dong, et al., Elevated serum CA19-9 level is a promising predictor for poor prognosis in patients with resectable pancreatic ductal adenocarcinoma: A pilot study, World J. Surg. Oncol. 12 (1) (2014), 171.
[123] A. Lux, et al., c-Met and PD-L1 on circulating exosomes as diagnostic and prognostic markers for pancreatic cancer, Int. J. Mol. Sci. 20 (13) (2019) 3305.
[124] J. Yu, et al., Plasma protein biomarkers for early detection of pancreatic ductal adenocarcinoma, Int J. Cancer 148 (8) (2021) 2048–2058.
[125] Y. Zhu, et al., Diagnostic value of various liquid biopsy methods for pancreatic cancer: A systematic review and meta-analysis, Medicine 99 (3) (2020), e18581.
[126] S.A. Melo, et al., Glypican-1 identifies cancer exosomes and detects early pancreatic cancer, Nature 523 (7559) (2015) 177–182.
[127] A.E. Frampton, et al., Glypican-1 is enriched in circulating-exosomes in pancreatic cancer and correlates with tumor burden, Oncotarget 9 (27) (2018) 19006–19013.
[128] P. Moutinho-Ribeiro, et al., Exosomal glypican-1 discriminates pancreatic ductal adenocarcinoma from chronic pancreatitis, Dig. Liver Dis. 54 (7) (2022) 871–877.
[129] J. Castillo, et al., Surfaceome profiling enables isolation of cancer-specific exosomal cargo in liquid biopsies from pancreatic cancer patients, Ann. Oncol. 29 (1) (2018) 223–229.
[130] X. Lai, et al., A microRNA signature in circulating exosomes is superior to exosomal glypican-1 levels for diagnosing pancreatic cancer, Cancer Lett. 393 (2017) 86–93.
[131] T. Kitagawa, et al., Circulating pancreatic cancer exosomal RNAs for detection of pancreatic cancer, Mol. Oncol. 13 (2) (2019) 212–227.
[132] W. Zhou, et al., Pancreatic cancer-targeting exosomes for enhancing immunotherapy and reprogramming tumor microenvironment, Biomaterials 268 (2021), 120546.
[133] S. Shang, et al., Exosomal miRNA-1231 derived from bone marrow mesenchymal stem cells inhibits the activity of pancreatic cancer, Cancer Med 8 (18) (2019) 7728–7740.
[134] Y. Zhou, et al., Bone marrow mesenchymal stem cells-derived exosomes for penetrating and targeted chemotherapy of pancreatic cancer, Acta Pharm. Sin. B 10 (8) (2020) 1563–1575.
[135] S. Kamerkar, et al., Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer, Nature 546 (7659) (2017) 498–503.
[136] Y. Shiravand, et al., Immune checkpoint inhibitors in cancer therapy, Curr. Oncol. 29 (5) (2022) 3044–3060.
[137] Z. Nsingwane, et al., Immunotherapeutic strategies in pancreatic ductal adenocarcinoma (PDAC): Current perspectives and future prospects, Mol. Biol. Rep. 47 (8) (2020) 6269–6280.
[138] C. Kole, et al., Immunotherapy in combination with well-established treatment strategies in pancreatic cancer: Current insights, Cancer Manag. Res. Volume 14 (2022) 1043–1061.
[139] S.B. Kemp, M. Pasca Di Magliano, H.C. Crawford, Myeloid cell mediated immune suppression in pancreatic cancer, Cell. Mol. Gastroenterol. Hepatol. 12 (5) (2021) 1531–1542.
[140] R.C. Sterner, R.M. Sterner, CAR-T cell therapy: Current limitations and potential strategies, Blood Cancer J. 11 (4) (2021), 69.
[141] W. Fu, et al., CAR exosomes derived from effector CAR-T cells have potent antitumour effects and low toxicity, Nat. Commun. 10 (1) (2019).
[142] S. Kawamura, et al., Exosome-encapsulated microRNA-4525, microRNA-451a and microRNA-21 in portal vein blood is a high-sensitive liquid biomarker for the selection of high-risk pancreatic ductal adenocarcinoma patients, J. Hepato-Biliary-Pancreat. Sci. 26 (2) (2019) 63–72.