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Pancreatic ductal adenocarcinoma microenvironment: Soluble factors and cancer associated fibroblasts as modulators of NK cell functions

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ABSTRACT

Pancreatic Ductal Adenocarcinoma (PDAC) is the most frequent pancreatic cancer and represents one of the most aggressive human neoplasms. Typically identified at advance stage disease, most PDAC tumors are unresectable and resistant to standard therapies.

The immunosuppressive microenvironment in PDAC impedes tumor control but a greater understanding of the complex stromal interactions within the tumor microenvironment (TME) and the development of strategies capable of restoring antitumor effector immune responses could be crucial to fight this aggressive tumor and its spread.

Natural Killer (NK) cells play a crucial role in cancer immunosurveillance and represent an attractive target for immunotherapies, both as cell therapy and as a pharmaceutical target.

This review describes some crucial components of the PDAC TME (collagens, soluble factors and fibroblasts) that can influence the presence, phenotype and function of NK cells in PDAC patients tumor tissue. This focused overview highlights the therapeutic relevance of dissecting the complex stromal composition to define new strategies for NK cell-based immunotherapies to improve the treatment of PDAC.

List of abbreviations

aCAFs	antigen presenting fibroblasts
ADCC	antibody-dependent cell-mediated cytotoxicity
CAFs	cancer associated fibroblasts
CAR	chimeric antigen receptors
csCAFs	complement secreting CAFs
DCs	dendritic cells
ECM	extracellular matrix
EpMT	epithelial-to-mesenchymal transition
FAP	fibroblast activation protein
FGL1	fibrinogen-like protein-1
iCAFs	inflammatory fibroblasts
IDO	indoleamine-2,3-dioxygenase
iKIRs	Inhibitory KIRs
ITIM	immunoreceptor tyrosine-based inhibitory motifs
KIRs	Killer-cell immunoglobulin-like receptors
LAG-3	Lymphocyte activation gene-3
LAIR	leukocyte-associated immunoglobulin-like receptor
MDSC	Myeloid-derived suppressor cells
MIF	macrophage migration inhibitory factor
MMPs	matrix metalloproteases
myCAFs	myofibroblasts
NCR	natural cytotoxicity receptor
NK	natural killer
PB	peripheral blood
PD-1	programmed death-1
PDAC	pancreatic ductal adenocarcinoma
PDGF	platelet-derived growth factor
PDGFRα	platelet-derived growth factor receptor α
PSCs	pancreatic stellate cells
Siglec	sialic acid-binding immunoglobulin-like lectins
TAM	tumor-associated macrophage
TGF	transforming growth factor
TIGIT	T cell immunoreceptor with immunoglobulin and ITIM domain

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TILs	tumor-infiltrating lymphocytes
TIM-3	T-cell immunoglobulin and mucin domain
TME	tumor microenvironment
TNF	tumor necrosis factor
Tregs	regulatory T cells
trNK	cells tissue-resident NK cells

1. Introduction

Pancreatic Ductal Adenocarcinoma (PDAC) is the most frequent malignancy in the pancreas (more than 90% of pancreatic cancers) and represents the sixth-leading cause of cancer related death worldwide [1]. PDAC shows a heterogeneity of clinical and biomolecular manifestations often leading to delayed diagnoses. Late disease recognition, rapid progression and early metastases create a distressing synergy resulting in a very low 5-year survival rate of about 13% [2,3]. The current lack of adequate tools for early diagnosis and the estimate of a rapid increase in the number of cases worldwide (https://gco.iarc.who.int/tomorrow/en/dataviz/bars?cancers=13&years=2035) underline how it is necessary to multiply efforts to understand the dynamics of tumor development and define new therapeutic approaches that limit its impact on human health. Low neoplastic cellularity together with dense and fibrotic stroma and immunosuppressive tumor microenvironment (TME) are considered hallmarks of PDAC [4,5]. In recent years, studies focusing on the PDAC OMICs have multiplied, revealing the high inter-tumoral and intra-tumor heterogeneity of PDAC [4,6]. This heterogeneity, which has led to the identification of different types of PDAC and to proposals for different tumor subclassifications [7–10], can only be partially influenced by distinct cancer cell genomes considering that PDAC genes main alterations substantially interest four main genes (CDKN2A, KRAS, SMAD4, TP53) [11].

Briefly, in 2011, Collisson and coworkers identified three PDAC subtypes, naming them as “classical”, “quasimesenchymal” and “exocrine-like”, depending on specific gene expression identified on laser-micro dissected sections from PDAC patients and exhibited by PDAC cell lines [7]. Later, in 2015, Moffitt and colleagues distinguished the malignant epithelial cell compartment in “classical” or “basal-like,” and the stroma in “normal” or “activated” based on the analysis of patients’ prognostic profile and gene expression. Patients in the basal-like group or expressing an activated signature of stromal cells showed a worse prognosis [8,12]. In 2016, based again on different RNA expression profiles, mainly related to transcriptional factors and downstream targets important in lineage specification/differentiation of pancreas differentiation/regeneration, Bailey and coworkers identified four subtypes of PDAC: (i) squamous; (ii) pancreatic progenitor; (iii) immunogenic; and (iv) aberrantly differentiated endocrine/exocrine [9]. Each subtype was associated with specific histological features and differential survival, revealing that squamous tumors were related to poor prognosis [12,13]. Even named in an alternative way, some of all described PDAC subtypes display significant overlap in the transcriptional profiles [10]. In summary, until now PDAC has been stratified into two “bona fide” tumor subtypes: “classical/progenitor” and “squamous/basal-like”, based on the transcriptional profile and the pathological features [14]. The classical/progenitor PDAC is associated with (i) an endodermal-pancreatic characteristic, (ii) an epithelial gene expression signature, and (iii) relies on lipid metabolism/fatty acid oxidation for its metabolic needs, whereas the squamous basal-like PDAC is associated with (i) a non-glandular structure, (ii) a mesenchymal gene expression profile and (iii) relies more on glycolysis for its metabolic needs [15].

Recently, additional papers dissected in more depth the spatial relationship between neoplastic cells and stroma, providing new insight for the identification of heterogeneity in TME composition [16,17] and strengthening the idea that reciprocal interactions between transformed epithelial cells and their surrounding microenvironment influence the development and evolution of PDAC [18]. In 2021, Grünwald and colleagues, by large-scale integration of histology-guided regional multiOMICs with clinical data and patient-derived preclinical models, reported the existence of two fundamental microenvironmental states, “deserted” and “reactive”, rooted in fibroblast plasticity, causing functional intratumoral heterogeneity of the pancreatic TME [16]. In particular, deserted subTEMs exhibited strong enrichment of the extracellular matrix (ECM) and ECM-related signaling, besides humoral immunity pathways, being inhabited by Cancer Associated Fibroblasts (CAFs) having high proliferation potential, and by malignant epithelial cells having high cellular metabolism (fatty acids, OxPhos) and protein secretion gene sets. Notably, these deserted subTEMs became more frequent upon chemotherapy with chemoprotective effect. On the other hand, reactive subTEMs are characterized by gene sets related to cellular stress response (e.g., hypoxia and metabolic stress), growth factors with activating or immunomodulatory functions (Fibroblast growth factor, FGF; Transforming growth factor, TGF; Platelet-derived growth factor, PDGF; WNT), cytokines (Tumor Necrosis Factor, TNF; interferon, IFN), and cellular immunity (e.g., innate immunity and TCR signaling) and are inhabited by CAFs with higher motility and exhibiting inflammation related gene sets (e.g. IL-2/STAT5, IL-6/JAK/STAT, IFNs, TNF-α, complement). In addition, reactive subTEMs are enriched with tumor cells exhibiting a basal-like gene signature and show a higher proliferative index. In short, deserted subTME appears to support tumor differentiation while a reactive subTME is favorable towards tumor progression through the involvement of proliferative, de-differentiated, squamous/basal-like tumor cell phenotypes. Interestingly, patient survival was not associated with main TME phenotypes but rather with intratumoral subTME co-occurrence, suggesting their combinatory effect on disease outcome [16].

Very recently, by molecular profiling at single cell level of histologically different tumor clusters, Natoli and colleagues identified three morphological and functional variants, called “morpho-biotypes”, coexisting in various proportions in all PDAC [19]. These morpho-biotypes, named “glandular”, “transitional”, and “undifferentiated” and reflecting the previous classifications only partially, identify cells with different gene expression programs which are organized in morphologically distinct and spatially discrete tumor areas and with different organization of ECM. In particular, the term “glandular” identifies areas characterized by classical ductal morphology and typical endodermal gene expression; “transitional” identifies areas with abortive ductal morphology, gene expression programs intermediate between those of endodermal and mesenchymal- or myofibroblast-like cells; and finally “undifferentiated” pinpoints areas with the lack of evident histological differentiation and a gene expression program typical of undifferentiated cell populations including a quite unexpected neuronal-like gene expression profile. In addition, translational morpho-biotype areas are characterized by thick collagen fibers in the proximity of tumor cells, an increased and disorganized production of components of cell basal membranes that result undefined up to be completely lost in the undifferentiated morpho-biotype [19].

Finally, by transcriptomic analyses, 4 distinct and prognostically relevant TME subtypes have been identified based on immune composition [17]: (i) “Immune Enriched” (IE); (ii) “Immune Enriched/Fibrotic” (IE/F); (iii) “Fibrotic” (F); and (iv) “Immune Depleted” (D). The IE subtype is characterized by the highest signal of antitumor immune components including T cells, B cells, NK cells, M1 macrophages and antitumor cytokines that overcome the signal, however high, of protumor immune components, including regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), Th2 and checkpoint inhibition. The IE/F subtype includes IE immune components but with the addition of high endothelial signaling, and the highest activation of the WNT signaling pathway. The F subtype is characterized by the highest enrichment of CAFs pathway and angiogenetic signaling, whereas the D subtypes express the highest proliferation gene signature. The F and D TME subtypes are enriched in basal-like/squamous tumor PDAC subtypes and are associated with a worse prognosis. Interestingly, the

authors also showed that TME, including fibroblast populations, varied based on the site of metastases: the majority of lung and peritoneum metastases showed the IE or the IE/F TME subtype, whereas the liver showed the D TME subtype. TME subtypes IE and IE/F have a more favorable prognosis and could potentially benefit from immunotherapeutic approaches rather than the other subtypes [17].

All together, these data highlight the therapeutic relevance of dissecting the complex stromal composition to define new strategies for immunotherapy in PDAC which still remain unsatisfying to date [20–22]. In this review, we describe the known PDAC TME components, mainly focusing on soluble factors and fibroblasts, which can condition presence, phenotype, and function of NK cells, i.e., innate lymphocytes known to be one of the most efficient antitumor effector cells. In PDAC, it has been estimated that less than 0.5% of tumor-infiltrating lymphocytes (TILs) are NK cells. Mainly due to their limited size population, few studies explored phenotype and function of tumor-associated NK cells, so their more detailed characterization is certainly advantageous to evaluate their role in PDAC progression control. In this regard, there are no definitive results on the correlation between the presence of NK cells and patients' survival, even if a more significant number of evidences leans more towards a correlation with prolonged survival [23–26].

2. Fibroblasts, CAFs and collagens

Fibroblasts are known as mesenchyme-derived differentiated cells, capable of producing and remodeling the connective tissue ECM, which provides mechanical support to all types of tissues, besides representing the pericellular environment for metabolic/respiratory exchanges and molecular signaling. Common shared functions exerted by all fibroblasts are (i) synthesis of ECM components, such as collagens, proteoglycans, elastin fibers, and a multitude of affiliated non-collagenous glycoproteins, collectively named "matrisome", (ii) active ECM remodeling, mainly controlled through the secretion of the matrix metalloproteases (MMPs), and (iii) secretion of various growth factors, adipokines, and cytokines [27].

In particular, the major "matrisome" component is represented by both fibrillar and non-fibrillar collagens, which form a 3D meshwork supporting cancer cells besides being involved in cell signaling between PDAC cells and ECM and exerting their biomechanic properties, which condition cell ability to escape from the tumor moving towards other body sites as well as more or less great permissiveness to cancer cell invasion. Although collagen amount and typology undergo time-related changes during tumor development, there is still not enough evidence on how specific collagen family members are involved in distinct protumorogenic or anti-tumorogenic performances from the earliest preclinical stages to the latest ones.

Type I collagen, by far the most abundant member in the body, was reported to promote the malignant phenotype of PDAC [28], besides accelerating tumor progression by increasing immune suppression [29].

Immunofluorescence staining of fibrillar collagens types I, II, III, and non-fibrillar collagen type IV revealed differential changes after acute treatments, being the fibrillar ones inversely correlated with cell survival whereas the non-fibrillar one, well known to be a major component of the cell basal laminae, being directly correlated with such cell responsiveness [30]. Consistently with the finding that turnover and degradation of collagen type IV is associated with the invasion by cancer cell metastasis, besides effects on angiogenesis and neovessel formation in 3D rat aorta model [31], these immunohistochemical data suggest that treatment-induced increase in collagen type IV amounts may be predictive of favorable treatment-dependent outcomes, including both a reduction in PDAC invasion and metastasis and inhibitory effects on angiogenesis. Instead, it remains unclear why and how collagen type II may be involved in PDAC, also considering that this type is normally restricted to cartilages and vitreous body.

Using proteomics to define multiple changes in ECM profiles in the course of PDAC progression in both the mouse models and in human patient samples, α1 and α2 chains of heterotrimeric collagen type I as well as α1 chains of homotrimeric collagen type III were found to be the most abundant collagenous components, representing more than 90% of the entire collagen mass at all PDAC stages, with a 2.6-fold increase during PDAC progression, while α1, α2, and α3 chains of heterotrimeric non-fibrillar collagen type VI were over-represented [32].

Another collagenous member acting in PDAP is non-fibrillar collagen type XII, which was revealed by temporal proteomic profiling of decellularized tumors. It has been shown to alter the organization of collagen type I, giving rise to a pro-invasive microenvironment supporting metastasis dissemination [33].

Returning to CAFs, these cells play a crucial role in tissue homeostasis, wound healing processes, fibrotic conditions, inflammation, and cancer progression [34]. Fibroblasts are endowed with an extraordinary versatility in gene transcriptional program that allows them to acquire a multiplicity of phenotypic and functional characteristics conditioned by the surrounding microenvironment, which is, in turn, conditioned by their own activity [27].

From this perspective, CAFs best express fibroblast attitude by gathering a multitude of different cell subsets characterized by pro- or anti- tumor functional activities, even in the context of the same tumor. It has been suggested that the high heterogeneity of CAFs is a mirror of precursor heterogeneity [34,35]. Adipose-derived mesenchymal cells [36], pericytes [37], endothelial cells [38], smooth muscle cells [39], $CCR2^{+}$ migrating monocytes [40], or epithelial cells undergone a complete epithelial-to-mesenchymal transition (EpMT) [41] have been identified as sources of CAFs. In PDAC, resident fibroblasts [42], pancreatic stellate cells (PSCs) [43], and mesenchymal stem cells [44] have been described to be commonly the three major sources of CAFs [45].

CAFs are perpetually activated fibroblasts [39] with a high capacity to synthesize ECM components in tumors, a phenomenon called "stromal desmoplasia" [46]. In PDAC, CAFs are one of the most abundant cell populations. They are considered the major contributors to the desmoplastic reaction by providing a kind of biomechanical shell that constrains and isolates tumor cells from the surrounding tissue, thus preventing the spreading of malignant cells. However, this highly abundant fibrotic reaction is a double-edged sword since it constitutes an "entry barrier", preventing the immune system effector cells from penetrating into the stroma, where they recognize and destroy malignant cells efficiently [5,47]. In addition, desmoplasia generates a substantial pressure that contributes to blood vessel collapse [48] which, in turn, results in a hypoxic [49] and nutrient deprived [50] microenvironment. Therefore, the simple definition of collagenous ECM producers does not reflect the complexity of the CAFs regulatory role in TME, considering that it additionally consists in (i) crosstalking with immune cells [51], (ii) promoting angiogenesis [52], (iii) providing metabolic support, and (iv) impacting tumor growth and therapy responses in numerous tumor types [53,54]. Different studies showed that CAFs can secrete immunomodulatory cytokines such as IL-10, TGF-β, TNF, IFN-γ, IL-6, CXCL9, CXCL10, and CXCL11 that can also recruit immune cells (e.g., T cells, macrophages, and NK cells) within the TME [55,56]. Multiple signal pathways instructing the transition from quiescent to activated fibroblasts, which can act in autocrine, paracrine, and endocrine manners were described, including specific responses to (i) soluble mediators such as TGF-β, PDGF, Wnt; (ii) inflammatory cytokines such as IL-1 and IL-6 and TNF-α; and (iii) biomechanical signals mediated through integrin-associated focal adhesions [47,57].

CAFs are commonly defined as cells non-expressing the markers typically used for the identification of epithelial cells (EpCAM), leukocytes (CD45), and endothelial cells (CD31), and showing distinctive morphology in comparison to normal fibroblasts [58] such as larger cytoplasmic area and jagged nuclei in light microscopy, and more intricate structures as revealed by electron microscopy, such as increased rough endoplasmic reticulum, abundant free ribosomes, large Golgi apparatus, and abundant stress fibers [58,59]

Nearly all CAFs express fibroblast activation protein (FAP) which is a type-II transmembrane serine protease with dipeptidase and gelatinase/collagenase activities, which are essential for ECM remodeling [60]. FAP, which expression can be induced by TGF-β [61], has been reported to be capable of affecting cell apoptosis, enhancing stromal cell proliferation, invasiveness, and participating in immune system suppression [62,63]. However, FAP can not be considered a helpful marker for CAFs identification since its expression is not confined to cancer stromal cells. Namely, higher amounts of FAP can be detected on the surface of different carcinoma cells where its expression was closely correlated with poor prognosis [62].

Differently from initial evidence suggesting CAFs to be a homogenous population, in recent years through pivotal single-cell RNA-sequencing studies and using murine models, two main subtypes of CAFs have been described in PDAC: myofibroblasts (myCAFs) and inflammatory fibroblasts (iCAFs) [64]. These two subtypes differ in morphological, behavioral, and functional properties but are joined by the absence of specific lineage markers, which makes their origin unambiguous [65]. MyCAFs are TGF-β activated fibroblasts expressing high levels of α-smooth protein and very low/lacked expression of inflammatory cytokines (α-SMA^{high}, IL-6^{low}), (i) reside closely to tumor cells, (ii) have contractile properties, and (iii) are believed to restrain PDAC tumor growth. Otherwise, iCAFs are induced by IL-1/NF-κB/JAK/STAT pathway [66], besides exhibiting very low expression of α-SMA and high production of cytokines such as IL-6, IL-11, and LIF (α-SMA^{low}, IL-6^{high}), highly expressing platelet-derived growth factor receptor α (PDGFRα), and being arranged more distant distributed from the malignant cells [45,64,67]. These two subsets can transition from one phenotype to another, indicating that the distinctions between CAF subtypes may be fluid and context-dependent [65,68]. In a genetically engineered mouse model, PDAC lesion was demonstrated to present heterogenous exosome secretion identified by Rab27a expression [69]. Interestingly, PDAC lesions characterized by efficient secretion of exosomes (Rab27a^{high}) are surrounded by iCAFs, contrary to others (Rab27a^{low}) which are surrounded by myCAFs. Therefore, exosome secretion, in addition to various biological processes promoting tumor progression, could modulate CAFs phenotype and impact in their spatial distribution within PDAC tumors [69,70].

Different preclinical studies showed that depletion of α-SMA^{+} CAFs reduces fibrotic stroma and increases the tumor aggressiveness [71]. In addition, an increased myCAFs/iCAFs ratio obtained inhibiting the IL-1/JAK signaling pathway, resulted in a better prognosis, supporting an opposite role of iCAFs compared to myCAFs in PDAC evolution [65,66].

Although myCAFs and iCAFs are the dominant populations in PDAC cancer stroma, many other CAFs subtypes were described, such as the so-called antigen presenting fibroblasts (aCAFs), which are derived from mesothelial cells (both IL-1 and TGF-β can induce mesothelial-to-CAF transition) and express molecules of the Major Histocompatibility Complex (MHC) of class II and CD74 but no classic costimulatory molecules (CD80, CD86, CD40) [72]. It has been shown that aCAFs directly bind naive CD4^{+} T cells, inducing them into Tregs in an antigen-specific manner promoting immunosuppression [73]. A further supposed immune-modulating subset of CAFs, named complement secreting CAFs (csCAFs), was detected by Chen and colleagues in the early stages of PDAC and collected cells able to express many components of the complement system [74]. Finally, recent evidence suggests that expression of CD105/endoglin, an accessory receptor for TGF-β, distinguishes between CAFs able to accelerate (CD105^{+}) or suppress (CD105^{-}) tumor growth if co-injected with malignant cells in murine model [75]. However, further investigation is needed to understand the origin and the impact of these subsets *in vivo* and the role of each CAF subpopulation in crosstalk with tumor and immune cells.

3. Conventional and tissue-resident NK cells

NK cells are cytotoxic cells of innate immunity that represent a first line of defense against invading pathogens and tumors. Indeed they can rapidly kill transformed cells via non-MHC-restricted actions [76]. While such innate immunity can often stop infections at a subclinical level, in more severe cases it can contain the infection allowing the intervention of adaptive immunity, which can conduct a more efficient response thanks to the involvement of T and B lymphocytes. Indeed, NK cell-mediated cytotoxicity and cytokine release can influence functions of additional innate immune cells (such as dendritic cells and macrophages) and confer NK cells a regulatory function, capable of affecting the HLA-restricted T and B cell responses. Unlike T and B cells, NK cells do not express receptors encoded by rearranging genes. However, they might be self-reactive, like T and B cells [77,78]. For this reason, NK cells must undergo an "education" process to make them effective against transformed cells while also ensuring self-tolerance [79–81]. Through this process, only NK cells expressing inhibitory receptors specific for self-HLA class I molecules acquire full functional potential, while "autoreactive" NK cells maintain a hyporesponsive state. The NK cell education is a process necessary to ensure self-tolerance. Indeed, during their development, NK cells randomly acquire a set of self-HLA-I inhibitory receptors, and, at the same time, the HLA molecules recognized by some of these receptors are inherited independently of the receptor genes. Therefore, due to the education process, most mature and functional NK cells are equipped with at least one inhibitory receptor for self-HLA class I antigens.

As they mature, NK cells can migrate from the bone marrow into the blood and then to peripheral tissues. Indeed, their presence in most tissues depends on their ability to circulate between lymphatic and non-lymphoid organs. Thus, thanks to their powerful effector functions and tissue distribution, NK cells can play a crucial role in many types of diseases, including infectious diseases and cancer [78].

For many years, NK cell studies have focused on analyzing NK cells in peripheral blood (called PB-NK cells or conventional NK cells). NK cells were known as effector cells circulating in the blood that could infiltrate inflamed or otherwise diseased tissues. Traditionally, two main subsets of NK cells have been distinguished in peripheral blood (PB) based on the cell surface density of CD56 and CD16 molecules: CD56^{bright}CD16-$^{dim/-}$ and CD56^{dim}CD16^{+} [82,83]. The former is believed to be less mature but capable of producing high levels of cytokines (e.g., IFN-γ and TNF-α). In contrast, the latter is more mature and cytotoxic thanks to their high content of perforin and granzymes.

Only recently, researchers have begun to explore the diversity of NK cells present in peripheral tissues, indicating that they are a mixture of cells recirculating from the blood to the tissues and cells expressing specific markers (such as CD69, CD103 and CD49a and others) capable of retaining them in the tissues (these cells are called tissue-resident NK cells, trNK cells) [84,85]. Indeed, CD69, a protein that is also associated with lymphocyte activation, binds the sphingosine-1-phosphate receptor 1 (S1P1), promoting its internalization and degradation, and thus blocking its interaction with sphingosine-1-phosphate (S1P), whose higher gradient in the blood compared to tissues promotes lymphocyte egress from tissues into circulation [86]. CD103 mediates cell adhesion to epithelial cells by binding to its ligand, E-cadherin, a cell-to-cell junction protein. CD49a is a α-1 integrin that binds collagen and laminin [87].

The migration of NK cells into inflamed tissues is controlled by different types of molecules, such as adhesion molecules and chemokine receptors, and by the concentration gradient of the various chemokines present in the tissues. The main two NK cell subsets (CD56^{dim} and CD56^{bright} NK cells) express different types of chemokine receptors and, thus, different migration capabilities. Indeed, CD56^{dim} NK cells expressing mostly CXCR1 and CX3CR1 can migrate into inflamed tissues, whereas CD56^{bright} NK cells expressing CD62L, CCR7, CCR5, and CXCR3 can migrate into secondary lymphoid organs [88,89].

Furthermore, CD56^{bright} and CD56^{dim} NK cells could be differentially recruited to the tumor due to their different repertoire of chemokine receptors [90,91]. However, since NK cell density in solid tumors is usually extremely low, and since in some tumors the presence of a "high" number of tumor-associated NK cells correlates with a more favorable clinical outcome, it could be relevant to deepen our knowledge on the factors that regulate the trafficking of NK cells into the tumor in order to design new approaches capable of favoring their entry into the tumor and improving the efficacy of NK cell-based immunotherapies [90].

The antitumor effect of NK cells is regulated by a broad spectrum of germline-encoded inhibitory and activating receptors expressed on their surface [92]. Indeed, HLA-I molecules recognition on healthy cells by specific inhibitory NK receptors prevents the NK cell-mediated attack. Human NK cells also express several receptors that can trigger their function upon interaction with specific ligands on the surface of transformed, virus-infected, or stressed cells. In normal conditions, the interaction between the inhibitory receptors and their specific HLA-I ligands inactivate NK cells, thus preventing cytolytic activity against healthy cells. During cancer progression, the transformed cells decrease or even lose the expression of HLA-I on their surface and acquire the expression of the ligands for activating NK receptors. Indeed, these ligands are generally absent or expressed in small amounts in normal cells, while they are expressed de novo or upregulated at the cell surface in stressed normal cells, particularly on tumor cells.

In humans, the HLA-specific inhibitory receptors allow the NK cell tolerance toward healthy autologous cells and are mainly represented by Killer-cell immunoglobulin-like receptors (KIRs) and CD94/NKG2A [92]. Inhibitory KIRs (iKIRs) are type-I molecules of the Ig superfamily characterized by 2 or 3 extracellular domains and a long cytoplasmic tail, whereas CD94/NKG2A heterodimer is composed of type-II proteins, belonging to the C-type lectin superfamily. The main iKIRs recognize epitopes shared by distinct groups of HLA class I molecules, referred to as KIR-ligands (KIR-L): KIR2DL1 is specific for HLA-C^{K80} allotypes (HLA-C2 epitope), KIR2DL2/L3 recognize HLA-C^{N80} allotypes (HLA-C1 epitope), KIR3DL1 binds to HLA-B and HLA-A molecules sharing the Bw4 epitope, and KIR3DL2 recognizes HLA-A*03 and -A*11. Differently, CD94/NKG2A is specific for the non-classical HLA-E molecules that are characterized by limited polymorphism. Upon receptor engagement, the immunoreceptor tyrosine-based inhibitory motifs (ITIM) localized in the cytoplasmic tail of the inhibitory receptors become phosphorylated, recruit tyrosine phosphatases, and then deliver an inhibitory signal [93].

During NK cell development, immature stages primarily express CD94/NKG2A, and KIRs are acquired upon maturation. In peripheral blood, NKG2A is expressed on CD56^{bright} NK cells and on a subset of CD56^{dim} NK cells, whereas KIRs are expressed on a large proportion of CD56^{dim} NK cells. At later stages of differentiation CD56^{dim} KIR^{+} NK cells also express high levels of CD57, lose the expression of CD94/NKG2A and decrease their proliferative capacity [94,95].

Major activating NK receptors are represented by the three members of the Natural Cytotoxicity Receptor (NCR) family (NKp46, NKp30, and NKp44), and by NKG2D. NKp46 and NKp30, expressed on virtually all resting NK cells, are associated with CD3-ζ and/or FcεRI-γ, whereas NKp44, acquired upon NK cell activation, signals through KARAP/DAP12 adapter molecule. NKG2D, expressed not only by NK cells but also by cytotoxic T cells, is a homodimer that, upon recognition of MICA/B and ULBPs on virally infected, stressed, and tumor cells, transduces the activating signal through the DAP10 adapter protein. NK cells are also equipped with numerous activating co-receptors, including DNAM-1, NKp80, 2B4 and NTB-A [76]. In addition, CD56^{dim} NK cells are characterized by the expression of CD16, the type III low affinity receptor for IgG (FcγRIIIa) [96], that can mediate antibody-dependent cell-mediated cytotoxicity (ADCC) thanks to its association with CD3-ζ and FcεRI-γ [97].

4. Changes in NK phenotype and function in TME

Tumor-associated cells and tumor cells can inhibit the NK cell-mediated antitumor response by establishing cell-to-cell contacts and releasing different types of soluble molecules (such as cytokines, inflammatory mediators, and reactive oxygen species) (Fig. 1).

Among the various soluble factors present in the TME, TGF-β exerts a master and pleiotropic role for immunosuppression supporting the differentiation of mononuclear cells into MDSCs, preventing infiltration of cytotoxic T cells into the tumor, and inducing the differentiation/proliferation of T regulatory cells. TGF-β also plays a crucial role in suppressing the NK antitumor immune response by down-regulating the surface expression of the NKG2D and NKp30 activating receptors [98,99]. In pancreatic cancer, the TGF-β1 pathway is considered a critical pathway for the pathogenesis of the disease resulting altered in 47% of cases of PDAC. The overexpression of TGF-β has been related to adverse outcomes such as shorter patient survival rate and metastasis [98,100] and, among the various functions, TGF-β is related to conversion of fibroblasts or endothelial cells into myCAFs [101] which produce fibrotic stroma and to induction of immunosuppressive cytokines production (IL-10, TGF-β, IL-35) from immune cells (Treg cells, TAMs and B cells).

Besides TGF-β, other soluble factors in PDAC TME such as IL-10, macrophage migration inhibitory factor (MIF), MUC-16, L-kynurenine, and adenosine, can influence NK cell functions. Generally, IL-10 is known to have a dual role, affecting or enhancing NK cells [102–104]; MIF can down-regulate NKG2D expression; MUC-16 can interfere both the formation of the NK/tumor immunological synapses [105] and ADCC; L-kynurenine prevent the up-regulation of NKp46 and NKG2D expression and function [106]; adenosine can interfere with NKG2D expression and with proliferation, maturation, and cytotoxicity of NK cells [107].

IL-10 can be released by CAFs or TAM; it has also been recently described in PDAC as secreted by infiltrating CD38^{+}CD19^{+} B cells and causing inhibition of NK cells [108].

Divergent roles for MIF have been described. Recent single cell RNAseq analyses from PDAC tissue suggest that MIF could co-partecipate in HLA-class-I and co-stimulatory molecules up-regulation on the dendritic cell surface, favoring the antigen presentation to T cells and cross-talk with NK cells [26], suggesting an antitumor role. Differently, MIF is considered a prosurvival factor in different cell types, and its overexpression in pancreatic cancer cells, PSCs, and CAFs has been correlated with poor prognosis [109]. In PSCs and CAFs, in particular, MIF seems to constitute a survival advantage under growth factor deprivation and a promoter of cancer cell growth.

In PDAC, MUC-16 high expression correlates with faster tumor progression, more metastasis, aggressive subtypes like basal-like and squamous tumors, shorter survival [110,111], and CAFs differentiation, but inversely with NK cell infiltration [112]. In this regard, NK cells can bind membrane bound and soluble forms of MUC-16 by the inhibitory receptor sialic acid-binding immunoglobulin-like lectins (Siglec)-9 (see below) [113].

About indoleamine-2,3-dioxygenase (IDO), the enzyme that catalyzes the initial, rate-limiting step in the degradation of the essential aminoacid tryptophan producing L-kynurenine, it has been demonstrated to be overexpressed in PDAC malignant cells and to affect T cell function *in vitro* [114]. The activity of IDO can affect NK cell function in PDAC TME as described in melanoma, hepatocellular and colorectal carcinoma [115–117]. Extracellular adenosine is mainly generated by the coordinated action of the cell-surface ectonucleotidases CD39 and CD73. Induced by tissue hypoxia, inflammation, tissue repair and specific oncogenic pathways, the adenosinergic axis is a broadly immunosuppressive pathway that regulates both innate and adaptive immune responses[118]. CD73 has been demonstrated to be expressed at high levels in TILs, especially Tregs [119]; in different kinds of solid tumors, such as melanomas and prostate cancers [120,121], in CAFs derived from colorectal cancer [122] and in stromal cells in pancreatic cancer

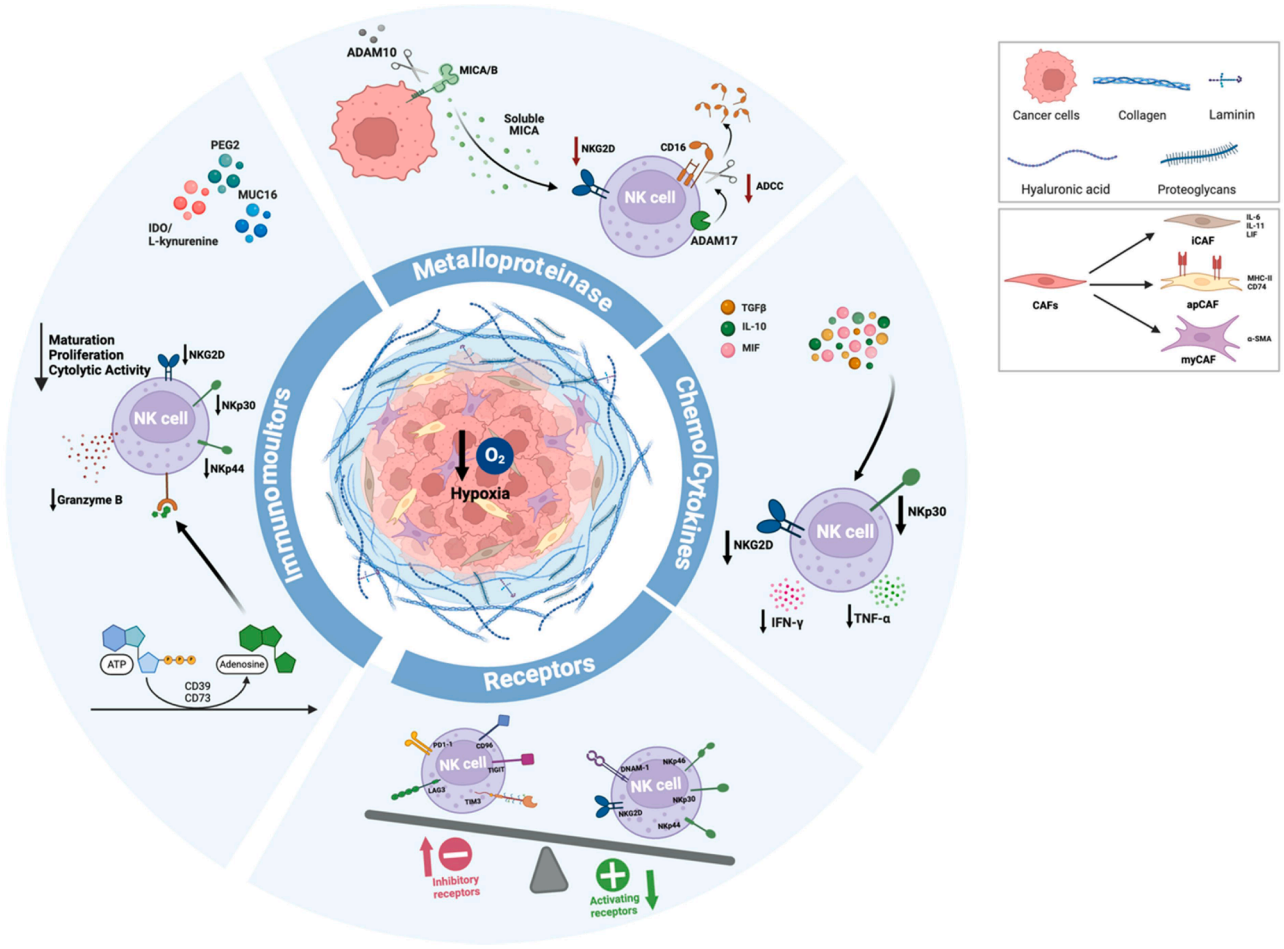

Fig. 1. PDAC TME affect NK cell function. The picture summarize cell-to-cell contacts, immunomodulators, metalloproteinases, cytokines and chemokines that can modulate NK cell phenotype and function in PDAC TME. Created with BioRender.com.

[123] but its role and detailed expression in PDAC needs further evaluation. Of note, CD73 is highly expressed in amoeboid PDAC cells and drives their invasive, metastatic, and immunomodulatory traits [124], correlates with increased disease aggressiveness and decreased number of $CD4^+$, $CD8^+$ and $CD21^+$ TILs [125].

Dysregulation of nutrient profiles and hypoxia, additional hallmarks of PDAC TME, induce resident cells to face numerous adaptations [49, 126]. NK cells usually utilize a variety of metabolic fuels such as glucose, glutamine, and arginine to promote specific metabolic and effector functions. Glucose is their preferred metabolic fuel and is crucial to support glycolysis and oxidative phosphorylation needed for activation, cytokine production, degranulation, and target elimination [127]. Low glucose levels and lactate accumulation are due to increased glucose consumption by cancer cells and CAFs, and, consequently, low TME pH reduces IFN-γ production capacity of NK cells [128,129].

Hypoxia drives a multitude of biological behavior adaptations in malignant and stromal cells. Under hypoxic conditions, NKG2D, perforin, and granzyme B expression in NK cells is severely impaired, thus suppressing NK cell immunotoxicity [126]. In addition, hypoxia promotes cancer cell metalloproteinase ADAM10 expression through HIF-1α, leading to shedding of MICA (NKG2D ligand) from cancer cell surfaces, thereby attenuating NK cell-mediated lysis [49,130,131].

The action of metalloproteases can also directly influence the expression of NK cell activating receptors through proteolytic cleavage, and therefore, their removal from the cell surface, as in the case of CD16 whose expression is regulated by ADAM17 [132].

In addition, CAFs in PDAC TME can attenuate the cytotoxic activity of NK cells against tumor cells, as demonstrated for T cells [133], through the secretion of prostaglandin E2 that induce the down-modulation of activating receptors (NKp30, NKp44 and NKG2D) on the NK cell surface [134].

Chronic exposure to soluble factors and cell-to-cell contacts in the TME can profoundly modify the phenotype of NK cells, inducing an increased expression of immune checkpoints (IC), such as Programmed Death-1 (PD-1), T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT), CD96, Lymphocyte activation gene-3 (LAG3) or T-cell immunoglobulin and mucin domain (TIM-3), a reduced expression of the activating NK receptors and a lower ability to proliferate, degranulate and release cytokines. Indeed, these additional inhibitory receptors recognize cell surface or extracellular ligands and contribute to the physiological control of immune responses and tolerance. In pathological conditions, as in the TME, these receptors can be upregulated and/or de-novo acquired, thus mediating the inhibition of antitumor responses and facilitating tumor escape.

In particular, PD-1 is an inhibitory receptor originally discovered on T cells. It plays an important role in maintaining peripheral tolerance and T-cell homeostasis. Still, it is also expressed on additional types of immune cells within TME, including activated monocytes, dendritic cells (DCs), NK cells, and B cells [135–137]. Upon interaction with its ligands, PD-L1 and PD-L2, often upregulated on tumor cells, it can inhibit immune cell functions, contributing to immune escape [138]. High levels of PD-1 ligands affecting T cell functionality [139] have been demonstrated on CAFs [140] as well as on pancreatic cancer cells [141], suggesting a possible further inhibitory function on NK cells.

TIGIT and CD96 are inhibitory receptors that share the same ligands (PVR/CD155 and Nectin-2/CD112) with the activating receptor DNAM-

1. Thus, TIGIT and CD96 can exert an inhibitory effect by competing with DNAM-1 for binding to their ligands, often upregulated on cancer cells as demonstrated in tissue speciments of PDAC patients [142,143]. CD96 also binds to CD112. Upregulation of both TIGIT and its ligands have been described in multiple cancer types [144].

LAG-3 is a co-inhibitory receptor mainly expressed on T and NK cells but also on other immune cells, such as Treg, NKT, B cells, and DCs. LAG-3 recognizes several molecules on target cells, such as MHC of class II, C-type lectin receptor LSECtin and fibrinogen-like protein-1 (FGL1). In PDAC, tumor infiltrating LAG-3^{+} T cells, the majority CD8^{+} [139], are associated with poor disease-free survival [145]. LAG-3 expression leads to decreased cytotoxic CD8^{+} T cell function and increased Treg suppression activity while its specific role in regulating NK-cell function is not yet fully understood.

TIM-3 is an inhibitory receptor expressed on many immune cells, including NK cells. In particular, TIM-3 is expressed on resting NK cells, mainly restricted to the CD56^{dim} NK cell subset, but it can be upregulated on CD56^{bright} NK cells upon cytokine stimulation [146]. TIM-3 receptor can recognize several ligands, such as galectin-9, phosphatidylserine on apoptotic cells, HMGB1, and CEACAM1 [147]. High frequencies of circulating and/or tumor infiltrating TIM-3^{+} NK cells have been found in different types of solid tumors, and if NK cells inhabit the TME of PDAC, they could be inhibited by the high expression of CEACAM1 [148,149].

In this regard, single-cell RNA-seq analysis combined with other complementary approaches suggests that in PDAC TME, NK cells could be equipped with a multiple immune checkpoint receptor repertoires similar to tumor-infiltrating CD8^{+} T cells [150]. Indeed, NK cells revealed elevated mRNA expression of CD47, TIGIT, TNFRSF18 and LAG3, as well as modest of PDCD1. Interestingly, highly variable gene expression analysis highlighted heterogeneity among NK cell subpopulations in pancreatic non-tumor and tumor tissues. In advanced disease specimens, NK cells showed increased GZMA, TIGIT, and HAVCR2 expression compared to non-tumor samples.

The leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1, CD305) is an IC broadly expressed on the cell surface of immune cells, such as T, B, NK cells, monocytes, and dendritic cells [151,152]. Upon binding to collagen [153] and molecules with collagen-like domains [154,155] it transduces an inhibitory signal. Thus, collagens expressed at the TME level may not only shape an ECM-rich and fibrotic tumor niche, but also promote immune evasion through their direct interaction with LAIR-1. Interestingly, the natural soluble homolog of LAIR-1, called LAIR-2, shares 83% identity with the extracellular region of LAIR-1 and binds to collagens with higher affinity than LAIR-1, thus functioning as a natural antagonist of LAIR-1 [156,157].

Changes in glycosylation are a general hallmark of tumor progression, and PDAC tumor cells are characterized by increased sialylation. Overexpression of sialic acids can induce immunomodulatory properties via binding to Siglec receptors, a group of cell surface proteins that can bind to sialic acids [158]. Among them, Siglec-7 is an inhibitory receptor mostly confined to NK cells, but is also expressed on monocytes, a minor fraction of CD8^{+} T cells, and granulocytes. It preferentially binds to α-2, 8-disialyl and branched α-2,6-sialyl residues that are highly expressed on several tumor cell lines and primary tumor cells. Similarly to a subset of T cells and TAMs, a subpopulation of NK cells, both in peripheral blood and tumors, can also express Siglec-9, an inhibitory receptor recognizing α2-3-linked sialic acid. It has been demonstrated that the overexpression of sialic acids on PDAC tumor cells and CAFs contributes to an immunosuppressive microenvironment affecting T and NK cell functions by promoting TAM differentiation via the interaction with their Siglec receptors [159,160]. However, it could be of interest evaluate the possible expression of Siglec receptors and the direct inhibitory effects of sialic acids on NK cells infiltrating PDAC tumor tissue.

Although NK cells figure well equipped to efficiently degrade a wide range of ECM structures [90,161] they show great difficulty crossing a microenvironment rich in thick collagen fibers. The NK cell scarcity in PDAC TME has also been attributed to the migration inability towards tumor because of their reduced expression of the chemokine receptor CXCR2, which binds to CXCL chemokines released from PDAC cells [162]. NK cells inhabiting the PDAC microenvironment result functionally impaired, as shown by their failure to fully upregulate IFN-γ and granzyme B content upon stimulation with IL-2 compared to NK cells derived from peripheral blood [163] and as suggested by the strong down-modulation of some activating receptors, in particular CD16, NKp30, and DNAM-1, compared to NK cells from the corresponding peripheral blood samples and NKG2D with respect to healthy donors [164]. Downmodulation of activating NK receptors can also occur following their chronic stimulation by ligands expressed on tumor cells [165]. In this case, the engagement of the activating NK receptor by the corresponding ligand induces both the receptor endocytosis, and, therefore, the decrease in the ability of NK cells to recognize and kill tumor cells [78].

Focusing on direct and indirect NKs/CAFs interactions, *in vitro* experiments revealed that, upon culture with PSC isolated from tumor specimens, the NK cell line NK92 reduced the production of IFN-γ differently from NK92 cells cocultured with quiescent normal PSCs [164], therefore suggesting a mechanism of immune suppression mediated by activated PSC. More recently, in 2022 and 2024 AACR meetings, two research groups have presented abstracts describing respectively that (1) PSC express membrane bound MICA, ULBP-2, and NKp44-ligands and are susceptible to NK mediated killing [166], and that (2) activated PSC are less susceptible to NK mediated lysis. In addition, cocultured cells showed a bi-directional interaction: PSCs showed a myo-fibroblastic shift and NK cells modulated NKG2A and TIM-3 expression [167].

Interestingly, in 2020, Francescone et colleagues discovered an increased expression of the neural glutamatergic synaptic stabilizing protein NetG1 in CAFs [168]. This protein interacts with the ligand NetG1 (NGL1) found in PDAC cells, suggesting its involvement in the TME. The interaction between NetG1 and NGL1, facilitated by direct physical contact between CAFs and PDAC tumor cells, combined with the secretion of glutamate and glutamine by CAFs, plays a critical role in supporting the survival of PDAC tumor cells under nutrient-depleted conditions. Furthermore, they demonstrated that NetG1 contributes to the establishment of an immunosuppressive milieu that shields PDAC cells from NK cell-mediated cytotoxicity, significantly preventing NK cell activation and function by inducing the release of immunosuppressive cytokines, including TGF-β in high amounts.

5. Future perspectives

Primary treatments for PDAC are surgery, in about 15-20% of cases, and chemotherapy, but in the last years, emerging approaches have been developed, including new targeted and immune-based therapies. Unfortunately, patients' overall survival rate has not significantly improved. The strong immunosuppressive microenvironment is considered the limiting factor for the successful clinical outcomes of immunotherapeutic approaches that aim to increase the infiltration and functions of cytotoxic T cells. Encouraging clinical results have been achieved by combining chemotherapy with targeting one or more critical points of dysfunctional TME and immune checkpoint inhibitors [21, 169,170].

Shifting activated CAFs towards quiescent CAFs and potentiating NK cell infiltration and functions could be additional interesting therapeutic approaches to be implemented. Also, NK cells genetically modified with chimeric antigen receptors (CAR) specific for tumor-associated antigens, promoting homing and trafficking to the tumor site, overcoming the immunosuppressive action of TGF-β in the TME (e.g., by deleting the TGF-β receptor), and finally metabolically optimizing NK cells could be combined to exploit NK cell antitumor response fully.

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Simona Carlomagno: Conceptualization, Investigation, Writing – original draft. **Chiara Setti:** Visualization, Writing – review & editing. **Fulvia Ortolani:** Investigation, Writing – review & editing. **Simona Sivori:** Conceptualization, Funding acquisition, Investigation, Writing – original draft.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

[1] F. Bray, M. Laversanne, H. Sung, J. Ferlay, R.L. Siegel, I. Soerjomataram, A. Jemal, Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. 74 (2024) 229–263, https://doi.org/10.3322/caac.21834.

[2] R.L. Siegel, A.N. Giaquinto, A. Jemal, Cancer statistics, 2024, CA Cancer J. Clin. 74 (2024) 12–49, https://doi.org/10.3322/caac.21820.

[3] O. Partyka, M. Pajewska, D. Kwaśniewska, A. Czerw, A. Deptała, M. Budzik, E. Cipora, I. Gąska, L. Gazdowicz, A. Mielnik, K. Sygit, M. Sygit, E. Krzych-Fałta, D. Schneider-Matyka, S. Grochans, A.M. Cybulska, J. Drobnik, E. Bandurska, W. Ciećko, P. Ratajczak, K. Kamecka, M. Marczak, R. Kozłowski, Overview of pancreatic cancer epidemiology in europe and recommendations for screening in high-risk populations, Cancers 15 (2023) 3634, https://doi.org/10.3390/cancers15143634.

[4] J.J. Swietlik, S. Bärthel, C. Falcomatà, D. Fink, A. Sinha, J. Cheng, S. Ebner, P. Landgraf, D.C. Dieterich, H. Daub, D. Saur, F. Meissner, Cell-selective proteomics segregates pancreatic cancer subtypes by extracellular proteins in tumors and circulation, Nat. Commun. 14 (2023) 2642, https://doi.org/10.1038/s41467-023-38171-8.

[5] M.H. Sherman, G.L. Beatty, Tumor microenvironment in pancreatic cancer pathogenesis and therapeutic resistance, Annu. Rev. Pathol. Mech. Dis. 18 (2023) 123–148, https://doi.org/10.1146/annurev-pathmechdis-031621-024600.

[6] N. Elebo, E.A. Abdel-Shafy, S. Cacciatore, E.E. Nweke, Exploiting the molecular subtypes and genetic landscape in pancreatic cancer: the quest to find effective drugs, Front. Genet. 14 (2023), https://doi.org/10.3389/fgene.2023.1170571.

[7] E.A. Collisson, A. Sadanandam, P. Olson, W.J. Gibb, M. Truitt, S. Gu, J. Cooc, J. Weinkle, G.E. Kim, L. Jakkula, H.S. Feiler, A.H. Ko, A.B. Olshen, K. L. Danenberg, M.A. Tempero, P.T. Spellman, D. Hanahan, J.W. Gray, Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy, Nat. Med. 17 (2011) 500–503, https://doi.org/10.1038/nm.2344.

[8] R.A. Moffitt, R. Marayati, E.L. Flate, K.E. Volmar, S.G.H. Loeza, K.A. Hoadley, N. U. Rashid, L.A. Williams, S.C. Eaton, A.H. Chung, J.K. Smyla, J.M. Anderson, H. J. Kim, D.J. Bentrem, M.S. Talamonti, C.A. Iacobuzio-Donahue, M. A. Hollingsworth, J.J. Yeh, Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma, Nat. Genet. 47 (2015) 1168–1178, https://doi.org/10.1038/ng.3398.

[9] Australian Pancreatic Cancer Genome Initiative, P. Bailey, D.K. Chang, K. Nones, A.L. Johns, A.M. Patch, M.C. Gingras, D.K. Miller, A.N. Christ, T.J.C. Bruxner, M. C. Quinn, C. Nourse, L.C. Murtaugh, I. Harliwong, S. Idrisoglu, S. Manning, E. Nourbakhsh, S. Wani, L. Fink, O. Holmes, V. Chin, M.J. Anderson, S. Kazakoff, C. Leonard, F. Newell, N. Waddell, S. Wood, Q. Xu, P.J. Wilson, N. Cloonan, K. S. Kassahn, D. Taylor, K. Quek, A. Robertson, L. Pantano, L. Mincarelli, L. N. Sanchez, L. Evers, J. Wu, M. Pinese, M.J. Cowley, M.D. Jones, E.K. Colvin, A. M. Nagrial, E.S. Humphrey, L.A. Chantrill, A. Mawson, J. Humphris, A. Chou, M. Pajic, C.J. Scarlett, A.V. Pinho, M. Giry-Laterriere, I. Rooman, J.S. Samra, J. G. Kench, J.A. Lovell, N.D. Merrett, C.W. Toon, K. Epari, N.Q. Nguyen, A. Barbour, N. Zeps, K. Moran-Jones, N.B. Jamieson, J.S. Graham, F. Duthie, K. Oien, J. Hair, R. Grützmann, A. Maitra, C.A. Iacobuzio-Donahue, C. L. Wolfgang, R.A. Morgan, R.T. Lawlor, V. Corbo, C. Bassi, B. Rusev, P. Capelli, R. Salvia, G. Tortora, D. Mukhopadhyay, G.M. Petersen, D.M. Munzy, W.E. Fisher, S.A. Karim, J.R. Eshleman, R.H. Hruban, C. Pilarsky, J.P. Morton, O.J. Sansom, A. Scarpa, E.A. Musgrove, U.M.H. Bailey, O. Hofmann, R.L. Sutherland, D. A. Wheeler, A.J. Gill, R.A. Gibbs, J.V. Pearson, N. Waddell, A.V. Biankin, S. M. Grimmond, Genomic analyses identify molecular subtypes of pancreatic cancer, Nature 531 (2016) 47–52, https://doi.org/10.1038/nature16965.

[10] S.N Kalimuthu, G.W. Wilson, R.C. Grant, M. Seto, G. O'Kane, R. Vajpeyi, F. Notta, S. Gallinger, R. Chetty, Morphological classification of pancreatic ductal adenocarcinoma that predicts molecular subtypes and correlates with clinical outcome, Gut 69 (2020) 317–328, https://doi.org/10.1136/gutjnl-2019-318217.

[11] A. Hayashi, J. Hong, C.A. Iacobuzio-Donahue, The pancreatic cancer genome revisited, Nat. Rev. Gastroenterol. Hepatol. 18 (2021) 469–481, https://doi.org/10.1038/s41575-021-00463-z.

[12] V. Ruta, C. Naro, M. Pieraccioli, A. Leccese, L. Archibugi, E. Cesari, V. Panzeri, C. Allgöwer, P.G. Arcidiacono, M. Falconi, C. Carbone, G. Tortora, F. Borrelli, F. Attili, C. Spada, G. Quero, S. Alfieri, C. Doglioni, A. Kleger, G. Capurso, C. Sette, An alternative splicing signature defines the basal-like phenotype and predicts worse clinical outcome in pancreatic cancer, Cell Rep. Med. 5 (2024) 101411, https://doi.org/10.1016/j.xcrm.2024.101411.

[13] J. Martinez-Useros, M. Martin-Galan, J. Garcia-Foncillas, The match between molecular subtypes, histology and microenvironment of pancreatic cancer and its relevance for chemoresistance, Cancers 13 (2021) 322, https://doi.org/10.3390/cancers13020322.

[14] Cancer Genome Atlas Research Network. Electronic address: andrew_aguirre@dfci.harvard.edu, Cancer genome atlas research network, integrated genomic characterization of pancreatic ductal adenocarcinoma, Cancer Cell 32 (2017), https://doi.org/10.1016/j.ccell.2017.07.007, 185-203.e13.

[15] Y. Ohara, W. Tang, H. Liu, S. Yang, T.H. Dorsey, H. Cawley, P. Moreno, R. Chari, M.R. Guest, A. Azizian, J. Gaedcke, M. Ghadimi, N. Hanna, S. Ambs, S.P. Hussain, SERPINB3-MYC axis induces the basal-like/squamous subtype and enhances disease progression in pancreatic cancer, Cell Rep. 42 (2023) 113434, https://doi.org/10.1016/j.celrep.2023.113434.

[16] B.T. Grünwald, A. Devisme, G. Andrieux, F. Vyas, K. Aliar, C.W. McCloskey, A. Macklin, G.H. Jang, R. Denroche, J.M. Romero, P. Bavi, P. Bronsert, F. Notta, G. O'Kane, J. Wilson, J. Knox, L. Tamblyn, M. Udaskin, N. Radulovich, S. E. Fischer, M. Boerries, S. Gallinger, T. Kislinger, R. Khokha, Spatially confined sub-tumor microenvironments in pancreatic cancer, Cell 184 (2021), https://doi.org/10.1016/j.cell.2021.09.022, 5577-5592.e18.

[17] B. George, O. Kudryashova, A. Kravets, S. Thalji, S. Malarkannan, R. Kurzrock, E. Chernyavskaya, M. Gusakova, D. Kravchenko, D. Tychinin, E. Savin, L. Alekseeva, A. Butusova, A. Bagaev, N. Shin, J.H. Brown, I. Sethi, D. Wang, B. Taylor, T. McFall, M. Kamgar, W.A. Hall, B. Erickson, K.K. Christians, D. B. Evans, S. Tsai, Transcriptomic-based microenvironment classification reveals precision medicine strategies for pancreatic ductal adenocarcinoma, Gastroenterology 166 (2024), https://doi.org/10.1053/j.gastro.2024.01.028, 859-871.e3.

[18] A.D. Rhim, P.E. Oberstein, D.H. Thomas, E.T. Mirek, C.F. Palermo, S.A. Sastra, E. N. Dekleva, T. Saunders, C.P. Becerra, I.W. Tattersall, C.B. Westphalen, J. Kitajewski, M.G. Fernandez-Barrena, M.E. Fernandez-Zapico, C. Iacobuzio-Donahue, K.P. Olive, B.Z. Stanger, Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma, Cancer Cell 25 (2014) 735–747, https://doi.org/10.1016/j.ccr.2014.04.021.

[19] P. Di Chiaro, L. Nacci, F. Arco, S. Brandini, S. Polletti, A. Palamidessi, B. Donati, C. Soriani, F. Gualdrini, G. Frigè, L. Mazzarella, A. Ciarrocchi, A. Zerbi, P. Spaggiari, G. Scita, S. Rodighiero, I. Barozzi, G.R. Diaferia, G. Natoli, Mapping functional to morphological variation reveals the basis of regional extracellular matrix subversion and nerve invasion in pancreatic cancer, Cancer Cell 42 (2024), https://doi.org/10.1016/j.ccell.2024.02.017, 662-681.e10.

[20] G.L. Beatty, S. Eghbali, R. Kim, Deploying immunotherapy in pancreatic cancer: defining mechanisms of response and resistance, Am. Soc. Clin. Oncol. Educ. Book (2017) 267–278, https://doi.org/10.1200/EDBK_175232.

[21] C.J. Halbrook, C.A. Lyssiotis, M. Pasca di Magliano, A. Maitra, Pancreatic cancer: advances and challenges, Cell 186 (2023) 1729–1754, https://doi.org/10.1016/j.cell.2023.02.014.

[22] C. Musiu, F. Lupo, A. Agostini, G. Lionetto, M. Bevere, S. Paiella, C. Carbone, V. Corbo, S. Ugel, F. De Sanctis, Cellular collusion: cracking the code of immunosuppression and chemo resistance in PDAC, Front. Immunol. 15 (2024) 1341079, https://doi.org/10.3389/fimmu.2024.1341079.

[23] J.R.M. Van Audenaerde, G. Roeyen, P.K. Darcy, M.H. Kershaw, M. Peeters, E.L. J. Smits, Natural killer cells and their therapeutic role in pancreatic cancer: a systematic review, Pharmacol. Ther. 189 (2018) 31–44, https://doi.org/10.1016/j.pharmthera.2018.04.003.

[24] S. Karakhanova, E. Ryschich, B. Mosl, S. Harig, D. Jäger, J. Schmidt, W. Hartwig, J. Werner, A.V. Bazhin, Prognostic and predictive value of immunological parameters for chemoradioimmunotherapy in patients with pancreatic adenocarcinoma, Br. J. Cancer 112 (2015) 1027–1036, https://doi.org/10.1038/bjc.2015.72.

[25] J. Persky, S.M. Cruz, M.A. Darrow, S.J. Judge, Y. Li, R.J. Bold, A.N. Karnezis, K. E. Matsukuma, L. Qi, R.J. Canter, Characterization of natural killer and cytotoxic T-cell immune infiltrates in pancreatic ductal adenocarcinoma, J. Surg. Oncol. 129 (2024) 885–892, https://doi.org/10.1002/jso.27581.

[26] S. Go, C. Demetriou, S. Hughes, S. Lanfredini, G. Valenzano, H. Ferry, E. Arbe-Barnes, S. Sivakumar, R. Bashford-Rogers, M.R. Middleton, S. Mukherjee, J. Morton, K. Jones, E. O'Neill, Tissue-resident NK cells support survival in pancreatic cancer through promotion of cDC1-CD8T activity, eLife (2024), https://doi.org/10.7554/eLife.92672.1.

[27] M.V. Plikus, X. Wang, S. Sinha, E. Forte, S.M. Thompson, E.L. Herzog, R. R. Driskell, N. Rosenthal, J. Biernaskie, V. Horsley, Fibroblasts: origins, definitions, and functions in health and disease, Cell 184 (2021) 3852–3872, https://doi.org/10.1016/j.cell.2021.06.024.

[28] T. Armstrong, G. Packham, L.B. Murphy, A.C. Bateman, J.A. Conti, D.R. Fine, C. D. Johnson, R.C. Benyon, J.P. Iredale, Type I collagen promotes the malignant phenotype of pancreatic ductal adenocarcinoma, Clin. Cancer Res. 10 (2004) 7427–7437, https://doi.org/10.1158/1078-0432.CCR-03-0825.

[29] Y. Chen, J. Kim, S. Yang, H. Wang, C.J. Wu, H. Sugimoto, V.S. LeBleu, R. Kalluri, Type I collagen deletion in αSMA+ myofibroblasts augments immune suppression

and accelerates progression of pancreatic cancer, Cancer Cell 39 (2021), https://doi.org/10.1016/j.ccell.2021.02.007, 548-565.e6.
[30] G. Obaid, M. Eroy, J. Zhao, S. Bano, M. Mino-Kenudson, T. Hasan, Immunofluorescence profiling of collagen subtypes is a predictor of treatment outcomes in pancreatic cancer, J.f Photochem. Photobiol. B Biol. 250 (2024) 112811, https://doi.org/10.1016/j.jphotobiol.2023.112811.
[31] E. Bonanno, M. Iurlaro, J.A. Madri, R.F. Nicosia, Type IV collagen modulates angiogenesis and neovessel survival in the rat aorta model, *In Vitro* Cell. Dev. Biol. Anim. 36 (2000) 336–340, https://doi.org/10.1290/1071-2690(2000)036<0336:TICMAA>2.0.CO;2.
[32] C. Tian, K.R. Clauser, D. Öhlund, S. Rickelt, Y. Huang, M. Gupta, D.R. Mani, S. A. Carr, D.A. Tuveson, R.O. Hynes, Proteomic analyses of ECM during pancreatic ductal adenocarcinoma progression reveal different contributions by tumor and stromal cells, Proc. Natl. Acad. Sci. 116 (2019) 19609–19618, https://doi.org/10.1073/pnas.1908626116.
[33] M. Papanicolaou, A.L. Parker, M. Yam, E.C. Filipe, S.Z. Wu, J.L. Chitty, K. Wyllie, E. Tran, E. Mok, A. Nadalini, J.N. Skhinas, M.C. Lucas, D. Herrmann, M. Nobis, B. A. Pereira, A.M.K. Law, L. Castillo, K.J. Murphy, A. Zaratzian, J.F. Hastings, D. R. Croucher, E. Lim, B.G. Oliver, F.V. Mora, B.L. Parker, D. Gallego-Ortega, A. Swarbrick, S. O'Toole, P. Timpson, T.R. Cox, Temporal profiling of the breast tumour microenvironment reveals collagen XII as a driver of metastasis, Nat. Commun. 13 (2022) 4587, https://doi.org/10.1038/s41467-022-32255-7.
[34] G. Biffi, D.A. Tuveson, Diversity and biology of cancer-associated fibroblasts, Physiol. Rev. 101 (2021) 147–176, https://doi.org/10.1152/physrev.00048.2019.
[35] M.H. Sherman, M.P. di Magliano, Cancer-associated fibroblasts: lessons from pancreatic cancer, Annu. Rev. Cancer Biol. 7 (2023) 43–55, https://doi.org/10.1146/annurev-cancerbio-061421-035400.
[36] Y. Miyazaki, T. Oda, N. Mori, Y.S. Kida, Adipose-derived mesenchymal stem cells differentiate into pancreatic cancer-associated fibroblasts *in vitro*, FEBS Open Bio 10 (2020) 2268–2281, https://doi.org/10.1002/2211-5463.12976.
[37] K. Hosaka, Y. Yang, T. Seki, C. Fischer, O. Dubey, E. Fredlund, J. Hartman, P. Religa, H. Morikawa, Y. Ishii, M. Sasahara, O. Larsson, G. Cossu, R. Cao, S. Lim, Y. Cao, Pericyte–fibroblast transition promotes tumor growth and metastasis, Proc. Natl. Acad. Sci. USA 113 (2016), https://doi.org/10.1073/pnas.1608384113.
[38] E.M. Zeisberg, S. Potenta, L. Xie, M. Zeisberg, R. Kalluri, Discovery of endothelial to mesenchymal transition as a source for carcinoma-associated fibroblasts, Cancer Res. 67 (2007) 10123–10128, https://doi.org/10.1158/0008-5472.CAN-07-3127.
[39] R.J. McAnulty, Fibroblasts and myofibroblasts: their source, function and role in disease, Int. J. Biochem. Cell Biol. 39 (2007) 666–671, https://doi.org/10.1016/j.biocel.2006.11.005.
[40] K. Ino, M. Masuya, I. Tawara, E. Miyata, K. Oda, Y. Nakamori, K. Suzuki, K. Ohishi, N. Katayama, Monocytes infiltrate the pancreas via the MCP-1/CCR2 pathway and differentiate into stellate cells, PLoS ONE 9 (2014) e84889, https://doi.org/10.1371/journal.pone.0084889.
[41] O.W. Petersen, H.L. Nielsen, T. Gudjonsson, R. Villadsen, F. Rank, E. Niebuhr, M. J. Bissell, L. Rønnov-Jessen, Epithelial to mesenchymal transition in human breast cancer can provide a nonmalignant stroma, Am. J. Pathol. 162 (2003) 391–402, https://doi.org/10.1016/S0002-9440(10)63834-5.
[42] N. Nair, A.S. Calle, M.H. Zahra, M. Prieto-Vila, A.K.K. Oo, L. Hurley, A. Vaidyanath, A. Seno, J. Masuda, Y. Iwasaki, H. Tanaka, T. Kasai, M. Seno, A cancer stem cell model as the point of origin of cancer-associated fibroblasts in tumor microenvironment, Sci. Rep. 7 (2017) 6838, https://doi.org/10.1038/s41598-017-07144-5.
[43] A. Vonlaufen, S. Joshi, C. Qu, P.A. Phillips, Z. Xu, N.R. Parker, C.S. Toi, R. C. Pirola, J.S. Wilson, D. Goldstein, M.V. Apte, Pancreatic stellate cells: partners in crime with pancreatic cancer cells, Cancer Res. 68 (2008) 2085–2093, https://doi.org/10.1158/0008-5472.CAN-07-2477.
[44] G. Ishii, T. Sangai, T. Oda, Y. Aoyagi, T. Hasebe, N. Kanomata, Y. Endoh, C. Okumura, Y. Okuhara, J. Magae, M. Emura, T. Ochiya, A. Ochiai, Bone-marrow-derived myofibroblasts contribute to the cancer-induced stromal reaction, Biochem. Biophys. Res. Commun. 309 (2003) 232–240, https://doi.org/10.1016/S0006-291X(03)01544-4.
[45] P. Manoukian, M. Bijlsma, H. Van Laarhoven, The cellular origins of cancer-associated fibroblasts and their opposing contributions to pancreatic cancer growth, Front. Cell Dev. Biol. 9 (2021) 743907, https://doi.org/10.3389/fcell.2021.743907.
[46] R. Saúde-Conde, A. Arçay Öztürk, K. Stosic, O. Azurmendi Senar, J. Navez, C. Bouchart, T. Arsenijevic, P. Flamen, J.L. Van Laethem, Cancer-associated fibroblasts in pancreatic ductal adenocarcinoma or a metaphor for heterogeneity: from single-cell analysis to whole-body imaging, Biomedicines 12 (2024) 591, https://doi.org/10.3390/biomedicines12030591.
[47] P. Sarantis, E. Koustas, A. Papadimitropoulou, A.G. Papavassiliou, M. V. Karamouzis, Pancreatic ductal adenocarcinoma: treatment hurdles, tumor microenvironment and immunotherapy, WJGO 12 (2020) 173–181, https://doi.org/10.4251/wjgo.v12.i2.173.
[48] V. Longo, O. Brunetti, A. Gnoni, S. Cascinu, G. Gasparini, V. Lorusso, D. Ribatti, N. Silvestris, Angiogenesis in pancreatic ductal adenocarcinoma: a controversial issue, Oncotarget 7 (2016) 58649–58658, https://doi.org/10.18632/oncotarget.10765.
[49] Z. Chen, F. Han, Y. Du, H. Shi, W. Zhou, Hypoxic microenvironment in cancer: molecular mechanisms and therapeutic interventions, Signal Transduct. Target. Ther. 8 (2023) 70, https://doi.org/10.1038/s41392-023-01332-8.
[50] J.J. Kamphorst, M. Nofal, C. Commisso, S.R. Hackett, W. Lu, E. Grabocka, M. G. Vander Heiden, G. Miller, J.A. Drebin, D. Bar-Sagi, C.B. Thompson, J. D. Rabinowitz, Human pancreatic cancer tumors are nutrient poor and tumor cells actively scavenge extracellular protein, Cancer Res. 75 (2015) 544–553, https://doi.org/10.1158/0008-5472.CAN-14-2211.
[51] X. Mao, J. Xu, W. Wang, C. Liang, J. Hua, J. Liu, B. Zhang, Q. Meng, X. Yu, S. Shi, Crosstalk between cancer-associated fibroblasts and immune cells in the tumor microenvironment: new findings and future perspectives, Mol. Cancer 20 (2021) 131, https://doi.org/10.1186/s12943-021-01428-1.
[52] D. Unterleuthner, P. Neuhold, K. Schwarz, L. Janker, B. Neuditschko, H. Nivarthi, I. Crncec, N. Kramer, C. Unger, M. Hengstschläger, R. Eferl, R. Moriggl, W. Sommergruber, C. Gerner, H. Dolznig, Cancer-associated fibroblast-derived WNT2 increases tumor angiogenesis in colon cancer, Angiogenesis 23 (2020) 159–177, https://doi.org/10.1007/s10456-019-09688-8.
[53] R. Kalluri, The biology and function of fibroblasts in cancer, Nat. Rev. Cancer 16 (2016) 582–598, https://doi.org/10.1038/nrc.2016.73.
[54] S. Su, J. Chen, H. Yao, J. Liu, S. Yu, L. Lao, M. Wang, M. Luo, Y. Xing, F. Chen, D. Huang, J. Zhao, L. Yang, D. Liao, F. Su, M. Li, Q. Liu, E. Song, CD10+GPR77+ cancer-associated fibroblasts promote cancer formation and chemoresistance by sustaining cancer stemness, Cell 172 (2018), https://doi.org/10.1016/j.cell.2018.01.009, 841-856.e16.
[55] K. Louault, R.R. Li, Y.A. DeClerck, Cancer-associated fibroblasts: understanding their heterogeneity, Cancers 12 (2020) 3108, https://doi.org/10.3390/cancers12113108.
[56] A. Morgan, M. Griffin, L. Kameni, D.C. Wan, M.T. Longaker, J.A. Norton, Medical biology of cancer-associated fibroblasts in pancreatic cancer, Biology 12 (2023) 1044, https://doi.org/10.3390/biology12081044.
[57] D. Yang, J. Liu, H. Qian, Q. Zhuang, Cancer-associated fibroblasts: from basic science to anticancer therapy, Exp. Mol. Med. 55 (2023) 1322–1332, https://doi.org/10.1038/s12276-023-01013-0.
[58] A. Stylianou, V. Gkretsi, T. Stylianopoulos, Transforming growth factor-β modulates pancreatic cancer associated fibroblasts cell shape, stiffness and invasion, Biochim. Biophys. Acta (BBA) Gen. Subj. 1862 (2018) 1537–1546, https://doi.org/10.1016/j.bbagen.2018.02.009.
[59] Q. Qin, R. Yu, J.E. Eriksson, H. Tsai, H. Zhu, Cancer-associated fibroblasts in pancreatic ductal adenocarcinoma therapy: challenges and opportunities, Cancer Lett. 591 (2024) 216859, https://doi.org/10.1016/j.canlet.2024.216859.
[60] L. Juillerat-Jeanneret, P. Tafelmeyer, D. Golshayan, Fibroblast activation protein-α in fibrogenic disorders and cancer: more than a prolyl-specific peptidase? Expert Opin. Ther. Targets 21 (2017) 977–991, https://doi.org/10.1080/14728222.2017.1370455.
[61] M. Huang, M. Fu, J. Wang, C. Xia, H. Zhang, Y. Xiong, J. He, J. Liu, B. Liu, S. Pan, F. Liu, TGF-β1-activated cancer-associated fibroblasts promote breast cancer invasion, metastasis and epithelial-mesenchymal transition by autophagy or overexpression of FAP-α, Biochem. Pharmacol. 188 (2021) 114527 https://doi.org/10.1016/j.bcp.2021.114527.
[62] M. Shi, Expression of fibroblast activation protein in human pancreatic adenocarcinoma and its clinicopathological significance, WJG 18 (2012) 840, https://doi.org/10.3748/wjg.v18.i8.840.
[63] M. Kraman, P.J. Bambrough, J.N. Arnold, E.W. Roberts, L. Magiera, J.O. Jones, A. Gopinathan, D.A. Tuveson, D.T. Fearon, Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein–α, Science 330 (2010) 827–830, https://doi.org/10.1126/science.1195300.
[64] D. Öhlund, A. Handly-Santana, G. Biffi, E. Elyada, A.S. Almeida, M. Ponz-Sarvise, V. Corbo, T.E. Oni, S.A. Hearn, E.J. Lee, I.I.C. Chio, C.I. Hwang, H. Tiriac, L. A. Baker, D.D. Engle, C. Feig, A. Kultti, M. Egeblad, D.T. Fearon, J.M. Crawford, H. Clevers, Y. Park, D.A. Tuveson, Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer, J. Exp. Med. 214 (2017) 579–596, https://doi.org/10.1084/jem.20162024.
[65] T. Zhang, Y. Ren, P. Yang, J. Wang, H. Zhou, Cancer-associated fibroblasts in pancreatic ductal adenocarcinoma, Cell Death Dis. 13 (2022) 897, https://doi.org/10.1038/s41419-022-05351-1.
[66] G. Biffi, T.E. Oni, B. Spielman, Y. Hao, E. Elyada, Y. Park, J. Preall, D.A. Tuveson, IL1-induced JAK/STAT signaling is antagonized by TGFβ to shape CAF heterogeneity in pancreatic ductal adenocarcinoma, Cancer Discov. 9 (2019) 282–301, https://doi.org/10.1158/2159-8290.CD-18-0710.
[67] A. Brichkina, P. Polo, S.D. Sharma, N. Visestamkul, M. Lauth, A quick guide to CAF subtypes in pancreatic cancer, Cancers 15 (2023) 2614, https://doi.org/10.3390/cancers15092614.
[68] H. Huang, R.A. Brekken, Recent advances in understanding cancer-associated fibroblasts in pancreatic cancer, Am. J. Physiol. Cell Physiol. 319 (2020) C233–C243, https://doi.org/10.1152/ajpcell.00079.2020.
[69] B. Adem, N. Bastos, C.F. Ruivo, S. Sousa-Alves, C. Dias, P.F. Vieira, I.A. Batista, B. Cavadas, D. Saur, J.C. Machado, D. Cai, S.A. Melo, Exosomes define a local and systemic communication network in healthy pancreas and pancreatic ductal adenocarcinoma, Nat. Commun. 15 (2024) 1496, https://doi.org/10.1038/s41467-024-45753-7.
[70] S.K. Hsu, M. Jadhao, W.T. Liao, W.T. Chang, I.L. Lin, C.C. Chiu, The role of exosomes in pancreatic ductal adenocarcinoma progression and their potential as biomarkers, Cancers 15 (2023) 1776, https://doi.org/10.3390/cancers15061776.
[71] B.C. Özdemir, T. Pentcheva-Hoang, J.L. Carstens, X. Zheng, C.C. Wu, T. R. Simpson, H. Laklai, H. Sugimoto, C. Kahlert, S.V. Novitskiy, A. De Jesus-Acosta, P. Sharma, P. Heidari, U. Mahmood, L. Chin, H.L. Moses, V.M. Weaver, A. Maitra, J.P. Allison, V.S. LeBleu, R. Kalluri, Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas

cancer with reduced survival, Cancer Cell 28 (2015) 831–833, https://doi.org/10.1016/j.ccell.2015.11.002.
[72] E. Elyada, M. Bolisetty, P. Laise, W.F. Flynn, E.T. Courtois, R.A. Burkhart, J. A. Teinor, P. Belleau, G. Biffi, M.S. Lucito, S. Sivajothi, T.D. Armstrong, D. D. Engle, K.H. Yu, Y. Hao, C.L. Wolfgang, Y. Park, J. Preall, E.M. Jaffee, A. Califano, P. Robson, D.A. Tuveson, Cross-species single-cell analysis of pancreatic ductal adenocarcinoma reveals antigen-presenting cancer-associated fibroblasts, Cancer Discov. 9 (2019) 1102–1123, https://doi.org/10.1158/2159-8290.CD-19-0094.
[73] H. Huang, Z. Wang, Y. Zhang, R.N. Pradhan, D. Ganguly, R. Chandra, G. Murimwa, S. Wright, X. Gu, R. Maddipati, S. Müller, S.J. Turley, R.A. Brekken, Mesothelial cell-derived antigen-presenting cancer-associated fibroblasts induce expansion of regulatory T cells in pancreatic cancer, Cancer Cell 40 (2022), https://doi.org/10.1016/j.ccell.2022.04.011, 656-673.e7.
[74] K. Chen, Q. Wang, M. Li, H. Guo, W. Liu, F. Wang, X. Tian, Y. Yang, Single-cell RNA-seq reveals dynamic change in tumor microenvironment during pancreatic ductal adenocarcinoma malignant progression, EBioMedicine 66 (2021) 103315, https://doi.org/10.1016/j.ebiom.2021.103315.
[75] C. Hutton, F. Heider, A. Blanco-Gomez, A. Banyard, A. Kononov, X. Zhang, S. Karim, V. Paulus-Hock, D. Watt, N. Steele, S. Kemp, E.K.J. Hogg, J. Kelly, R. F. Jackstadt, F. Lopes, M. Menotti, L. Chisholm, A. Lamarca, J. Valle, O.J. Sansom, C. Springer, A. Malliri, R. Marais, M. Pasca Di Magliano, S. Zelenay, J.P. Morton, C. Jørgensen, Single-cell analysis defines a pancreatic fibroblast lineage that supports anti-tumor immunity, Cancer Cell 39 (2021), https://doi.org/10.1016/j.ccell.2021.06.017, 1227-1244.e20.
[76] M. Vitale, C. Cantoni, M. Della Chiesa, G. Ferlazzo, S. Carlomagno, D. Pende, M. Falco, A. Pessino, L. Muccio, A. De Maria, E. Marcenaro, L. Moretta, S. Sivori, An historical overview: the discovery of how nK cells can kill enemies, recruit defense troops, and more, Front. Immunol. 10 (2019) 1415, https://doi.org/10.3389/fimmu.2019.01415.
[77] E. Vivier, D.H. Raulet, A. Moretta, M.A. Caligiuri, L. Zitvogel, L.L. Lanier, W. M. Yokoyama, S. Ugolini, Innate or adaptive immunity? The example of natural killer cells, Science 331 (2011) 44–49, https://doi.org/10.1126/science.1198687.
[78] L. Quatrini, M. Della Chiesa, S. Sivori, M.C. Mingari, D. Pende, L. Moretta, Human NK cells, their receptors and function, Eur. J. Immunol. 51 (2021) 1566–1579, https://doi.org/10.1002/eji.202049028.
[79] N. Anfossi, P. André, S. Guia, C.S. Falk, S. Roetynck, C.A. Stewart, V. Breso, C. Frassati, D. Reviron, D. Middleton, F. Romagné, S. Ugolini, E. Vivier, Human NK cell education by inhibitory receptors for MHC Class I, Immunity. 25 (2006) 331–342, https://doi.org/10.1016/j.immuni.2006.06.013.
[80] M.T. Orr, L.L. Lanier, Natural killer cell education and tolerance, Cell 142 (2010) 847–856, https://doi.org/10.1016/j.cell.2010.08.031.
[81] J.E. Boudreau, K.C. Hsu, Natural killer cell education in human health and disease, Curr. Opin. Immunol. 50 (2018) 102–111, https://doi.org/10.1016/j.coi.2017.11.003.
[82] M.A. Cooper, T.A. Fehniger, M.A. Caligiuri, The biology of human natural killer-cell subsets, Trends Immunol. 22 (2001) 633–640, https://doi.org/10.1016/S1471-4906(01)02060-9.
[83] M.A. Caligiuri, Human natural killer cells, Blood 112 (2008) 461–469, https://doi.org/10.1182/blood-2007-09-077438.
[84] A.G. Freud, B.L. Mundy-Bosse, J. Yu, M.A. Caligiuri, The broad spectrum of human natural killer cell diversity, Immunity 47 (2017) 820–833, https://doi.org/10.1016/j.immuni.2017.10.008.
[85] N.K. Björkström, H.G. Ljunggren, J. Michaëlsson, Emerging insights into natural killer cells in human peripheral tissues, Nat. Rev. Immunol. 16 (2016) 310–320, https://doi.org/10.1038/nri.2016.34.
[86] H. Chen, Y. Qin, M. Chou, J.G. Cyster, X. Li, Transmembrane protein CD69 acts as an S1PR1 agonist, eLife 12 (2023) e88204, https://doi.org/10.7554/eLife.88204.
[87] P. Dogra, C. Rancan, W. Ma, M. Toth, T. Senda, D.J. Carpenter, M. Kubota, R. Matsumoto, P. Thapa, P.A. Szabo, M.M. Li Poon, J. Li, J. Arakawa-Hoyt, Y. Shen, L. Fong, L.L. Lanier, D.L. Farber, Tissue determinants of human NK cell development, function, and residence, Cell 180 (2020), https://doi.org/10.1016/j.cell.2020.01.022, 749-763.e13.
[88] G. Bernardini, F. Antonangeli, V. Bonanni, A. Santoni, Dysregulation of chemokine/chemokine receptor axes and NK cell tissue localization during diseases, Front. Immunol. 7 (2016), https://doi.org/10.3389/fimmu.2016.00402.
[89] M. Lima, M. Leander, M. Santos, A.H. Santos, C. Lau, M.L. Queirós, M. Gonçalves, S. Fonseca, J. Moura, M. dos A. Teixeira, A. Orfao, Chemokine receptor expression on normal blood CD56^{+} NK-cells elucidates cell partners that comigrate during the innate and adaptive immune responses and identifies a transitional NK-cell population, J. Immunol. Res. (2015) e839684, https://doi.org/10.1155/2015/839684, 2015.
[90] G.H. Ran, Y.Q. Lin, L. Tian, T. Zhang, D.M. Yan, J.H. Yu, Y.C. Deng, Natural killer cell homing and trafficking in tissues and tumors: from biology to application, Signal Transduct. Target. Ther. 7 (2022) 205, https://doi.org/10.1038/s41392-022-01058-z.
[91] R. Castriconi, P. Carrega, A. Dondero, F. Bellora, B. Casu, S. Regis, G. Ferlazzo, C. Bottino, Molecular mechanisms directing migration and retention of natural killer cells in human tissues, Front. Immunol. 9 (2018) 2324, https://doi.org/10.3389/fimmu.2018.02324.
[92] S. Sivori, P. Vacca, G. Del Zotto, E. Munari, M.C. Mingari, L. Moretta, Human NK cells: surface receptors, inhibitory checkpoints, and translational applications, Cell. Mol. Immunol. 16 (2019) 430–441, https://doi.org/10.1038/s41423-019-0206-4.
[93] D. Pende, M. Falco, M. Vitale, C. Cantoni, C. Vitale, E. Munari, A. Bertaina, F. Moretta, G. Del Zotto, G. Pietra, M.C. Mingari, F. Locatelli, L. Moretta, Killer Ig-like receptors (KIRs): their role in NK cell modulation and developments leading to their clinical exploitation, Front. Immunol. 10 (2019) 1179, https://doi.org/10.3389/fimmu.2019.01179.
[94] S. Lopez-Vergès, J.M. Milush, S. Pandey, V.A. York, J. Arakawa-Hoyt, H. Pircher, P.J. Norris, D.F. Nixon, L.L. Lanier, CD57 defines a functionally distinct population of mature NK cells in the human CD56dimCD16+ NK-cell subset, Blood 116 (2010) 3865–3874, https://doi.org/10.1182/blood-2010-04-282301.
[95] N.K. Björkström, P. Riese, F. Heuts, S. Andersson, C. Fauriat, M.A. Ivarsson, A. T. Björklund, M. Flodström-Tullberg, J. Michaëlsson, M.E. Rottenberg, C. A. Guzmán, H.G. Ljunggren, K.J. Malmberg, Expression patterns of NKG2A, KIR, and CD57 define a process of CD56dim NK-cell differentiation uncoupled from NK-cell education, Blood 116 (2010) 3853–3864, https://doi.org/10.1182/blood-2010-04-281675.
[96] I. Anegón, M.C. Cuturi, G. Trinchieri, B. Perussia, Interaction of Fc receptor (CD16) ligands induces transcription of interleukin 2 receptor (CD25) and lymphokine genes and expression of their products in human natural killer cells, J. Exp. Med. 167 (1988) 452–472, https://doi.org/10.1084/jem.167.2.452.
[97] S. Sivori, R. Meazza, C. Quintarelli, S. Carlomagno, M.Della Chiesa, M. Falco, L. Moretta, F. Locatelli, D. Pende, NK cell-based immunotherapy for hematological malignancies, JCM 8 (2019) 1702, https://doi.org/10.3390/jcm8101702.
[98] E. Batlle, J. Massagué, Transforming growth factor-β signaling in immunity and cancer, Immunity 50 (2019) 924–940, https://doi.org/10.1016/j.immuni.2019.03.024.
[99] R. Castriconi, C. Cantoni, M.Della Chiesa, M. Vitale, E. Marcenaro, R. Conte, R. Biassoni, C. Bottino, L. Moretta, A. Moretta, Transforming growth factor β1 inhibits expression of NKp30 and NKG2D receptors: consequences for the NK-mediated killing of dendritic cells, Proc. Natl. Acad. Sci. USA 100 (2003) 4120–4125, https://doi.org/10.1073/pnas.0730640100.
[100] J. Dardare, A. Witz, J.L. Merlin, P. Gilson, A. Harlé, SMAD4 and the TGFβ pathway in patients with pancreatic ductal adenocarcinoma, IJMS 21 (2020) 3534, https://doi.org/10.3390/ijms21103534.
[101] Q. Zhou, S. Xia, F. Guo, F. Hu, Z. Wang, Y. Ni, T. Wei, H. Xiang, D. Shang, Transforming growth factor-β in pancreatic diseases: mechanisms and therapeutic potential, Pharmacol. Res. 142 (2019) 58–69, https://doi.org/10.1016/j.phrs.2019.01.038.
[102] Z. Wang, D. Guan, J. Huo, S.K. Biswas, Y. Huang, Y. Yang, S. Xu, K.P. Lam, IL-10 enhances human natural killer cell effector functions via metabolic reprogramming regulated by mTORC1 signaling, Front. Immunol. 12 (2021) 619195, https://doi.org/10.3389/fimmu.2021.619195.
[103] G.M. Konjević, A.M. Vuletić, K.M. Mirjačić Martinović, A.K. Larsen, V.B. Jurišić, The role of cytokines in the regulation of NK cells in the tumor environment, Cytokine 117 (2019) 30–40, https://doi.org/10.1016/j.cyto.2019.02.001.
[104] S. Mocellin, The dual role of IL-10, Trends Immunol. 24 (2003) 36–43, https://doi.org/10.1016/S1471-4906(02)00009-1.
[105] J.A. Gubbels, M. Felder, S. Horibata, J.A. Belisle, A. Kapur, H. Holden, S. Petrie, M. Migneault, C. Rancourt, J.P. Connor, M.S. Patankar, MUC16 provides immune protection by inhibiting synapse formation between NK and ovarian tumor cells, Mol. Cancer 9 (2010) 11, https://doi.org/10.1186/1476-4598-9-11.
[106] M.D. Chiesa, S. Carlomagno, G. Frumento, M. Balsamo, C. Cantoni, R. Conte, L. Moretta, A. Moretta, M. Vitale, The tryptophan catabolite l-kynurenine inhibits the surface expression of NKp46- and NKG2D-activating receptors and regulates NK-cell function, Blood 108 (2006) 4118–4125, https://doi.org/10.1182/blood-2006-03-006700.
[107] A.M. Chambers, J. Wang, K.B. Lupo, H. Yu, N.M. Atallah Lanman, S. Matosevic, Adenosinergic signaling alters natural killer cell functional responses, Front. Immunol. 9 (2018) 2533, https://doi.org/10.3389/fimmu.2018.02533.
[108] H. Zhu, J. Xu, W. Wang, B. Zhang, J. Liu, C. Liang, J. Hua, Q. Meng, X. Yu, S. Shi, Intratumoral CD38+CD19+B cells associate with poor clinical outcomes and immunosuppression in patients with pancreatic ductal adenocarcinoma, EBioMedicine 103 (2024) 105098, https://doi.org/10.1016/j.ebiom.2024.105098.
[109] V. Suresh, P. Dash, S. Suklabaidya, K.C. Murmu, P.K. Sasmal, G.M. Jogdand, D. Parida, M. Sethi, B. Das, D. Mohapatra, S. Saha, P. Prasad, A. Satoskar, S. Senapati, MIF confers survival advantage to pancreatic CAFs by suppressing interferon pathway-induced p53-dependent apoptosis, FASEB J. 36 (2022) e22449, https://doi.org/10.1096/fj.202101953R.
[110] A. Shimizu, S. Hirono, M. Tani, M. Kawai, K.I. Okada, M. Miyazawa, Y. Kitahata, Y. Nakamura, T. Noda, S. Yokoyama, H. Yamaue, Coexpression of MUC16 and mesothelin is related to the invasion process in pancreatic ductal adenocarcinoma, Cancer Sci. 103 (2012) 739–746, https://doi.org/10.1111/j.1349-7006.2012.02214.x.
[111] X. Chen, I.K. Sandrine, M. Yang, J. Tu, X. Yuan, MUC1 and MUC16: critical for immune modulation in cancer therapeutics, Front. Immunol. 15 (2024), https://doi.org/10.3389/fimmu.2024.1356913.
[112] R. Chirravuri-Venkata, V. Dam, R.K. Nimmakayala, Z.W. Alsafwani, N. Bhyravbhatla, I. Lakshmanan, M.P. Ponnusamy, S. Kumar, M. Jain, D. Ghersi, S.K. Batra, MUC16 and TP53 family co-regulate tumor-stromal heterogeneity in pancreatic adenocarcinoma, Front. Oncol. 13 (2023) 1073820, https://doi.org/10.3389/fonc.2023.1073820.
[113] J.A. Belisle, S. Horibata, G.A. Jennifer, S. Petrie, A. Kapur, S. André, H.J. Gabius, C. Rancourt, J. Connor, J.C. Paulson, M.S. Patankar, Identification of Siglec-9 as the receptor for MUC16 on human NK cells, B cells, and monocytes, Mol. Cancer 9 (2010) 118, https://doi.org/10.1186/1476-4598-9-118.

[114] H. Jonescheit, H.H. Oberg, D. Gonnermann, M. Hermes, V. Sulaj, C. Peters, D. Kabelitz, D. Wesch, Influence of indoleamine-2,3-dioxygenase and its metabolite kynurenine on γδ T cell cytotoxicity against ductal pancreatic adenocarcinoma cells, Cells 9 (2020) 1140, https://doi.org/10.3390/cells9051140.
[115] M. Balsamo, F. Scordamaglia, G. Pietra, C. Manzini, C. Cantoni, M. Boitano, P. Queirolo, W. Vermi, F. Facchetti, A. Moretta, L. Moretta, M.C. Mingari, M. Vitale, Melanoma-associated fibroblasts modulate NK cell phenotype and antitumor cytotoxicity, Proc. Natl. Acad. Sci. 106 (2009) 20847–20852, https://doi.org/10.1073/pnas.0906481106.
[116] T. Li, Y. Yang, X. Hua, G. Wang, W. Liu, C. Jia, Y. Tai, Q. Zhang, G. Chen, Hepatocellular carcinoma-associated fibroblasts trigger NK cell dysfunction via PGE2 and IDO, Cancer Lett. 318 (2012) 154–161, https://doi.org/10.1016/j.canlet.2011.12.020.
[117] T. Li, S. Yi, W. Liu, C. Jia, G. Wang, X. Hua, Y. Tai, Q. Zhang, G. Chen, Colorectal carcinoma-derived fibroblasts modulate natural killer cell phenotype and antitumor cytotoxicity, Med. Oncol. 30 (2013) 663, https://doi.org/10.1007/s12032-013-0663-z.
[118] B. Allard, D. Allard, L. Buisseret, J. Stagg, The adenosine pathway in immuno-oncology, Nat. Rev. Clin. Oncol. 17 (2020) 611–629, https://doi.org/10.1038/s41571-020-0382-2.
[119] A. Ohta, M. Sitkovsky, Extracellular adenosine-mediated modulation of regulatory T cells, Front. Immunol. 5 (2014), https://doi.org/10.3389/fimmu.2014.00304.
[120] J. Reinhardt, J. Landsberg, J.L. Schmid-Burgk, B.B. Ramis, T. Bald, N. Glodde, D. Lopez-Ramos, A. Young, S.F. Ngiow, D. Nettersheim, H. Schorle, T. Quast, W. Kolanus, D. Schadendorf, G.V. Long, J. Madore, R.A. Scolyer, A. Ribas, M. J. Smyth, P.C. Tumeh, T. Tüting, M. Hölzel, MAPK signaling and inflammation link melanoma phenotype switching to induction of CD73 during immunotherapy, Cancer Res. 77 (2017) 4697–4709, https://doi.org/10.1158/0008-5472.CAN-17-0395.
[121] B.G. Leclerc, R. Charlebois, G. Chouinard, B. Allard, S. Pommey, F. Saad, J. Stagg, CD73 expression is an independent prognostic factor in prostate cancer, Clin. Cancer Res. 22 (2016) 158–166, https://doi.org/10.1158/1078-0432.CCR-15-1181.
[122] M. Yu, G. Guo, L. Huang, L. Deng, C.S. Chang, B.R. Achyut, M. Canning, N. Xu, A. S. Arbab, R.J. Bollag, P.C. Rodriguez, A.L. Mellor, H. Shi, D.H. Munn, Y. Cui, CD73 on cancer-associated fibroblasts enhanced by the A2B-mediated feedforward circuit enforces an immune checkpoint, Nat. Commun. 11 (2020) 515, https://doi.org/10.1038/s41467-019-14060-x.
[123] K. Tahkola, M. Ahtiainen, I. Kellokumpu, J.P. Mecklin, J. Laukkarinen, J. Laakkonen, I. Kenessey, S. Jalkanen, M. Salmi, J. Böhm, Prognostic impact of CD73 expression and its relationship to PD-L1 in patients with radically treated pancreatic cancer, Virchows Arch. 478 (2021), https://doi.org/10.1007/s00428-020-02888-4.
[124] R. Samain, O. Maiques, J. Monger, H. Lam, J. Candido, S. George, N. Ferrari, L. KohIhammer, S. Lunetto, A. Varela, J.L. Orgaz, F. Vilardell, J.J. Olsina, X. Matias-Guiu, D. Sarker, A. Biddle, F.R. Balkwill, J. Eyles, R.W. Wilkinson, H. M. Kocher, F. Calvo, C.M. Wells, V. Sanz-Moreno, CD73 controls Myosin II–driven invasion, metastasis, and immunosuppression in amoeboid pancreatic cancer cells, Sci. Adv. 9 (2023) eadi0244, https://doi.org/10.1126/sciadv.adi0244.
[125] J. Zhao, L.M.S. Soto, H. Wang, M.H. Katz, L.R. Prakash, M. Kim, C.W.D. Tzeng, J. E. Lee, R.A. Wolff, Y. Huang, I.I. Wistuba, A. Maitra, H. Wang, Overexpression of CD73 in pancreatic ductal adenocarcinoma is associated with immunosuppressive tumor microenvironment and poor survival, Pancreatology 21 (2021) 942–949, https://doi.org/10.1016/j.pan.2021.03.018.
[126] M. Hasmim, Y. Messai, L. Ziani, J. Thiery, J.H. Bouhris, M.Z. Noman, S. Chouaib, Critical role of tumor microenvironment in shaping NK cell functions: implication of hypoxic stress, Front. Immunol. 6 (2015), https://doi.org/10.3389/fimmu.2015.00482.
[127] S. Borde, S. Matosevic, Metabolic adaptation of NK cell activity and behavior in tumors: challenges and therapeutic opportunities, Trends Pharmacol. Sci. 44 (2023) 832–848, https://doi.org/10.1016/j.tips.2023.08.009.
[128] A. Brand, K. Singer, G.E. Koehl, M. Kolitzus, G. Schoenhammer, A. Thiel, C. Matos, C. Bruss, S. Klobuch, K. Peter, M. Kastenberger, C. Bogdan, U. Schleicher, A. Mackensen, E. Ullrich, S. Fichtner-Feigl, R. Kesselring, M. Mack, U. Ritter, M. Schmid, C. Blank, K. Dettmer, P.J. Oefner, P. Hoffmann, S. Walenta, E.K. Geissler, J. Pouyssegur, A. Villunger, A. Steven, B. Seliger, S. Schreml, S. Haferkamp, E. Kohl, S. Karrer, M. Berneburg, W. Herr, W. Mueller-Klieser, K. Renner, M. Kreutz, LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK cells, Cell Metab. 24 (2016) 657–671, https://doi.org/10.1016/j.cmet.2016.08.011.
[129] Y. Zhu, X. Li, L. Wang, X. Hong, J. Yang, Metabolic reprogramming and crosstalk of cancer-related fibroblasts and immune cells in the tumor microenvironment, Front. Endocrinol. 13 (2022), https://doi.org/10.3389/fendo.2022.988295.
[130] Z.L. Ou, Z. Luo, W. Wei, S. Liang, T.L. Gao, Y.B. Lu, Hypoxia-induced shedding of MICA and HIF1A-mediated immune escape of pancreatic cancer cells from NK cells: role of circ_0000977/miR-153 axis, RNA Biol. 16 (2019) 1592–1603, https://doi.org/10.1080/15476286.2019.1649585.
[131] A. Märten, M. von Lilienfeld-Toal, M.W. Büchler, J. Schmidt, Soluble MIC is elevated in the serum of patients with pancreatic carcinoma diminishing γδ T cell cytotoxicity, Int. J. Cancer 119 (2006) 2359–2365, https://doi.org/10.1002/ijc.22186.
[132] R. Romee, B. Foley, T. Lenvik, Y. Wang, B. Zhang, D. Ankarlo, X. Luo, S. Cooley, M. Verneris, B. Walcheck, J. Miller, NK cell CD16 surface expression and function is regulated by a disintegrin and metalloprotease-17 (ADAM17), Blood 121 (2013) 3599–3608, https://doi.org/10.1182/blood-2012-04-425397.
[133] L. Gorchs, C. Fernández Moro, P. Bankhead, K.P. Kern, I. Sadeak, Q. Meng, E. Rangelova, H. Kaipe, Human pancreatic carcinoma-associated fibroblasts promote expression of co-inhibitory markers on CD4+ and CD8+ T-cells, Front. Immunol. 10 (2019), https://doi.org/10.3389/fimmu.2019.00847.
[134] D. Gao, L. Fang, C. Liu, M. Yang, X. Yu, L. Wang, W. Zhang, C. Sun, J. Zhuang, Microenvironmental regulation in tumor progression: interactions between cancer-associated fibroblasts and immune cells, Biomed. Pharmacother. 167 (2023) 115622, https://doi.org/10.1016/j.biopha.2023.115622.
[135] L.M. Francisco, P.T. Sage, A.H. Sharpe, The PD-1 pathway in tolerance and autoimmunity, Immunol. Rev. 236 (2010) 219–242, https://doi.org/10.1111/j.1600-065X.2010.00923.x.
[136] T. Okazaki, S. Chikuma, Y. Iwai, S. Fagarasan, T. Honjo, A rheostat for immune responses: the unique properties of PD-1 and their advantages for clinical application, Nat. Immunol. 14 (2013) 1212–1218, https://doi.org/10.1038/ni.2762.
[137] S. Pesce, M. Greppi, G. Tabellini, F. Rampinelli, S. Parolini, D. Olive, L. Moretta, A. Moretta, E. Marcenaro, Identification of a subset of human natural killer cells expressing high levels of programmed death 1: a phenotypic and functional characterization, J. Allergy Clin. Immunol. 139 (2017), https://doi.org/10.1016/j.jaci.2016.04.025, 335-346.e3.
[138] M.E. Keir, M.J. Butte, G.J. Freeman, A.H. Sharpe, PD-1 and its ligands in tolerance and immunity, Annu. Rev. Immunol. 26 (2008) 677–704, https://doi.org/10.1146/annurev.immunol.26.021607.090331.
[139] Q. Meng, Z. Liu, E. Rangelova, T. Poiret, A. Ambati, L. Rane, S. Xie, C. Verbeke, E. Dodoo, M. Del Chiaro, M. Löhr, R. Segersvärd, M.J. Maeurer, Expansion of tumor-reactive T cells from patients with pancreatic cancer, J. Immunother. 39 (2016) 81, https://doi.org/10.1097/CJI.0000000000000111.
[140] H. Zhang, X. Yue, Z. Chen, C. Liu, W. Wu, N. Zhang, Z. Liu, L. Yang, Q. Jiang, Q. Cheng, P. Luo, G. Liu, Define cancer-associated fibroblasts (CAFs) in the tumor microenvironment: new opportunities in cancer immunotherapy and advances in clinical trials, Mol. Cancer 22 (2023) 159, https://doi.org/10.1186/s12943-023-01860-5.
[141] Y. Zhang, X. Chen, S. Mo, H. Ma, Z. Lu, S. Yu, J. Chen, PD-L1 and PD-L2 expression in pancreatic ductal adenocarcinoma and their correlation with immune infiltrates and DNA damage response molecules, J. Pathol. Clin. Res. 8 (2022) 257–267, https://doi.org/10.1002/cjp2.259.
[142] Y.P. Peng, C.H. Xi, Y. Zhu, L.D. Yin, J.S. Wei, J.J. Zhang, X.C. Liu, S. Guo, Y. Fu, Y. Miao, Altered expression of CD226 and CD96 on natural killer cells in patients with pancreatic cancer, Oncotarget 7 (2016) 66586–66594, https://doi.org/10.18632/oncotarget.11953.
[143] S. Liang, Z. Yang, D. Li, X. Miao, L. Yang, Q. Zou, Y. Yuan, The Clinical and Pathological Significance of Nectin-2 and DDX3 Expression in Pancreatic Ductal Adenocarcinomas, Dis. Markers (2015) e379568, https://doi.org/10.1155/2015/379568, 2015.
[144] P. Zhang, X. Liu, Z. Gu, Z. Jiang, S. Zhao, Y. Song, J. Yu, Targeting TIGIT for cancer immunotherapy: recent advances and future directions, Biomark. Res. 12 (2024) 7, https://doi.org/10.1186/s40364-023-00543-z.
[145] L. Seifert, I. Plesca, L. Müller, U. Sommer, M. Heiduk, J. von Renesse, D. Digomann, J. Glück, A. Klimova, J. Weitz, M. Schmitz, A.M. Seifert, LAG-3-expressing tumor-infiltrating T cells are associated with reduced disease-free survival in pancreatic cancer, Cancers 13 (2021) 1297, https://doi.org/10.3390/cancers13061297.
[146] M.K. Gleason, T.R. Lenvik, V. McCullar, M. Felices, M.S. O'Brien, S.A. Cooley, M. R. Verneris, F. Cichocki, C.J. Holman, A. Panoskaltsis-Mortari, T. Niki, M. Hirashima, B.R. Blazar, J.S. Miller, Tim-3 is an inducible human natural killer cell receptor that enhances interferon gamma production in response to galectin-9, Blood 119 (2012) 3064–3072, https://doi.org/10.1182/blood-2011-06-360321.
[147] Y. He, J. Cao, C. Zhao, X. Li, C. Zhou, F.R. Hirsch, TIM-3, a promising target for cancer immunotherapy, OTT 11 (2018) 7005–7009, https://doi.org/10.2147/OTT.S170385.
[148] F. Gebauer, D. Wicklein, J. Horst, P. Sundermann, H. Maar, T. Streichert, M. Tachezy, J.R. Izbicki, M. Bockhorn, U. Schumacher, Carcinoembryonic antigen-related cell adhesion molecules (CEACAM) 1, 5 and 6 as biomarkers in pancreatic cancer, PLoS ONE 9 (2014) e113023, https://doi.org/10.1371/journal.pone.0113023.
[149] N. Sauer, N. Janicka, W. Szlasa, B. Skinderowicz, K. Kołodzińska, W. Dwernicka, M. Oślizło, J. Kulbacka, V. Novickij, K. Karłowicz-Bodalska, TIM-3 as a promising target for cancer immunotherapy in a wide range of tumors, Cancer Immunol. Immunther. 72 (2023) 3405–3425, https://doi.org/10.1007/s00262-023-03516-1.
[150] N.G. Steele, E.S. Carpenter, S.B. Kemp, V.R. Sirihorachai, S. The, L. Delrosario, J. Lazarus, E.D. Amir, V. Gunchick, C. Espinoza, S. Bell, L. Harris, F. Lima, V. Irizarry-Negron, D. Paglia, J. Macchia, A.K.Y. Chu, H. Schofield, E. J. Wamsteker, R. Kwon, A. Schulman, A. Prabhu, R. Law, A. Sondhi, J. Yu, A. Patel, K. Donahue, H. Nathan, C. Cho, M.A. Anderson, V. Sahai, C.A. Lyssiotis, W. Zou, B.L. Allen, A. Rao, H.C. Crawford, F. Bednar, T.L. Frankel, M. Pasca Di Magliano, Multimodal mapping of the tumor and peripheral blood immune landscape in human pancreatic cancer, Nat. Cancer 1 (2020) 1097–1112, https://doi.org/10.1038/s43018-020-00121-4.
[151] L. Meyaard, G.J. Adema, C. Chang, E. Woollatt, G.R. Sutherland, L.L. Lanier, J. H. Phillips, LAIR-1, a novel inhibitory receptor expressed on human mononuclear leukocytes, Immunity 7 (1997) 283–290, https://doi.org/10.1016/S1074-7613(00)80530-0.

[152] A. Poggi, N. Pella, L. Morelli, F. Spada, V. Revello, S. Sivori, R. Augugliaro, L. Moretta, A. Moretta, p40, a novel surface molecule involved in the regulation of the non-major histocompatibility complex-restricted cytolytic activity in humans, Eur. J. Immunol. 25 (1995) 369–376, https://doi.org/10.1002/eji.1830250210.
[153] R.J. Lebbink, T. de Ruiter, J. Adelmeijer, A.B. Brenkman, J.M. van Helvoort, M. Koch, R.W. Farndale, T. Lisman, A. Sonnenberg, P.J. Lenting, L. Meyaard, Collagens are functional, high affinity ligands for the inhibitory immune receptor LAIR-1, J. Exp. Med. 203 (2006) 1419–1425, https://doi.org/10.1084/jem.20052554.
[154] M. Son, F. Santiago-Schwarz, Y. Al-Abed, B. Diamond, C1q limits dendritic cell differentiation and activation by engaging LAIR-1, Proc. Natl. Acad. Sci. 109 (2012) E3160–E3167, https://doi.org/10.1073/pnas.1212753109.
[155] M.J.M. Olde Nordkamp, M. van Eijk, R.T. Urbanus, L. Bont, H.P. Haagsman, L. Meyaard, Leukocyte-associated Ig-like receptor-1 is a novel inhibitory receptor for surfactant protein D, J. Leukoc. Biol. 96 (2014) 105–111, https://doi.org/10.1189/jlb.3AB0213-092RR.
[156] R.J. Lebbink, M.C.W. van den Berg, T. de Ruiter, N. Raynal, J.A.G. van Roon, P. J. Lenting, B. Jin, L. Meyaard, The soluble leukocyte-associated ig-like receptor (LAIR)-2 antagonizes the collagen/LAIR-1 inhibitory immune interaction1, J. Immunol. 180 (2008) 1662–1669, https://doi.org/10.4049/jimmunol.180.3.1662.
[157] M.J.M.O. Nordkamp, J.A.G. van Roon, M. Douwes, T. de Ruiter, R.T. Urbanus, L. Meyaard, Enhanced secretion of leukocyte-associated immunoglobulin-like receptor 2 (LAIR-2) and soluble LAIR-1 in rheumatoid arthritis: LAIR-2 is a more efficient antagonist of the LAIR-1–collagen inhibitory interaction than is soluble LAIR-1, Arthritis Rheum. 63 (2011) 3749–3757, https://doi.org/10.1002/art.30612.
[158] K. Boelaars, L. Goossens-Kruijssen, D. Wang, C.M. De Winde, E. Rodriguez, D. Lindijer, B. Springer, I. Van Der Haar Àvila, A. De Haas, L. Wehry, L. Boon, R. E. Mebius, N. Van Montfoort, M. Wuhrer, J.M.M. Den Haan, S.J. Van Vliet, Y. Van Kooyk, Unraveling the impact of sialic acids on the immune landscape and immunotherapy efficacy in pancreatic cancer, J. Immunother. Cancer 11 (2023) e007805, https://doi.org/10.1136/jitc-2023-007805.
[159] E. Rodriguez, K. Boelaars, K. Brown, R.J. Eveline Li, L. Kruijssen, S.C.M. Bruijns, T. van Ee, S.T.T. Schetters, M.H.W. Crommentuijn, J.C. van der Horst, N.C.T. van Grieken, S.J. van Vliet, G. Kazemier, E. Giovannetti, J.J. Garcia-Vallejo, Y. van Kooyk, Sialic acids in pancreatic cancer cells drive tumour-associated macrophage differentiation via the Siglec receptors Siglec-7 and Siglec-9, Nat. Commun. 12 (2021) 1270, https://doi.org/10.1038/s41467-021-21550-4.
[160] K. Boelaars, E. Rodriguez, Z.R. Huinen, C. Liu, D. Wang, B.O. Springer, K. Olesek, L. Goossens-Kruijssen, T. van Ee, D. Lindijer, W. Tak, A. de Haas, L. Wehry, J. P. Nugteren-Boogaard, A. Mikula, C.M. de Winde, R.E. Mebius, D.A. Tuveson, E. Giovannetti, M.F. Bijlsma, M. Wuhrer, S.J. van Vliet, Y. van Kooyk, Pancreatic cancer-associated fibroblasts modulate macrophage differentiation via sialic acid-Siglec interactions, Commun. Biol. 7 (2024) 1–15, https://doi.org/10.1038/s42003-024-06087-8.
[161] K. Edsparr, P.H. Basse, R.H. Goldfarb, P. Albertsson, Matrix metalloproteinases in cytotoxic lymphocytes impact on tumour infiltration and immunomodulation, Cancer Microenviron. 4 (2011) 351–360, https://doi.org/10.1007/s12307-010-0057-0.
[162] S.A. Lim, J. Kim, S. Jeon, M.H. Shin, J. Kwon, T.J. Kim, K. Im, Y. Han, W. Kwon, S. W. Kim, C. Yee, S.J. Kim, J.Y. Jang, K.M. Lee, Defective localization with impaired tumor cytotoxicity contributes to the immune escape of NK cells in pancreatic cancer patients, Front. Immunol. 10 (2019), https://doi.org/10.3389/fimmu.2019.00496.
[163] Q. Huang, M. Huang, F. Meng, R. Sun, Activated pancreatic stellate cells inhibit NK cell function in the human pancreatic cancer microenvironment, Cell. Mol. Immunol. 16 (2019) 87–89, https://doi.org/10.1038/s41423-018-0014-2.
[164] F. Marcon, J. Zuo, H. Pearce, S. Nicol, S. Margielewska-Davies, M. Farhat, B. Mahon, G. Middleton, R. Brown, K.J. Roberts, P. Moss, NK cells in pancreatic cancer demonstrate impaired cytotoxicity and a regulatory IL-10 phenotype, Oncoimmunology 9 (2020) 1845424, https://doi.org/10.1080/2162402X.2020.1845424.
[165] E. Ferretti, S. Carlomagno, S. Pesce, L. Muccio, V. Obino, M. Greppi, A. Solari, C. Setti, E. Marcenaro, M.Della Chiesa, S. Sivori, Role of the main non HLA-specific activating NK receptors in pancreatic, colorectal and gastric tumors surveillance, Cancers 12 (2020) 3705, https://doi.org/10.3390/cancers12123705.
[166] Z.X. Malchiodi, A. Fitzgerald, E. Galvano, L.M. Weiner, Abstract 6169: identification of molecules mediating natural killer cell-pancreatic stellate cell interactions, Cancer Res. 82 (2022) 6169, https://doi.org/10.1158/1538-7445.AM2022-6169.
[167] R.E. Fincham, P. Periasamy, C.R. Joseph, J. Meng, J.C.T. Lim, F. Wee, K. Stasinos, M.R. Goulart, J. Ye, L.Y. Chong, D. Goh, J.P. Yeong, H.M. Kocher, Abstract 1584: the interplay between natural killer cells and pancreatic stellate cells in pancreatic ductal adenocarcinoma, Cancer Res. 84 (2024) 1584, https://doi.org/10.1158/1538-7445.AM2024-1584.
[168] R. Francescone, D. Barbosa Vendramini-Costa, J. Franco-Barraza, J. Wagner, A. Muir, A.N. Lau, L. Gabitova, T. Pazina, S. Gupta, T. Luong, D. Rollins, R. Malik, R.J. Thapa, D. Restifo, Y. Zhou, K.Q. Cai, H.H. Hensley, Y. Tan, W.D. Kruger, K. Devarajan, S. Balachandran, A.J. Klein-Szanto, H. Wang, W.S. El-Deiry, M. G. Vander Heiden, S. Peri, K.S. Campbell, I. Astsaturov, E. Cukierman, Netrin G1 promotes pancreatic tumorigenesis through cancer-associated fibroblast–driven nutritional support and immunosuppression, Cancer Discov. 11 (2021) 446–479, https://doi.org/10.1158/2159-8290.CD-20-0775.
[169] M.H. O'Hara, E.M. O'Reilly, G. Varadhachary, R.A. Wolff, Z.A. Wainberg, A. H. Ko, G. Fisher, O. Rahma, J.P. Lyman, C.R. Cabanski, R. Mick, P.F. Gherardini, L.J. Kitch, J. Xu, T. Samuel, J. Karakunnel, J. Fairchild, S. Bucktrout, T. M. LaVallee, C. Selinsky, J.E. Till, E.L. Carpenter, C. Alanio, K.T. Byrne, R. O. Chen, O.C. Trifan, U. Dugan, C. Horak, V.M. Hubbard-Lucey, E.J. Wherry, R. Ibrahim, R.H. Vonderheide, CD40 agonistic monoclonal antibody APX005M (sotigalimab) and chemotherapy, with or without nivolumab, for the treatment of metastatic pancreatic adenocarcinoma: an open-label, multicentre, phase 1b study, Lancet Oncol. 22 (2021) 118–131, https://doi.org/10.1016/S1470-2045(20)30532-5.
[170] B. Bockorny, T. Macarulla, V. Semenisty, E. Borazanci, J. Feliu, M. Ponz-Sarvise, D.G. Abad, P. Oberstein, A. Alistar, A. Muñoz, R. Geva, C. Guillén-Ponce, M. S. Fernandez, A. Peled, M. Chaney, I. Gliko-Kabir, L. Shemesh-Darvish, D. Ickowicz, E. Sorani, S. Kadosh, A. Vainstein-Haras, M. Hidalgo, Motixafortide and pembrolizumab combined to nanoliposomal irinotecan, fluorouracil, and folinic acid in metastatic pancreatic cancer: the COMBAT/KEYNOTE-202 trial, Clin. Cancer Res. 27 (2021) 5020–5027, https://doi.org/10.1158/1078-0432.CCR-21-0929.