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Tailoring Natural Killer cell immunotherapy to the tumour microenvironment

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Abstract

Natural killer (NK) cells are cytotoxic and cytokine-secreting cells that can mediate potent anti-tumour activity. Accumulating evidence indicates that NK cell functions are severely compromised within the confines of the tumour microenvironment thus impairing the efficacy and development of NK cell-based therapies. Here we review the various cellular and molecular pathways that tumours have supplanted to evade NK cell surveillance. We highlight novel strategies designed to alleviate or circumvent the immunosuppressive conditions of the tumour microenvironment in order to emancipate NK cell function and stifle the inexorable growth and metastasis of malignant cells.

Keywords

Natural Killer cell; immunosuppression; tumour microenvironment; inhibitory receptors; checkpoint blockade; immunotherapy

1. Introduction

NK cells are recognised as the founding member of the growing family of innate lymphoid cells (ILCs). Together NK cells and ILC1 are a subset of ILCs that express the transcription factor (TF) T-bet and secrete IFN- γ upon activation. Even though the different ILC family subsets have been characterised primarily by their cytokine secretion profiles, NK cells are the sole ILC population endowed with specialised cytotoxic effector function. Thus, NK cells are envisaged as the innate equivalent of CD8⁺ T cells, whereas ILC1 have been proposed to be the innate equivalent of T-helper 1 CD4⁺ T cells. In contrast to other lymphocytes, NK cells do not require prior immunisation to attain cytolytic activity and can lyse tumour cells either by direct ‘natural’ cellular cytotoxicity or via antibody-dependent cellular cytotoxicity (ADCC) [1]. NK cells can also secrete cytokines, such as IFN- γ and TNF- α , that can inhibit tumour cell proliferation, tumour angiogenesis, and metastage

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carcinogenesis [2]. The ability to selectively manipulate NK cell functions *in vivo* would provide powerful therapeutic tools for diseases, such as cancer.

NK cell activity is regulated by an array of activating and inhibitory germ-line encoded cell-surface receptors that can distinguish healthy cells from virus-infected or transformed cells [3-5]. For example, ligands expressed on healthy cells engage inhibitory NK receptors thus sparing normal tissues from NK cell attack [6-8]. The downregulation of inhibitory ligands and the expression of ligands for activating receptors by cancer cells can trigger NK cells to kill these abnormal cells and secrete cytokines that influence subsequent adaptive immune responses [9-11]. Thus, blocking inhibitory receptor-ligand interactions with antibodies (e.g. 'checkpoint blockade' therapy) or augmenting activating receptor-ligand pathways offers real potential to release NK cells from inhibition to generate anti-tumour activity [12].

NK cells are therefore attractive targets for the development of new cancer immunotherapies. In support of this, higher NK cell infiltration of some cancers is associated with a more favorable prognosis [13], an inverse correlation exists between the cytotoxic activity of NK cells and cancer incidence [14], and enhanced tumour growth is observed following NK cell depletion or in mice that carry genetic deficiencies in NK cell receptors [15,16]. Despite possessing many desirable anti-tumour functions the efficacy of NK cell-based immunotherapies has yet to reach maximum potential in human clinical trials [17]. Several barriers to the successful development of NK cell-based cancer therapies exist not least for solid tumours [18]. Following the three stages of the immunoediting process - elimination, equilibrium and escape - tumour cells are eventually selected that can establish a predominantly immunosuppressive and pro-angiogenic tumour microenvironment [19]. The tumour microenvironment is characterised by a complex network of tumour, immune and stromal cells, embedded in extracellular matrix (ECM) that collaborates to accomplish the proliferation, migration, and dissemination of malignant cells. The precise physiological mechanisms employed by tumour cells in order to establish and maintain this immunosuppressive niche are only now beginning to be understood (Figure 1).

The immunosuppressive functions of tumour-resident T regulatory cells (Treg), tumour-associated macrophages (TAM), and myeloid-derived suppressor cells (MDSC) are well documented and have been reviewed in detail before [12,20-22]. In this review, we will discuss the properties of the tumour microenvironment that act to inhibit NK cell functions focussing on the cellular and molecular mechanisms that are less often emphasised, such as the modification of the cancer cell glycocalyx and the manipulation of the ECM by tumour cells, and how these might impact the therapeutic use and design of NK cell-based anti-cancer treatments.

2. The cancer cell glycocalyx

Eukaryotic cells are covered by a surface layer of glycans referred to as the 'glycocalyx', which serves to contribute to cell-cell recognition, communication, and intercellular adhesion. The glycocalyx of cancer cells is generally highly robust which can promote integrin clustering and growth factor signalling, and mechanotransduction thus enhancing

tumour cell motility and metastasis. Cancer cells may also manipulate the glycocalyx to avoid NK cell recognition.

2.1. Sialic acid recognition

Sialic acids are sugars found on the periphery of cell surface glycans. A characteristic shared by many tumour cells is the capacity to express a high density of sialic acid-rich cell surface glycoproteins through the epigenetic or genetic dysregulation of glycan synthesis pathways. Such 'hypersialylation' of cell surface glycans is correlated with poor patient survival and decreased tumour immunogenicity in a variety of cancers potentially by interacting with lectin receptors on the surface of immune cells. The sialic acid-binding Ig-like lectins (Siglecs) are a family of inhibitory cell surface receptors expressed in mammals, amphibians and fish [23]. All human NK cells constitutively express Siglec-7. In contrast, a subset of CD56^{dim} NK cells was recently reported to selectively express Siglec-9. Evidence has accumulated that NK cells may play a role in selecting for cancer cell hypersialylation because tumours that developed in *Ifng*^{-/-} failed to develop a hypersialylated surface phenotype [24], and there is a correlation between the sialylation state of various target cell-lines and resistance to NK cell-mediated cytotoxicity [25-27]. Thus, cell surface hypersialylation is hypothesised to offer a selective advantage for malignant cells under evolutionary pressure from NK cell immunosurveillance by increasing the density of sialic acid ligands for inhibitory Siglec receptors.

Susceptible cancer cells have been engineered to express synthetic glycopolymers containing sialic acid (Sia polymer) ligands for Siglec-7 [28]. Cancer cells engineered with Sia polymers induced Siglec-7 recruitment to the NK-target cell immunological synapse, induction of inhibitory Siglec-7 signaling, and protection from NK cell lysis [28]. A different study found the sialic acid ligands for Siglec-7 and -9 were expressed on a wide range of different primary tumours and protected the tumour cells by dampening NK cell cytotoxicity and IFN- γ secretion [29]. Interestingly, this latter study identified a subset of circulating Siglec-9⁺ CD56^{dim} NK cells with enhanced chemotactic responses that was reduced in patients with colon adenocarcinoma and malignant melanoma. These results suggest that cancer cells have exploited cell surface sialylation to evade NK cell activity. Therapeutic interventions designed to target tumour-associated sialosides or block inhibitory Siglec function may provide a promising new avenue for cancer immunotherapy. To this end, a recent study showed that recombinant sialidase fused to the human epidermal growth factor receptor 2 (HER2)-specific antibody trastuzumab desialylated tumour cells in a HER2-dependent manner, reduced tumour cell binding to inhibitory NK cell Siglecs, and enhanced ADCC [30]. Yet another approach has been to synthesise small sialic acid derivatives that bind with high affinity to Siglec-7, although it remains unclear whether these molecules can block Siglec receptor function in cytotoxicity assays [31].

2.2. Heparan sulfate proteoglycans (HSPGs)

HSPGs play vital roles in every step of tumour progression allowing cancer cells to proliferate, elude immunosurveillance and invade neighboring tissues, and metastasise to distal sites away from the primary site of tumour growth. Heparan sulfate (HS) and hyaluronic acid (HA) are the principal glycosaminoglycan components of the glycocalyx

[32]. Like sialic acid, HS and HA are consistently elevated on cancer cells where they are associated with tumour growth, angiogenesis, and metastasis. For example, cell surface HS acts as a mechanosensor for interstitial flow by upregulating the secretion of matrix metalloproteinases (MMPs) that can degrade the ECM and enhance metastasis [32]. In contrast, HS incorporated into the ECM enhances barrier protection by limiting cellular migration and metastases. Heparanase can thus promote tumour growth by cleaving HS chains of proteoglycan to release HS-bound angiogenic growth factors and degrading the ECM to facilitate tumour cell invasion and metastasis [33]. Several cancers including breast, lung, brain, pancreatic, skin, and colorectal cancers display aberrant regulation of key HS biosynthetic enzymes such as 3-*O*- and 6-*O*-Sulfotransferases, and catabolic enzymes such as heparanase and the HS endosulfotases, SULF1 and SULF2 [34]. These results suggest that NK cells may have evolved to express activating receptors that may function to survey aberrant changes in the glycocalyx of malignant cells and the surrounding ECM.

The activating NK cell receptors NKp30, NKp44, NKp46, and KIR2DL4, have all been reported to bind to HS to regulate NK cytotoxicity and IFN- γ secretion [35,36]. Binding of HS to NKp30 remains controversial [37] and may depend on the glycosylation state of the NKp30 ectodomain [38]. Of note, the only definitive binding partner for NKp30 has been shown to be B7-H6 [39,40]. *Cis* interactions with the HS proteoglycan, syndecan-4, at the NK cell surface was shown to modulate the functions of NKp44 and KIR2DL4 [36,41]. In both cases, treatment with soluble HS *in trans* was proposed to disrupt *cis* association with syndecan-4 and potentiate NKp44 and KIR2DL4 signaling resulting in greater NK cell secretion of IFN- γ [36,41]. Since NKp44 and NKp46 only recognised cancer cells exhibiting certain 6-*O*-Sulfonation and *N*-Acetylation patterns, cancer cells expressing increased extracellular heparanase and aberrant HS 6-*O*-endosulfatase activity exhibit diminished NK cell recognition [42].

Cancer cell over-expression of telomere repeats binding factor 2 (TRF2), a key factor in telomere protection, can upregulate expression of the gene for heparan sulfate-glucosamine 3-*O*-sulfotransferase 4 (HS3ST4) by binding to the interstitial telomeric sequence (ITS) located within the HS3ST4 intron [43]. The targeted reduction of HS3ST4 expression using short hairpin RNAs in cancer cells resulted in the increased tumor infiltration of activated NK cells and TRF2 over-expression correlated with decreased NK cell density during the early development of human colon cancer. Thus, sulfation of cell surface HS proteoglycans by HS3ST4 modified the tumours capacity to be eliminated by NK cells perhaps by influencing the binding of cytokines and chemokines to the tumour ECM, although it is possible that changes in tumour cell-surface recognition by activating or inhibitory NK cell receptors could also have been influenced by differences in HS sulfation.

Potent anti-tumour HS mimetics, such as PG545, have now been developed that are designed to suppress angiogenesis and metastasis by inhibiting heparanase and competing for the HS-binding domain of angiogenic growth factors [44]. Interestingly, PG545 exerts additional immunostimulatory effects that are critically dependent on TLR9-dependent NK cell activation [45]. PG545 treatment increased the lysosomal accumulation of the TLR9 ligand CpG DNA in dendritic cells (DCs) leading to the enhanced IL-12 production that was critical for PG545-mediated NK cell control in a murine model of T cell lymphoma. PG545

did not bind TLR9 directly suggesting that an as yet unidentified molecule expressed by DCs may bind to PG545 to enhance CpG-TLR9 signaling. It would be interesting to elucidate the precise molecular basis for the immunostimulatory effects of PG545 on DCs, which could provide valuable new insights for NK cell-based cancer immunotherapies and beyond.

3. Tumour manipulation of the ECM

Aberrant expression of ECM collagens or collagen-modifying enzymes, such as lysyl oxidase (LOX) is elevated in many different types of tumours [46] and is associated with poor prognosis. For example, LOX-dependent collagen cross-linking mediated focal adhesion and growth factor signaling that supported tumour cell survival, growth and invasion, and the recruitment of stromal cells that contribute to tumour progression [47]. Tumour modification of the ECM may also promote the suppression of NK cell activity. For example, the Leukocyte-associated Immunoglobulin-like receptor 1 (LAIR-1) encodes a 32 kDa transmembrane glycoprotein with a single immunoglobulin-like domain and a cytoplasmic tail containing two ITIM (Figure 1) that was identified in a screen for molecules that inhibit NK cell function [48]. Collagen was identified as a LAIR-1 ligand [49,50]. Secreted and cell surface collagens expressed by tumour cells engaged LAIR-1 and diminished NK cell cytotoxicity [51]. In contrast to a possible inhibitory function, ECM components, such as collagen, may promote the recruitment of adoptively transferred NK cells to sites of tumour growth [52]. Melanoma lung metastases that were well-vascularised and characterised by high amounts of ECM components, such as collagen, laminin and fibronectin, were efficiently infiltrated by adoptively transferred, IL-2-activated NK cells, in contrast, to tumours that were resistant to infiltration that were poorly vascularised and contained fewer ECM components. It will be interesting to determine what classes of ECM collagens and whether LAIR-1 expression correlates with the enhanced recruitment, infiltration and functional activation of NK cells in different types of solid tumours, or whether inhibiting LOX activity can potentiate NK cell anti-tumour activity.

4. The complement system

Complement proteins have been shown to mediate anti-neoplastic activity by promoting the formation of the membrane attack complex and complement-dependent cytotoxicity (CDC). CDC plays an important role in modulating the anti-tumour activity of many monoclonal antibodies (mAbs) that can opsonise tumour cells. However, an increasing number of studies suggest a pathogenic role for the complement system in tumourigenesis [53]. Cancer cells can increase expression of membrane bound regulators of the complement system, such as CD46, CD55, and CD59, that may limit local complement activation in the tumour microenvironment in order to protect cancer cells from CDC and promote cancer resistance to therapeutic mAbs [54,55]. Blockade of CD55 and CD59, and a rodent-specific membrane-bound complement regulator, Crry, unexpectedly enhanced NK cell-mediated ADCC even in the absence of complement [56-58]. Conversely, complement activation has been shown to augment cancer progression in part by regulating inflammation in the tumour microenvironment [59].

A therapeutic intervention designed to transiently deplete complement using cobra venom factor prior to tumour challenge increased the number of splenic and tumour-infiltrating NK cells and enhanced the anti-tumour cytotoxic T lymphocyte (CTL) response [60]. A model was proposed in which the expanded NK cell population leads to increased tumour cell lysis and antigen release facilitating DC maturation and antigen presentation and the enhanced generation of tumour-specific CD8⁺ T lymphocytes. Although the molecular mechanism responsible was not identified, this study demonstrated that complement proteins regulate NK cell activity to influence tumour growth. A direct interaction of complement factor P (CFP, properdin) with NKp46 was recently reported [61]. It will be interesting to delineate the pathways regulated by the CFP/NKp46 interaction in different cancers since NKp46 can mediate both positive and negative immunoregulation [62,63].

Complement proteins in the tumour microenvironment can also play a role in cancer progression independent of complement activation. C1q-deficient mice exhibit slower tumour growth and prolonged survival in the B16 lung metastasis model compared to wild-type mice by promoting cancer cell adhesion, migration and proliferation [64]. C1q (but not C4) is expressed in the stroma and vascular endothelium of several human malignant tumours [64]. Interestingly, C1q is a LAIR-1 ligand [65]. Tumour expression of C1q may therefore engage LAIR-1 to inhibit NK cell activity in addition to promoting tumour growth. LAIR-2 is a homologue of LAIR-1 encoded in the Leukocyte Receptor Complex [66]. LAIR-2 lacks a transmembrane domain and is secreted upon PBMC stimulation [67]. LAIR-2 binds to collagen [67] and C1q [65,68] with higher affinity than LAIR-1 and has been proposed to act as a soluble competitor for LAIR-1 [65,67-69]. The transcript for LAIR-2 is broadly expressed in peripheral blood mononuclear cells [67], although activated CD4⁺ T cells were shown to be the main producers of soluble LAIR-2 protein [69]. To add to this complex biology, a secreted isoform of LAIR-1 has also been reported [69]. The relative expression of transmembrane versus secreted LAIR-1, LAIR-2 and the respective collagen and C1q ligands within the tumour microenvironment could therefore have important outcomes for the functional activity of NK cells. In contrast to a possible role in solid tumours, LAIR1 is also expressed by myeloid cells and has been shown to be required for the development of acute myeloid leukaemia (AML). LAIR-1 activated a SHP-1-dependent CAMK1-CREB pathway that sustained AML stem cell survival and self-renewal [70]. A non-stimulatory mAb to LAIR-1 has recently been developed [71]. Similarly to the mode of action of the anti-SlamF7 (also known as CS1 or CRACC) Elotuzumab in multiple myeloma [72-74], the binding of a non-stimulatory mAb to LAIR-1 on AML cells may block the activation of a CAMK1-CREB pathway of AML stem cell survival and self-renewal and simultaneously render AML cells susceptible for NK cell ADCC.

5. Tumour resident cells that mediate immunosuppression

NK cells that can infiltrate solid tumours are often functionally impaired. The contribution of Treg, TAM, and MDSC to the maintenance of an immunosuppressive tumour microenvironment that favours cancer progression are well documented [12,20-22]. However, accumulating evidence indicates that other non-cancerous tumour-resident cells are also endowed with properties that can suppress NK cell functions.

5.1. Cancer-associated fibroblasts

Cancer-associated fibroblasts (CAFs) are the prominent stromal cell population in many types of human carcinomas and play a central role in tumour-stromal interactions by secreting several factors such as ECM proteins (e.g. collagens, laminin and fibronectin), MMPs, chemokines, vascularisation promoting factors (e.g. PDGFs and VEGF), and secreted growth factors (e.g. epidermal and fibroblast growth factors) that promote tumour cell proliferation, survival, angiogenesis, tissue invasion, and metastasis [75]. CAFs also directly secrete many immunosuppressive factors, such as TGF- β , that help maintain an immunosuppressive tumour microenvironment [76]. For example, TGF- β can downregulate cell surface expression of the activating NK cell receptor, NKG2D, by repressing expression of the NKG2D signaling adaptor, DAP10 [77]. In combination with IL-15 and IL-18, TGF- β induces the expression of different splice forms of NKp30 and NKp44 that are predicted to have inhibitory functions in cancer [78-81]. TGF- β has been shown to mediate the attenuation of NK cell function by repressing IL-15-dependent activation of the mTOR pathway [82].

More recent studies have uncovered additional mechanisms. CAFs can secrete PGE2 and the tryptophan catabolic enzyme, IDO that can downregulate the expression of NKG2D, perforin, and granzyme B potentially disarming NK cell cytotoxic potential [83,84]. A therapeutic strategy targeting CAFs, such as the genetic depletion of fibroblast activation protein- α (FAP)-expressing CAFs in Lewis lung carcinomas and in a subcutaneous model of pancreatic ductal adenocarcinoma (PDAC), caused the rapid necrosis of both cancer and stromal cells in immunogenic tumours via a process involving IFN- γ and TNF- α [85]. In contrast, another study found that fewer tumour-resident myofibroblasts in PDAC patients correlated with reduced survival and the genetic depletion of alpha smooth muscle actin-expressing myofibroblasts actually enhanced immunosuppression and accelerated pancreatic cancer resulting in reduced survival [86]. Thus, whilst it may be informative to determine if the therapeutic targeting CAFs can enhance NK and T cell activity caution should be taken for some solid tumours, such as PDAC.

5.2. Platelets

Platelets drive thrombus formation and coagulation at sites of vascular injury [87], but also function to promote the development of blood and lymphatic vessels, as well as the regeneration of blood vessels following vascular trauma [88,89]. Cancer is frequently characterised by a hypercoagulable state and a mean platelet volume can predict poor prognosis in some cancers [90,91]. Platelets are implicated in cancer progression and metastasis by preventing intra-tumoural haemorrhage, and promoting angiogenesis and the tethering and arrest of circulating tumour cells. Tumour cells can also subvert platelet function to evade NK cell immunosurveillance. By forming aggregates on the tumour cell surface, platelets can shield cancer cells from NK cells, thus facilitating tumour progression and metastasis [92,93]. Platelets can also bestow ligands for inhibitory NK cell receptors to the tumour cell surface, such as MHC class I complexes [94] and glucocorticoid-induced TNF-related ligand (GITRL) [95], as well as secreting soluble factors, such as TGF- β that can downregulate NKG2D [96], thereby impairing NK cell cytotoxicity and IFN- γ production. Mice administered anti-platelet antiserum to deplete platelets had >95%

reduction of metastasis of CFS1 and B16 tumour cells compared to animals with normal platelet numbers [92] suggesting that strategies to deplete platelets or target platelet function e.g. anticoagulants may provide a powerful adjunct to NK cell-based immunotherapies.

5.3. Regulatory NK cells

Although NK cells are implicated in cancer immunosurveillance they can also be culprits in suppressing T cell-mediated immunity [20]. A unique CD56⁺CD3⁻ innate lymphoid cell (ILC) population with the capacity to suppress the expansion and cytokine secretion properties of tumour-infiltrating lymphocytes (TILs) from high-grade ovarian serous carcinomas was recently described that corresponded with a striking reduction in the time to disease recurrence [97]. This novel population of regulatory CD56⁺CD3⁻ ILCs exhibited low cytotoxic activity, secreted IL-22, and expressed a transcriptional profile overlapping that of NK cells and other ILCs. Interestingly, the activating NKp46 receptor was highly expressed by the regulatory CD56⁺CD3⁻ cells and NKp46 function conferred the ability to suppress T cell expansion. Thus, depletion of tumour-resident regulatory NK cells or blocking regulatory NK receptors, such as NKp46, may provide a novel approach to cancer immunotherapy.

6. Tumour metabolism

Hypoxia is a prominent feature of solid tumours and results from inadequacies between the tumour microcirculation and the oxygen demands of the growing tumour mass. The resulting expression of the hypoxia-responsive TF HIF-1 α accentuates cancer cell glycolysis and the release of lactate [98]. Exposure of NK and T cells to high concentrations of lactate prevented upregulation of the TF NFAT resulting in diminished IFN- γ production and enhanced tumour growth [99]. The generation of adenosine is linked to the hypoxic tumour environment and is another mechanism whereby tumours suppress immune responses through the activation of A_{2A} receptors on NK cells and T cells [100,101]. The ectoenzyme CD39 catalyzes the degradation of ATP into AMP via the intermediate ADP. AMP can then act as a CD73 substrate, resulting in the production of immunosuppressive adenosine [102]. Adenosine deaminase is a major enzyme for the degradation of adenosine and can be localised to cell surfaces when bound to CD26. However, CD26 expression is reduced on tumour cells and so the tumour microenvironment can generate sufficiently high concentrations of adenosine to inhibit anti-tumour immune responses [101]. Interestingly, TGF- β has been shown to guide the differentiation of a unique population of CD73-expressing salivary gland ILCs with characteristics of both NK cells and ILC1, suggesting tissue-resident ILCs could potentially contribute to the local generation of adenosine in the tumour microenvironment [103]. Targetting the immunosuppressive activity of adenosine may be a promising therapeutic strategy because inhibition of A_{2A} adenosine receptor signaling in combination with immune checkpoint blockade can enhance CD8⁺ T and NK cell anti-metastatic activity [104,105].

7. Concluding Remarks

Whilst checkpoint blockade can induce durable responses in patients with a variety of solid tumours, it is unlikely that a strategy based on a single antibody or therapeutic intervention

will address all the necessary requirements to elicit an effective anti-tumour response. Combinatorial approaches have the enhanced potency needed to mediate the most significant tumour destruction and approaches that can nullify the molecular and cellular factors that maintain an immunosuppressive tumour microenvironment may thus improve the design and efficacy of NK cell-based therapies. Combining one or two checkpoint blockers with a co-stimulatory mAbs also results in improved anti-neoplastic therapy and reflects the ability of the co-stimulatory mAbs to promote NK cell functions. The encouraging success of chimeric antigen receptor (CAR) T cell adoptive therapies also makes it possible to graft the ectodomain of an inhibitory receptor (e.g. Siglec-7 or LAIR-1) on to chimeric antigen receptor (CAR) backbone thereby converting an inhibitory receptor/tumour ligand interaction into an activating receptor/tumour ligand interaction within the confines of the tumour microenvironment. Such an approach may 'turn the tables' on immunosuppressive tumours themselves and aid in the creation of novel CAR-T or -NK cell adoptive therapies. Thus, identifying the molecular and cellular factors that are critical for maintaining the immunosuppressive tumour microenvironment will drive the successful development of novel NK cell-based immunotherapies. Such immunosuppressive mechanisms will undoubtedly differ depending on the type of tumour, between different cancer patients, and even within the same tumour, which may be an important point to consider for the rational design of new NK cell therapeutic interventions.

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Highlights

- The microenvironment of solid tumours suppresses Natural Killer (NK) cell function and is a significant barrier to successful immunotherapy.
- Approaches that nullify the molecular and cellular factors maintaining an immunosuppressive tumour microenvironment will augment established immunotherapies, such as checkpoint blockade.
- Grafting the ectodomain of an inhibitory NK receptor on to a chimeric antigen receptor (CAR) backbone has real potential to convert an inhibitory receptor/tumour ligand interaction into an activating receptor/tumour ligand interaction and will aid the design of novel CAR-T and -NK cell adoptive therapies.
- Identification of the molecular and cellular factors critical for maintaining the immunosuppressive tumour microenvironment is fundamentally important for the rational design of new translational immunotherapies.
- Immunosuppressive mechanisms will undoubtedly differ depending on the type of tumour, between different cancer patients, and even within the same tumour, which is an important point to consider when designing new immunotherapies.

Figure 1. Overview of the endemic cellular and molecular factors that govern NK cell suppression in the tumour microenvironment

Solid tumours contain a complex network of tumour cells (light green), stromal cells, and tumour-infiltrating immune cells (NK cells, light purple), embedded in extracellular matrix (ECM; collagen, dark brown). In response to hypoxic conditions, proliferating tumour cells upregulate HIF-1 α that accentuates glycolysis and the generation of immunosuppressive lactate. Tumour cells modify their cell surface glycocalyx to become hypersialylated (light blue cloud) or over-express ECM components e.g. collagen that may engage inhibitory NK cell receptors encoding cytoplasmic Immunoreceptor Tyrosine-based Inhibition Motifs [3] (ITIM, red boxes), such as Siglec-7 and LAIR-1, respectively. Platelets (anucleated, light brown) coat tumour surfaces thus masking ligands (RAET/ULBPs; yellow, blue, magenta and cyan) for activating NK cell receptors like NKG2D or NKp46 that pair with adaptors encoding activating cytoplasmic signalling motifs [3] (green boxes), thus providing a protective shield from NK recognition. Cancer-associated fibroblasts (CAFs; nucleated, light brown) secrete soluble factors that promote angiogenesis (e.g. VEGF; blood vessels, red), tumour growth (e.g. Fibroblast Growth Factors, FGFs), and factors, such as TGF- β , prostaglandin E2 (PGE2) and indoleamine-2,3-dioxygenase (IDO) that can impair the cytotoxic and cytokine secreting functions of NK cells. TGF- β can guide the differentiation of CD73-expressing NK-like ILCs, suggesting tumour-resident CD73-expressing ILCs could potentially contribute to increased concentrations of adenosine in the tumour microenvironment. Finally, a novel population of regulatory NK cells (NK^{reg}) can secrete IL-22 and suppress the expansion and cytokine secretion properties of tumour-infiltrating lymphocytes via an NKp46-dependent mechanism.