Natural Killer (NK) cells in immunotherapy and perspectives in antitumour approaches

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Abstract. Natural Killer (NK) cells comprise a group of specialized innate lymphoid cells endowed with multiple cytotoxicity mechanisms while also harnessed with the ability to enhance other immune cells with cytokine production. This exclusive advantage of them to recognize and eliminate virally infected cells and tumour cells has been unmasked for decades, and previous clinical trials are also successfully tested for both efficacy and safety. With the emerging strategies in CAR-T cell therapy, such technologies can also be capable of further enhancing the viability of NK cell immunotherapy through cytokine armouring, chimeric antigen receptor (CAR) transduction, checkpoint inhibition, and co-stimulatory signals. Other than the conventional approach of engineering CAR to target tumour antigens, they are also capable of acting as blockers to the inhibitory compartments on tumour cells within the harsh environment to reduce the negative effects. Despite all these aspects, the tumour microenvironment (TME) is another essential facet when discussing cancer therapy owing to its characteristic setting that contributes immensely to immune evasion and immune function inhibition. In this review, I introduce the foundational mechanism for NK cytotoxicity and its signalling routes, discuss the impacts of TME on immune cells and their antitumour effects, evaluate possible strategies that overcome the current challenges, and propose a few potentially adoptive measures for future research in general immunotherapy from a perspective of molecular biology.

Keywords: Natural Killer (NK), Cancer Immunotherapy, Tumour Microenvironment (TME).

1. Introduction to Natural killer cell

The natural killer (NK) cell is one of the most essential groups of cells involved in human immunity, especially in the innate immune system. Unlike macrophages which are sentinels and residents in local tissues, NK cells are descendant of blood stem cells and flow around the body. When there are signs of inflammation like the secretion of pro-inflammatory cytokines, NK cells can also secrete cytokines that help with defending invading cells or particles. NK cells are viable for killing virus-infected cells, bacteria, parasites, and most importantly, tumour cells by inducing programmed cell death through secreting granzyme B and perforin. The mechanism for NK cells to function is a balance between activating and inhibitory receptors on the surface of NK cells: separately, activating receptors bind to target cell antigens for killing purposes; inhibitory receptors bind to the major histocompatibility complex type I (MHC I) which indicates the presence of self-antigen that balance the activating signal, leaving the target cells safe (Figure 1).

In contrast, NK cells also secrete a large quantity of cytokines that enable and enhance systematic defense against pathogens and tumour cells. Additionally, NK cells can be activated by interferon alpha (IFN-alpha) and interferon beta (IFN-beta). Among the receptors present on the surface of NK cells, CD 16 and CD 32, efficiently triggers the release of cytokines and cytotoxicity to activate NK cells to kill cells coated with antibody. This is one of the most essential activities of NK cells, through antibody-dependent
cellular cytotoxicity (ADCC), and this is highly relevant to tumour therapeutics using NK cells mediated by antibodies. In contrast, NK cells kill the marked “stressed cells”, viral-infected cells, or cancerous cells, which express extracellular receptor tyrosine kinases that are induced by cellular stress pathways. This mode of killing cells is known as “induced-self recognition”, which has been studied in Natural Killer Group 2D (NKG2D) to a large extent. NKG2D is the type of receptor that recognizes distinct structures compared to MHC I, upregulated when under various types of stress like activated p53 proteins (Natalie et al., 2022). The antitumour activity in NK cells is extensively guaranteed by the upregulation of ligands to activating receptors and downregulation in the expression of inhibitory ligands on tumour cells while secreting interferon-gamma (IFN-Gamma) and tumour necrosis factor-alpha (TNF-Alpha).

2. Receptor signaling of NK cells

When NK cells encounter the target cells, it would result in adhesion and conjugation, the formation of immune synapse, a dynamic interface formed for direct cell contact, in between, and as mentioned in the introduction, the cellular mechanism of NK cells is specifically dependent on the dynamic balance between the inhibitory and activating signalling through the binding of ligands on the target cells, the eventual outcome of the immune synapse would be largely dependent on the balance. (Figure 2)

![Figure 2. Table of receptors and ligands in NK cells](image)

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activating Receptors</td>
<td>NK44, NKG2D, CD160, CD56, CD94/NKG2A</td>
</tr>
<tr>
<td>Inhibitory Receptors</td>
<td>CD94/NKG2A, KL, LIR-1, CD94, TIGIT, LAG-3, PD-1</td>
</tr>
</tbody>
</table>

Positive (activating) signals are provided by corresponding ligands binding to a series of receptors like NKG2D, NKp46, NKp30, and NKp44 that are commonly expressed on pathogens or cells induced by cellular stress or viral infections. The activating format of killer cell immunoglobulin-like receptor (KIR) eventually triggers the NK mechanisms through cytokine secretions and cell cytotoxicity (Yilmaz et al., 2020b). On the contrary, NK cells also express inhibitory receptors which suppress NK cell functions by antagonizing the activating pathways through post-translational phosphorylation. The quintessential mechanism for the inhibitory KIR receptors is to suppress the activating pathways by the recognition of self-antigens expressed in healthy and normal cells; in this case, the ligand for the KIR receptor is presented on the MHC I molecules (Romagné & Vivier, 2011). The intracytoplasmic immunoreceptor tyrosine-based inhibition motifs are phosphorylated by tyrosine phosphatases after binding to the ligand. The products of the phosphorylation of a few signaling components are essential substrates for tyrosine kinases and protein tyrosine phosphatases, and, therefore, are the core of NK cell functions. The triggering of activating receptors on NK cells leads to the induction of phosphorylation of ITAM or kinase as well as the rearrangements of cytoskeleton networks to result in higher stability of activation. As microRNAs (miRNA) are molecules that control the gene expression of a particular cell, NK cells themselves can be activated by these various factors including cytokines and non-coding miRNAs. Within the immune synapse, the continual stimulatory signals trigger vigorous actin polymerization and microtubule organizing centre (MTOC) polarization that is quintessential for the latter process which lytic granules, containing perforin 1 and granzyme B for target cell apoptosis induction, are transported along the cytoskeleton network for subsequent release (Krzewski & Strominger, 2008) (Figure 3).

![Figure 3. NK cell function towards a typical cancerous cell.](image)

Both MHC I and stimulatory ligands are expressed on the cancerous cell. However, owing to the disruption in the process of MHC I formation within the cancerous cell, the cell may downregulate the expression of MHC I to evade CD8+ cell function (Cornel et al., 2020); however, this favours NK cell function because of the imbalance between the activating and inhibitory signals and trigger the following cascade effect. The activated NK cell may then start the synthesis of the cytolytic granule containing perforin and granzyme B which induces apoptosis within the cancer cell. Death ligands, FasL and TRAIL, can also be expressed by the NK cell, which binds to Fas and TRAIL-R that are expressed on the cancerous cell to mediate apoptosis. ADCC is also an important feature in which CD-16 may be expressed to recognize the antibody-coated cancer cell to mediate its cell death. The promotion of NK cell function and the overall immune response can be achieved by the secretion of a certain set of cytokines and chemokines like IFN-gamma and TNF-alpha.

Specifically, it is the granzyme B that cleaves the procaspases in the target cell to induce the classical pathway for apoptosis, and perforin 1 only facilitates the transfer of granzyme B to the cytosol. The discovery of corresponding ligands to the mentioned receptors of NKG2D and NKp30 suggests the receptors bind to rarely expressed antigens on normal cells while being observed
to be upregulated when encountering infections or carcinogenesis (Figure. 4)(SR Yoon et al., 2014).

Figure. 4 Signalling and interaction between natural killer cells and their target cells

Upon the elaboration above, strategies for cancer immunotherapy from using NK cells are essentially categorized as first, treating NK cells with cytokines to trigger cell proliferation, differentiation, and activation of corresponding activating and suppressing pathways; second, artificially manipulating the NK cells to adjust the balance between the activating and inhibitory pathways by, for example, the use of chimeric antigen receptor (CAR); third, antibodies can be adopted for the purpose of enhancing the NK cell functions through inhibiting the inhibitory pathway for the NK cells through blocking certain receptors like KIR (SR Yoon et al., 2014). Upon these potential measures to be taken in treating cancer, tumour cells are also adapted with their own new mechanism for immune evasion by, for instance, secreting immunosuppressive cytokines like IL-10 or transforming growth factor-beta (TGF-beta) to repress the NK cell functions, or either upregulating the expression of MHC-I complexes or downregulating the expression for tumour associated antigens to deactivate or evade the attacks from NK cells, correspondingly (Yilmaz et al., 2020b). Therefore, CAR is necessary for engineering the future NK cell therapy in terms of increasing specificity as well as to counteract the immune evasion mechanism from tumour cells.

3. NK cell therapy

Current NK cell therapies are about to either activate endogenous NK cell responses through NK cell stimulants or apply hematopoietic stem cell transplantation to adopt exogenous NK cells.

3.1 Endogenous NK cell activation

A consistent problem found in patients with cancers is non-functioning NK cells, therefore, activation for endogenous NK cells becomes extensively critical. Endogenous NK cell activations may be administered by reacting with cytokines, for example, IL-2, IL-12, IL-15, IL-18, IL-21, and Type I interferons, directly or indirectly. In addition to that, immunomodulators can also be administered to enhance NK cell activities against tumour cells. Thalidomide and lenalidomide can help to increase the peripheral NK cell counts as well as enhance the NK cell functions (SR Yoon et al., 2014). Specifically, during T cell activation, when APC present MHC to T cell receptor (TCR), the presence of accessory molecules on both APC and T cell are capable of providing secondary signals, which are necessary for activation and preventing T cell non-responsiveness; by applying thalidomide or any other immunomodulatory drugs (IMiDs) is effective in enhancing T cell receptor-mediated signaling to enhance immune responses against tumour cells (Bartlett et al., 2004). Inducing apoptosis in tumour cells and, at the same time, inhibiting the vascular endothelial growth factors responsible for angiogenesis all appear as evidence for thalidomide and general IMiDs to inhibit tumour growth. In terms of NK cells, IMiDs enhance the release of IL-2 and interferon-gamma, which effectively activate NK cells, through T cell stimulation and proliferation (Hayashi et al., 2005) (Bartlett et al., 2004). Alternative strategies for inducing endogenous NK cell activation take the principle of the balance between inhibitory and activating NK receptors. By blocking the inhibitory NK receptors, CD94/NKG2A-HLA-E complex or inhibitory KIRs on NK cells can reduce the inhibitory effect and, therefore, enhance NK cell activity (Creelan & Antonia, 2019). Conversely, by stimulating the upregulation of expression in activating NK receptors, NKG2D, or their ligands, ULBPs and MICA/B, on tumour cells could enhance NK activities (Sutherland et al., 2006). These effects could be achieved by using, for example, antibodies or medicinal drugs, which will downregulate the expression of MHC I complexes on tumour cells to augment NK cell activities (Shi et al., 2006).

3.2 Exogenous stem cell transplantation

Hematopoietic stem cell transplantation (HSCT) is a clinical therapy that focuses on haematological malignancies like leukaemia. Despite the chance of having a matched donor is extensively lower than to find a mismatched, allogeneic donor, clinical studies have shown that, by utilizing a mismatched donor, the survival rate after five years is higher (60% to 5%) and proportion with acute myeloid leukaemia relapse is lower (9% to 75%) when patients are given mismatched donor cells (Barao & Murphy, 2003). In allogeneic HSCT, a human leukocyte antigen (HLA)-mismatched donors are harvested and infused into the recipients. The mismatching could abnormally activate T cells in the recipient body, and T cells could start to attack the infused donor cells; this increases the probability of graft-versus-host-diseases (GVHD) (Ferrara et al., 2009).
However, because of the mismatching, T cells would also recognize the non-self-antigens presented on the haematological malignancies and kill them, also called graft-versus-tumour (GVT) effect. Due to the simultaneous existence of both GVHD and GVT which causes a disequilibrium, the current clinical strategy of utilizing HSCT is to alleviate the probability of GVHD and enhance the GVT effect (Fleischhauer & Beelen, 2016) (SR Yoon et al., 2014). Furthermore, on contrary to all the risks involved when using T cells, the ability to enhance the GVT effect while having no implication in GVHD in NK cells has been illustrated (Barao & Murphy, 2003). The same strategies are used in NK cell infusion. While the NK cells infused are capable of killing the APCs in the recipient’s body to reduce the chance of GVHD. Mismatching in HLA is allowed when infusing NK cells and it has a lower chance or no chance to cause GVHD compared to T cells (Elahi et al., 2021). Studies have also shown that missing one or more KIR ligands in the recipient’s body might actually reduce their chances of having a relapse and increase their survival rates (Miller et al., 2007).

4. Tumour microenvironment (TME)

Though the presence of CAR on NK cells can significantly increase the specificity for targeting tumours, targeting the right tumour cells or even targeting the actual tumour cells is still a great challenge. Due to the nature that NK cells immune cells including NK cells circulate through the bloodstream, CAR NK therapy has demonstrated great efficacy for haematological tumours like lymphoma and leukaemia since they can easily establish contact with cells in the plasma. For solid tumours like breast cancer, CAR NK cells will face greater challenges because they need to first travel through the bloodstream, passing through the anatomical structures in between the solid tumour and plasma to reach the correct site and contact with the tumour cells there (Yilmaz et al., 2020a). In addition, the harsh TME there including inhibitory cytokines and metabolic products secreted by tumours cell could deprive the CAR NK cell functions; other distractions throughout could be inhibitory ligands, immunosuppressive immune cells and the overall hypoxia environment could extensively inhibit the CAR NK cell activities to make them target on tumour cells. Typical immunosuppressive immune cells can be regulatory T cells, cancer-associated dendritic cells, and anti-inflammatory tumour-associated macrophages (M2-TAMs). Specifically, Macrophages are capable of secreting inhibitory cytokines like interleukine-10 (IL-10) and TGF-Beta and regulatory T cells can suppress immune response from dendritic cells and NK cells by blocking the release of IFN-Gamma and the expression of activating KIR and limit anti-tumour effects through TGF-Beta to mediate tumour responses (Figure 5) (Navin et al., 2020).

4.1 Ligand expression of cancer cells

Cancer cells express ligands to NKG2D through “a disintegrin and metalloproteinases” (ADAM) family that cleave MICA (MHC class I chain-related protein A) (expressed by cells under damage, stress or transformation; typically expressed by tumour cells) proteolytically to reduce the MICA surface density (Ghaidiali et al., 2017). Therefore, the blockage of ADAM should be effective. Additionally, ligands released by cancer cells like MICA/B may bind to NKG2D and downregulate its expression, which then alleviates NK cell function and promote immune evasion (Du et al., 2021c).

4.2 Immunosuppressive cytokines

Among all, Transforming Growth Factor-beta (TGF-beta) (TGF-beta prevents autoimmuneity through its properties of antiproliferative and anti-inflammatory among immune cells) is considered a potent immunosuppressive cytokine within TME. Activated TGF-beta may suppress the release of IFN-gamma after binding to NK cells because the triggered phosphorylation of SMAD2, SMAD3, and SMAD4 is utilized by TGF-beta to suppress IFN-gamma and T-bet/TBX21 (stimulator of IFN-gamma gene) (Yu et al., 2006). Ghiringhelli et al. experimented with membrane-bound TGF-beta expressed by T-reg cells and observed direct inhibitory effect on NK cell functions with downregulation of NKG2D receptors on NK cell surfaces. Conversely, the experiments in exacerbation of NK cell proliferation and in vivo cytotoxicity when T-reg
cells are depleted also proved the inhibition of the antitumour effect by T-reg cells (Ghiringingelli et al., 2005). Serine and threonine kinase mammalian target of rapamycin (mTOR) is also an important pathway in regulating NK cytotoxicity. This IL-15-induced mTOR would be suppressed by TGF-beta which leads to NK cell dysfunction (Viel et al., 2016). However, after exposure to high concentrations of IL-15, the mTOR pathway would be recovered to enhance NK cytotoxicity while also positively feeding back through increasing the expression of IL-15 receptors (Marçais et al., 2014). Additionally, an investigation of fructose-1,6-bisphosphatase (FBP1) gene expression by Cong et al. indicated its correlation with TME-derived TGF-beta. The result showed a gradual increase in TGF-beta while expression of FBP1 also increases to inhibit glycolysis in NK cells, thus suppressing their cytotoxic effects and mediating immune evasion (Cong et al., 2018). Wu et al. experimented on tumour infiltrating NK cells in Oesophagus squamous cell carcinoma (ESCC) and found the induction of the STAT 3 pathway by secretion of IL-6 and IL-8 from primary ESCC cells, which downregulated the activating receptors (NKP30 and NKG2D) on NK cell surfaces. Contrastively, the blockade of the STAT 3 pathway alleviated the attenuation of NK cytotoxicity by IL-6 and IL-8 (Wu et al., 2019).

4.3 NK regulatory cell (NKreg) dysfunction

Though NK cells have always been referred to as killer cells to implement cytotoxic functions, they also have regulatory effects, which implies a dampened antitumour effect. Human NK cells have differentiations in their degree of expression of CD-56, so they can be roughly categorized as CD56dim NK cells and CD56bright NK cells accordingly. CD56dim NK cells, with low expression of CD56, have greater cytotoxicity while CD56bright, with high expression of CD56, have weaker cytotoxicity and rather produce more cytokines for mediation (Sungur & Murphy, 2014). Consequently, there are also differences in the expression of ligand receptors like KIR and NKG2. In CD56bright NK cells, the expression of CD94-NKG2A is largely presented while being relatively low in CD56dim NK cells. The high expressions in inhibitory receptors, CD94-NKG2A, could then alleviate the overall antitumour functions (Cooper et al., 2001).

4.4 Reverse impacts for NK cell functions to antitumour effects

NKG2D is a potent ligand-receptor in executing NK cytotoxic function, while it also plays a role in pro-inflammation, which has been recognized as an important factor for tumorigenesis (Du et al., 2021d). Sheppard et al. demonstrated the stimulatory effects in the recruitment of CD8+ T cells by NKG2D receptors while CD8+ T cells indicated a partial downregulation of NKG2D. Overall, the exacerbation of local inflammation by the upregulation of NKG2D was proved (Sheppard et al., 2017).

5. Challenges and corresponding strategies for NK cell therapy

5.1 CAR transduced NK

A chimeric antigen receptor (CAR) is a synthetically programmed protein that consists of an extracellular antigen-binding domain and an intracellular protein complex that triggers a cascade of cellular activation. Most of the time, a single-chain variable fragment (scFv) derived from an antibody is used, but a ligand for a specific tumour receptor is sometimes used directly (Laskowski et al., 2022; Tilmaz et al., 2020). An intracellular signaling domain is a derivative of immunoreceptor tyrosine-based activation motifs (ITAMs) of the T-cell receptor (TCR) or another form of activating receptors in the cytoplasm. The recruitment of adaptor molecules and immune cells occurs after the phosphorylation of ITAMs, and therefore, further stimulates the downstream cellular pathways (Yilmaz et al., 2020).

CAR NK cells have been generated using umbilical cord blood (UCB) NK cells and is transduced to express the gene coded for anti-CD 19 CAR by Liu et al. (Myers & Miller, 2020) (Liu et al., 2017b). The whole design was an expression of the CAR, IL-15 (promotes T and NK cell proliferation and induces apoptosis inhibitor), and an inducible caspase-9 suicide switch which allowed immediate eradication of the genetically engineered cells in vivo triggered by a dimerizer, AP1903 (10nM). Through the clinical results for the patients with chronic lymphocytic leukaemia (CLL), the encouraging results of the effective elimination of CD-19 expressing leukaemia cells with no major adverse effects like cytokine release syndrome (Myers & Miller, 2020) (Liu et al., 2017b). Owing to the harsh TME in solid tumours, in which the ligands for activating receptors (NKG2D) on tumour cells are extensively downregulated, the antitumour activity of NK cells is impeded since the balance between activating signals and inhibitory signals is disrupted. However, specifically designed CAR can be utilized to act as a potent co-stimulatory and activating signal that binds specifically to cancerous cells. Though CAR-NK has yielded positive results in clinical trials, the lack of TME components might suggest the need for further investigation (Navin et al.).

In order to strengthen the cytolytic effects of NK cells, cytokine-based transgenes have also been experimented with to demonstrate an improvement in the NK cell activity but also the infiltration of other immune cells that enhance antitumour activities. Common gamma-chain cytokines like IL-2, IL-7, IL-15, and IL-21 play key roles in enhancing NK cell proliferation, functionality as well as survival (Meazza et al., 2011). Selecting the commonly expressed antigens on both T cells and NK cells, Conversely, these experiments were only done on haematologic tumours instead of solid tumours. Nevertheless, the use of CAR is not only limited in trigger the activating receptors on NK cells to promote its cellular functions but also allowed it to target a broader spectrum of antigens. Under this prerequisite, CAR can also be modified to target the specific inhibitory components in
the overall TME. It can be achieved in two ways: reducing the immunosuppression resulting from TME and also acting as a checkpoint inhibitor to enhance NK cell functions. In this case, one of the primary concerns on CAR construction is the similarity or overlap between the type of antigens expressed on solid tumour cells and normal cells, which leads to “off-target” cytotoxicity (Navin et al., 2020a). However, owing to the main characteristic – hypoxia – in the TME, CARs can be potentially engineered to only drive the expression of CAR when detecting the quintessential environment (Juillerat et al., 2017). Upon this strategy, not only the therapeutic effects could be improved but also the precision and specificity of CAR-NK.

5.2 Recombinant therapies
During a holistic course of cancer therapy, utilizing multiple strategies would apparently become one of the advanced therapeutic choices. Owing to the off-target effect that has been previously mentioned, utilizing both T cells and NK cells might appear to be a better solution in which CD8+, in this case, has experimentally demonstrated the essential role of CD8+ in the stimulation of the activating receptors, NKG2D, on the surface of NK/CAR-NK cells which eventually enhances the cellular functions. Interleukine-12 (IL-12) fundamentally triggers antigenic stimulation and is also involved in the maturation of T cells into T helper cells. It helps T cells and NK cells to release IFN-gamma and TNF-alpha; it plays a vital role in enhancing the cytolytic effects in both NK cells and CD8+ T cytotoxic lymphocytes (CD8+ cells); IL-12 also owns activity in anti-angiogenic, which suppresses blood vessel formations that serve as a typical characteristic of cancer: by secreting IFN-gamma, it then increases the level of inducible protein-10 (IP-10) that mediates the ultimate effect (Trinchieri, 2003). Retinoic acid early inducible gene 1 (RAE-1) family are ligands to NKG2D activating receptors and are usually expressed by cells involved in wound healing (Jung et al., 2012).

Jiemiao Hu et al. have experimentally proved the facilitated induction of NKG2D in NK cells by CD8+ cells using IL-12 and doxorubicin – a common anti-cancer drug (Figure. 6) (Hu et al., 2017a).

Positive results in NK cell functions have also been obtained through recombinant therapy. Fusing NK cell therapy with anti-cancer drugs, immune checkpoint inhibitors, or cancer vaccines has shown an increase in the CD56bright NK cell count in ALT-803-treated patients, which further proves the effectiveness of the strategy (Melaiu et al., 2020b).

5.3 Evasion from NK cell immunosurveillance
Immune evasion is considered a hallmark of established tumours. A large quantity of molecules produced in TME is immunosuppressive. For example, extracellular released molecules like adenosine and prostaglandins are common molecules produced in TME that significantly suppress NK cell functions. Suppressive cytokines like TGF-beta also suppress the expression of the activating receptors on NK cells, NKG2D and Nkp30. Another mechanism for escaping from NK immune surveillance is from ligands to immune checkpoint. Inhibitory receptors for MHC I are expressed on NK cells and, particularly, inhibitory receptors for immune checkpoints T cell immunoreceptor with immunoglobulin
and ITIM domains (TIGIT), CD-96, PD-1, SIRPalpha. Specifically, tumour cells upregulate ligands to these receptors to inhibit the antitumour effect; conversely, NK cells may also upregulate receptors like PD-1 and SIRPalpha when they are specifically located in tumour. Similar to the upregulation of certain inhibitory receptors on NK cells when in tumours, NK cells may also be in a hyporesponsive state in which they become desensitized. This desensitized state can be either caused by the acquirement of the dysfunctional state by NK cells within the tumour or by the overexpression of the ligands to activating receptors. A similar situation occurs in normal endothelial cells and other healthy cells where the ligands to activating receptors are extensively expressed, and, therefore, the NK cell function is conversely inhibited. Such ligands are massively developed within tumour vasculature, which has the same effect of mediating NK cell functions and impairing their antitumour effect. In order to reverse global desensitization, another soluble NKG2D ligand (which conventionally is thought to be inhibitory) MULT1 was found to promote NK activation and stimulate tumour rejection owing to its particularly high affinity to NKG2D (Du et al., 2021c).

6. Discussion

It is clear that NK cells truly play a vital role in the antitumour immune response. Upon all the tumour cells, clinical results for haematologic tumours like lymphoma have received encouraging results, whereas solid tumours are more challenging in terms of their special TME. The significant issue caused by TME has extensively encouraged more advancements and research in overcoming TME for treating solid tumours. Since TME dampens NK cell functions through inhibitory signals, several strategies have also been discussed to enhance NK cell functions by using, for example, CAR, IL treatments, and immune checkpoint inhibitors. Understanding more of the interactions between immune cells in positive effects like CD8+ cells may also improve the progression of solid tumour research significantly. TME places a huge barrier in between the path to effective cancer therapy. Current research has innovated through different strategies, but there is still a lack of clinical results which involved TME components. The inhibitory signals from TME remain significantly unconsidered. From this concern, future research should involve more comprehensive variables and conditions in testing antitumour activity among different measures.

In addition to CAR NK cells, genetic engineering in CAR can also allow it to express IL-15 in order to overcome the TME effect (Liu et al., 2017). Switch receptors also provide significant inspiration by either blunting the inhibitory signals or switching them to activating signals. Though this can be done by sharing some of the common ligands to inhibitory receptors on T and NK cells, this strategy is extensively dependent on the presence of certain inhibitory ligands on tumour cells (Navin et al., 2020b). In addition to the recent study, drugs can also be utilized to induce the expression of ligands to the activating receptors on tumour cells to enhance the NK cell functions overall. The ability for clinical monitoring of NK cell activity could significantly progress in successful treatments in real medical applications. While targeting the specific antigens presented by solid and haematologic tumours using CAR, it is also important to note the TME particularly. With sophisticated mechanisms for immune evasion, tumour cells are coated with checkpoints which deactivate immune cells like cytotoxic CD8+ cells as well as NK cells. Therefore, it is important to implement combined therapies with checkpoint inhibitors while enhancing immune cells by cytokine armouring and co-stimulatory signals to eventually harness the strategy.

While considering the unique characteristic of TME, environment-sensitive immune cells may be adopted in the research. While detecting hypoxia in vivo, a certain genetic circuit is promoted to synthesize cytokines and other molecules essential for recruitment. This activated genetic circuit can also be modified to produce immunity-enhancing cytokines to achieve in vivo cytokine armouring for more effective cytotoxicity. Another approach may be injecting residential immune cells with abundant vascular endothelial growth factor receptors (VEGFR) to reduce the signal transduction of VEGF sent by tumour cells for angiogenesis. In a harsher environment with nutrient depletion, tumour growth and metastasis might therefore be suppressed. Considering the dynamic state of plasma, injected cells might circulate throughout the body and reduce the efficiency of essential blood vessel growth, the mobility of the injected cells may need significant manipulation while a suicide circuit may also be implemented to ensure post-therapeutic safety which the cells no longer stay in the body and disrupt normal angiogenesis.

In addition, if customized DNA could be available and oncogenes are detected after diagnosis, another approach to treat cancer may be through gene knockouts. Using CRISPR/Cas9 or any other more effective genome editing mechanisms may be the ultimate treatment strategy to perform oncogene knockout or in vivo gene repair. This can be done by encapsulating modified CRISPR/Cas9 with nanostructured lipid carriers (NLCs) to transport this desired enzyme to the site of DNA, by detecting the precise position of the oncogene, either genome editing approaches could be performed. When adopting any further therapeutic approaches involving gene editing or genetically modified biological molecules, clinical safety is always the preliminary objective. Achieving a controllable error range that is low enough for implementation, despite ethical issues, can all contribute to our ultimate fight against cancer. By establishing a multidisciplinary team with different perspectives provided, researchers and clinicians would succeed in various emerging cancer therapy and blueprint a holistic path to clinics.

References


