Am J Cancer Res 2021;11(7):3445-3460
www.ajcr.us /ISSN:2156-6976/ajcr0129309

Review Article

Nab-paclitaxel promotes the cancer-immunity cycle as a potential immunomodulator

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Received December 30, 2020; Accepted June 23, 2021; Epub July 15, 2021; Published July 30, 2021

Abstract: Paclitaxel is a widely used anti-tumor chemotherapeutic drug. Solvent-based paclitaxel causes bone marrow suppression, allergic reactions, neurotoxicity and systemic toxicity, which are associated with non-specific cytotoxicity and side effects of fat-soluble solvents. Studies have explored various new nano-drug strategies of paclitaxel, including nanoparticle albumin-bound paclitaxel (nab-paclitaxel) to improve the water solubility and safety of paclitaxel. Nab-paclitaxel is a targeted solvent-free formulation that inhibits microtubule depolymerization to anticancer. It is easily taken up by tumor and immune cells owing to the nano-scaled size and superior biocompatibility. The internalized nab-paclitaxel exhibits significant immunostimulatory activities to promote cancer-immunity cycle. The aim of this study was to explore the synergistic effect of nab-paclitaxel in tumor antigen presentation, T cell activation, reversing the immunosuppressive pattern of tumor microenvironment (TME), and the synergistic effect with cytotoxic lymphocytes (CTLs) in clearance of tumor cells. The effects of nab-paclitaxel on modulation of cancer-immunity cycle, provides potential avenues for combined therapeutic rationale to improve efficacy of immunotherapy.

Keywords: Nab-paclitaxel, cancer-immunity cycle, immunomodulatory, anti-tumor

Introduction

Paclitaxel is a natural product derived from *Taxus* and a widely used anti-tumor chemotherapy drug [1]. In the past two decades, extensive research on its structure-activity relationship reported that its activity is closely related to the C-2, C-10, and C-13 side chains [2]. Notably, the active group formed by the side chains binds to the β-tubulin site to prevent the depolymerization of tubulin. As a microtubule stabilizer, paclitaxel disrupts microtubule dynamics and triggers mitotic arrest, ultimately inducing cell apoptosis [3]. Besides this classical mechanism, a novel paclitaxel potential has been reported in cancer therapy; where it promotes anticancer immunity by regulating various functions of immune cells. Based on reported pieces of literature, tumor cells pretreated with paclitaxel are more likely to exhibit immunogenicity potentially expanding the range of responding patients [4]. Nonetheless, paclitaxel is not a tumor-specific chemotherapeutic drug, with apparent tissue cytotoxicity and low water solubility. To improve its poor solubility, fat-soluble solvents as cremophor are applied for injection purposes; this type of paclitaxel may cause bone marrow suppression, allergic reactions, and neurotoxicity [5, 6]. In clinics, paclitaxel requires the administration of antihistamines and steroids in advance with a strict dose and frequency of administration [7]. Reduce the cytotoxicity of paclitaxel while maintaining its anti-tumor stimulation to immune cells remains challenging. For the safety and controllability of paclitaxel, scientists are continually searching for its novel nano-drug strategies, focusing on both tumor targeting and human tolerance.

Nab-paclitaxel is a 130 nm particle formulation comprising albumin nanoparticles and paclitaxel with non-covalent bonds [8]. Its lyophilized formulation comprising albumin and pacli-

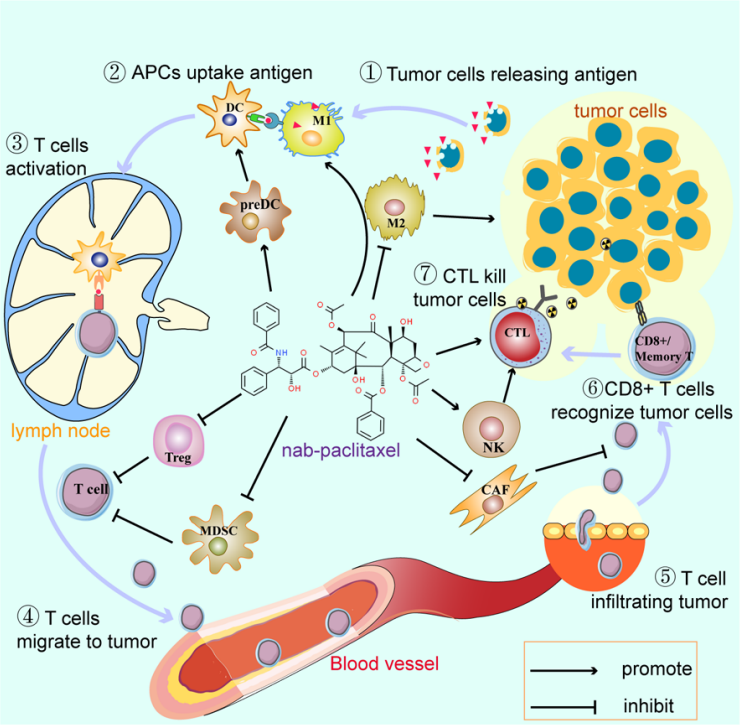

Figure 1. Nab-paclitaxel in the cancer-immunity cycle. The cancer-immunity cycle is a self-propagating cyclic process, starting from the release of antigen from cancer cells and ending with killing the cancer cells. The figure above describes the main seven steps, as well as the main cell types involved and the location of their activities. Nab-paclitaxel mainly involves four important steps in cancer-immunity cycle, including enhancing the antigen presentation ability of APCs, indirectly promoting T cell activation, reversing the immune suppression of TME, and cooperating with CTLs to kill tumor cells.

taxel is reconstituted in normal saline, forming a colloidal suspension for clinical use [7]. Food and Drug Administration (FDA) lists nab-paclitaxel as a vital drug for the treatment of non-small cell lung cancer, pancreatic, and breast cancers. For many fat-soluble and biologically intolerant drugs, albumin nanoparticles are efficient carriers that endow the drugs with water solubility and tumor-targeting properties. *In vivo*, the albumin receptor gp60 recognizes the albumin nanoparticles; nab-paclitaxel penetrates vascular endothelial cells through vesicle transport, enters the tumor tissue, and is finally retained in the tumor cells by binding secreted protein acidic and rich in cysteine (SPARC) [9]. The enriched nab-paclitaxel kills tumor cells and releases a colossal amount of antigen to stimulate anti-tumor immunity. This achieves similar or better responses of paclitaxel with appropriate biocompatibility, low toxicity, and immune-susceptibility accompanied by a dramatic increase in their permeability [10, 11].

Notably, anti-tumor immunity aims to amplify and broaden T cell responses. It is a self-propagating cyclic process; this includes signal transmission between immune cells and accumulation of immune-stimulatory factors [12]. First, antigen-presenting cells (APCs), which primarily comprise macrophages and dendritic cells (DCs), capture, process the antigens, and present them to T cells via the major histocompatibility complex class I/II (MHC I/II), thereby activating the naive T cells. The effector T cells then migrate to and infiltrate the tumor, where they recognize and kill tumor cells. Eventually, the dead tumor cells release additional antigens to activate immune cells. This cyclic process is widely known as the cancer-immunity cycle (**Figure 1**) [12, 13]. However, the interaction between cancer and the human immune system is complex and diverse. In TME, immune cells are often induced by tumor growth factors and chronic inflammatory environment differentiating into inhibitory or tolerogenic immune cells, which lose their normal function and even inhibit T cells [14-16]. Cancer-associated fibroblasts (CAFs) generate growth factors and extracellular matrix to assist tumor expansion; besides, they also form a physical barrier against T cell penetration and drugs [17-19]. Notably, these immune escape mechanisms are exploited by tumors. Interestingly, nab-paclitaxel breaks through the barriers established by CAFs and other tumor tissues, regulating the function and expression of various immune cells in TME [20]. The mononuclear phagocyte system (MPS), including monocytes and their developed macrophages, recognize albumin nanoparticles via scavenger receptors (SRs) and actively uptake nab-paclitaxel [21-23]. Paclitaxel internalized by monocytes exhibits an antigenic response similar to lipopolysac-

charide (LPS), which triggers maturation and cytokine expression of APCs [24-28]. Additionally, nab-paclitaxel blocks the immunosuppression of myeloid-derived suppressor cells (MDSCs) and regulatory T (Treg) cells, helping $CD8^+$ T cells differentiate into effector T cells and cooperates with CTLs to achieve apoptosis of tumor cells [29-31]. In summary, nab-paclitaxel might promote the cancer-immunity cycle by regulating the interaction between cancer and the immune system (**Figure 1**). This article reviews the unique immunomodulation mechanism of nab-paclitaxel. We also propose future perspectives in the research of nab-paclitaxel as a potential immunomodulator promoting the cancer-immunity cycle.

Nab-paclitaxel enhances the function of APCs

APCs are a class of messenger cells that transmit signals in adaptive immune response. Professional APCs are mainly composed of DCs, macrophages and B lymphocytes [32, 33]. In the process of antigen presentation, macrophages and DCs recognize and take up tumor antigens then process them into antigen peptides [34, 35]. The antigen peptide combines with MHC I/II on the surface of APCs to form an antigen-MHC complex, which is recognized by the T cell receptor (TCR) and passed to $CD4^+$/$CD8^+$ T cells [36, 37]. The intensity of T cell activation depends on the level of antigen-MHC complexes on APCs (signal 1), the expression of costimulatory molecules (signal 2) and cytokines such as IL-12 (signal 3) [38, 39]. Under the influence of chronic inflammatory environment and tumor growth factors, macrophages and DCs easily develop into immunosuppressive cells such as M2 macrophages and tolerogenic DCs, thereby losing the potential to activate T cells [40, 41]. MPS highly expresses SRs to identify and eliminate abnormal albumin, so nab-paclitaxel is easily taken up by monocytes (DCs/macrophage precursors) and macrophages [21, 22, 42]. The internalized paclitaxel acts as a positive stimulating factor to macrophages and DCs in TME.

Selective uptake of nab-paclitaxel by macrophages

Due to its superior water solubility and biocompatibility, human serum albumin is often used as a natural carrier of hydrophobic molecules [43, 44]. Nab-paclitaxel formulation is produced from high-pressure homogenization, where albumin and paclitaxel are combined to produce particles with an average diameter of 130 nm [45, 46]. When the albumin-paclitaxel complexes enter TME, the macrophages playing an anti-inflammatory and homeostatic role internalize the mutated serum albumin via phagocytosis. The selective uptake and internalization of nab-paclitaxel by these cells promote the accumulation of paclitaxel, which might awaken the immune function of these immunosuppressive macrophages [5, 23, 47].

Macrophages quickly detect and eliminate damaged or heterogeneous proteins, lipoproteins, and cells to maintain tissue homeostasis [22, 48]. Therefore, when nab-paclitaxel enters the blood and tissues, they might be cleared by macrophages in a specific approach to maintain homeostasis. SRs are expressed on the surfaces of macrophages and DCs; they are used to identify damaged cells and proteins [49, 50]. The affinity of SRs to endogenous components is implicated in the pathogenesis of many diseases by identifying and removing modified or damaged proteins, lipoproteins, and lipids [50]. Normally, macrophages do not clear serum albumin from blood tissue. Nevertheless, macrophages easily absorb serum albumin microspheres accumulated in surrounding tissues. Since the binding of drugs to serum albumin alters the structure of serum albumin, serum albumin forms a special domain as the SRs ligand [22, 51]. For instance, macrophages recognized malonylated bovine serum albumin by nucleolar proteins exhibiting scavenger receptor-like activity [49]. Reports indicate that nab-paclitaxel is internalized by macrophages mainly via phagocytosis [47]. Nonetheless, mechanisms by which SRs of macrophages recognize nab-paclitaxel remain unclarified. In TME, macrophages are often in an immunosuppressive state. Nab-paclitaxel uptake upregulates the expression of immunostimulatory cytokines in macrophages and synergizes with IFN-γ to promote expression of inducible nitric oxide synthase (iNOS), which enhances antigen presentation and tumor killing [23, 26, 47].

Nab-paclitaxel regulates the polarization of tumor-associated macrophages

Tumor-associated macrophages (TAMs) are the most abundant lymphocytes in tumor, pri-

marily differentiated from myeloid monocytes [52]. They are generally divided into two types, i.e., typical activated M1 macrophages and selectively activated M2 macrophages [53]. As innate immune cells, M1 macrophages directly eliminate tumor cells by secreting cytotoxic molecules and phagocytosis, while inducing adaptive anti-tumor immunity through T cell activation; however, M2 macrophages promote tumor growth and immune escape by secreting various growth factors (**Table 1**). Noteworthy, the functional phenotypes of macrophages are alternately activated and regulated by their exposure to different environments to maintain a balance between immune function and inflammatory inhibition [53, 54].

Nab-Paclitaxel regulates tumor-associated macrophage polarization due to the similar effect of LPS in stimulating macrophage activation [26]. One pathway suggests that paclitaxel directly binds to myeloid differentiation factor 2 (MD2) and TLR4, then the complex binds to myeloid differentiation factor88 (MyD88), promoting its activation [24, 25]. The activation of the MD2/TLR4/MyD88 pathway promotes the secretion of tumor necrosis factor (TNF) and NO by M1-like macrophages [23, 25]. In the production of inflammatory mediators, paclitaxel shares a TLR4-dependent/MyD88-independent pathway causing MAPK activation and nuclear translocation of NF-κB [26]. Evidence suggests that nab-paclitaxel upregulates the expression of immunostimulatory cytokines in macrophages, and synergizes with IFN-γ to promote iNOS expression in a TLR4-dependent manner [47]. Nab-paclitaxel uptake by M2-like macrophages is beneficial to the expression of M1-like phenotypic functional molecules in macrophages. Whether singly or combined with gemcitabine, nab-paclitaxel increases the *MHC II*$^+$ *CD80*$^+$ *CD86*$^+$ M1 Phagocyte population [25, 47]. Moreover, paclitaxel blocks the IL-4/STAT6-dependent M2 polarization, while driving cells to M1-profile via NF-κB activation [5, 47]. These phenomena reveal that nab-paclitaxel is implicated in the M1 polarization of macrophages. The ability of antigen recognition and presentation of TAMs is enhanced by up-regulating MHC II (first signal) and CD86, CD80 (costimulatory molecule, second signal). Meanwhile, nab-paclitaxel promotes the polarized M1 macrophages to secrete TNF and NO, causing tumor cell death and exposure of tumor-specific antigens. This expands the response of the cancer-immunity cycle.

Nab-paclitaxel enhances antigen presentation through regulation of DCs

Immature DCs phagocytize apoptotic cells, and then mature under the stimulation of factors such as interferon regulatory factor 8 and GMCSF [55]. Mature DCs process antigens then migrate to nearby lymph nodes, activating naive T cells in the lymph nodes [55]. Specific antigen peptides from tumor cells are usually presented with MHC II on DCs to CD4$^+$ T cells. When expressing appropriate costimulatory molecules, DCs present tumor antigen signals to CTLs, exerting their killing effect, closely related to the cross-presentation ability of DCs [56, 57]. In addition, the activation of NK cells is also related with DCs [58]. Mature DCs play an important role in anti-tumor immunity as a bridge between innate immunity and adaptive immunity (**Table 1**). In TME, due to the induction of immunostimulatory and tumor immunosuppressive factors, immature DCs may differentiate into tolerogenic rather than classical type DCs [56, 59]. Interestingly, myeloid-derived DCs are likely to absorb nab-paclitaxel like other members of MPS. Moreover, previous studies established that paclitaxel potentially promotes the maturation and antigen presentation ability of DCs.

Paclitaxel is implicated in the realization of three types of signals required by DCs to activate T cells, including antigen presentation, expression of costimulatory molecules, and IL-12 secretion. At a non-cytotoxic concentration, paclitaxel directly up-regulates the expression of antigen processing machinery components in DCs, improving the level of antigen processing by DCs [60]. Also, paclitaxel in a non-toxic dose increases the activity of Rac and RhoE, both derived from the Rho family of guanosine triphosphatases (Rho GTPases) in DCs [61]. Low molecular weight Rho GTPases regulate the migration and endocytosis of DCs and are vital actin regulators of DCs [62]. At a therapeutic dose, paclitaxel increases the level of phosphatidylserine (PS) exposure of tumor cells [63]. PS stimulates cells to induce Rac1 translocation and activation, then promotes the activation of Cdc42 and phosphorylation of mitogen-activated protein kinase via the Rac1/

Table 1. Roles of immune cells regulated by nab-paclitaxel in the cancer-immunity cycle

Cell types	Functional phenotype	Cytokines	Immune mechanism	Effects on tumor cells	Ref.
M1 macrophage	MHC II, CD14, CD80, CD86, CD11c, TLR4	IL-1, IL-6, IL-12, IL-23, ROS, iNOS/NO, TNF-α, IFN-I	defenses against pathogens, antigen uptake and presentation, activates Th1-type response	induces tumor cells apoptosis, phagocytoses apoptotic tumor cells	[48, 54]
M2 macrophage	SRs, CD204, CD206, CD11b, TLR4	IL-10, TGF-β, arginase/ ornithine, EGF/VEGF	promotes cell proliferation and repair, activates Th2-type response	promotes tumor immune escape, tumor angiogenesis, tumor growth	[35, 52, 54]
Classical type DC	MHC I/II, CD8, CD14, CD40, CD80, CD86, CD103, TLR4	IL-15, IL-12, IL-27, IFN-α	presents antigen to $CD4^+$/$CD8^+$ T cells, activates NK cells, promotes activation and proliferation of CTLs, enhances the cytotoxicity of Th1 cells	phagocytoses apoptotic tumor cells	[35, 58, 112, 113]
MDSC	CD11b, Ly6G, PD-1, PD-L1, CTLA-4, CD80	NO, Arg-1, ROS, TGF-β, IL-10, CCL4, CCL5	attracts Tregs to the tumor, inhibits T cell proliferation, induces T cell apoptosis	promotes tumor immune escape, tumor growth	[72, 76]
Treg cell	CD4, CD25, Foxp3, CTLA-4	IL-4, IL-10, IL-25, TGF-β, VEGF	induces DCs tolerance, inhibits effector T cells and NK cells	promotes tumor immune escape, tumor angiogenesis, tumor growth	[114, 115]
NK cell	CD16, NKp30, NKp44, NKp46, NKG2, DNAM1	CCL-5, XCL-1, XCL-2, IFN-γ, perforin, granzyme, TNF-α	recruits DCs to tumor, activate DCs, promotes Th1 cells polarization, induces $CD4^+$ T cell proliferation	induces tumor cells apoptosis	[86, 87, 116]
CTL	TCR, FasL, CX3CR1, CXCR5, CCR, PD-1	perforin, granzyme, TNF-α, IFN-γ	recruits additional myeloid cells, kills target cells specifically	kills tumor cells in rapid succession	[117, 118]

PS pathway [61]. This affects the changes in the actin skeleton within the cell. Paclitaxel potentially enhances the migration and endocytosis of DCs by influencing PS and low molecular weight Rho GTPases, hence promoting the activation of T cells in the nearby lymph nodes. Paclitaxel induces the expression of MHC II on DCs, which may be due to the effects of paclitaxel on mitochondrial function or tubulin arrangement [27]. Low-dose paclitaxel up-regulates the expression of costimulatory molecules including CD80, CD86 and CDc11 through the signal of TLR4/MD2. This prevents DC precursors (preDCs) from developing into tolerogenic DCs and promotes the maturation of DCs [28, 64]. Moreover, a low concentration of paclitaxel upregulates IL-12 expression from DCs via STAT4. When blocking the expression of IL-12, the ability of paclitaxel to upregulate MHC, CD80, CD86, and CD40 on the surface of DCs is significantly inhibited [60].

At present, intratumorally injection of DCs is a potential therapeutic strategy to induce an anti-tumor response. Nevertheless, due to the resistance of TME to immune response, DCs alone hardly produce a radical anti-tumor effect. As such, a combined treatment scheme is necessary to enhance the effect of DCs antigen presentation ability. Choi GS et al. previously combined intratumoral injection of DCs and paclitaxel in treating mouse fibrosarcoma. Consequently, the combination of paclitaxel chemotherapy and DC injection potentially triggered complete tumor regression. This was unlike using chemotherapy or DC alone to eradicate partial tumors [65, 66]. Paclitaxel promotes the maturation of DCs, ensuring that antigens are cross-presented to $CD8^+$ T cells. A high paclitaxel concentration initiates an effective mechanism of cytotoxic apoptosis, prompting tumor cells to release antigens; however, it also exhibits immunosuppressive effects. Nab-paclitaxel targets tumors via gp60 and SPARC, reduces the concentration of paclitaxel in peripheral blood/lymph and bone marrow, thereby reducing adverse reactions including bone marrow suppression. Nab-paclitaxel has a linear pharmacokinetic curve, providing safe and controllable clinical administration [7, 9]. These observations suggest that the combination of nab-paclitaxel and DCs exhibit a broader prospect for tumor treatment.

Nab-paclitaxel promotes activation of $CD8^+$ T cells by regulating related immune cells

CTLs are differentiated from $CD8^+$ T cells and play a specific killing role in tumor immunotherapy. When $CD8^+$ T cells are activated, they proliferate and differentiate into memory T cells and CTLs [67, 68], relying on FasL (a surface apoptotic protein-ligand) and cytotoxic factors including perforin and granzyme to achieve specific tumor-killing [69, 70]. Nonetheless, the activation and differentiation of $CD8^+$ T cells are regulated by numerous pathways. The number of $CD8^+$ T cells is significantly correlated with APCs because antigen presentation and costimulatory signals play a vital role in the proliferation and differentiation of $CD8^+$ T cells. Besides, IL-4, IL-12, TNF, and other cytokines secreted from related immune cells promote the function of $CD8^+$ T cells [37, 38, 71]. NK cells secrete cytokines including perforin to kill tumor cells and stimulate the maturation of $CD8^+$ T cells [41]. Conversely, MDSCs block the development of myeloid $CD8^+$ T cells by competitively consuming L-arginine and secreting NO, whereas Treg cells secrete IL-10 and TGF-β to inhibit differentiation and maturation of $CD8^+$ T cells [72, 73]. Reports indicate that paclitaxel at a low concentration is sufficient to regulate the functions of MDSC, Treg, and NK cells, thereby indicating a positive effect on $CD8^+$ T cell activation. However, the clinical therapeutic dose of paclitaxel suggests a concentration-dependent inhibition of CTLs and NK cells [74, 75]. In contrast with the solvent-based paclitaxel, nab-paclitaxel is easier to adjust the dosage of administration hence playing an immunomodulatory role. Therefore, the regulatory effect of nab-paclitaxel on MDSCs, Treg, and NK cells might actively promote the activation of $CD8^+$ T cells.

Nab-paclitaxel inhibits the function of MDSCs

Nab-paclitaxel is often associated with bone marrow suppression; however, bone marrow suppression is not completely equivalent to immunosuppression. The bone marrow hematopoiesis changes in cancer, causing the expansion of relatively immature and activated myeloid cells, known as MDSCs [72]. A low concentration of paclitaxel dose not significantly inhibits the proliferation of myeloid cells; however, it regulates the differentiation and immu-

nosuppressive function of MDSCs [30]. Immunosuppression from MDSCs plays a significant role in relieving acute inflammation, but in chronic inflammatory diseases, they aggravate cancer progression and chronic inflammation by inhibiting innate and adaptive immune responses [76]. Notably, aggregation of MDSCs is induced by various growth factors and pro-inflammatory cytokines (GM-CSF, M-CSF, IL-6, IL-1β, IL-13, etc.). Many transcription factors are implicated in the amplification of MDSCs, including STAT3 and CEPα [72, 77]. The immunosuppressive functions of MDSCs are linked to high levels of arginase 1 (ARG-1), NO, and ROS, which block T cell proliferation and even induce T cell apoptosis (**Table 1**). Therefore, therapeutic depletion of MDSC represents an attractive approach to cancer therapy. Paclitaxel is regarded as an important immunomodulator for depletion therapy of MDSCs.

Alexandra Sevko et al. discovered that the application of ultra-low concentration paclitaxel (mice were weekly injected i.p. with 1 mg/kg paclitaxel in 0.2 ml PBS three times) could significantly reduce the accumulation and immunosuppressive activity of tumor-infiltrating MDSCs without affecting bone marrow hematopoiesis. They found that paclitaxel could increase the frequency of total $CD8^+$ T cells in TME. This was believed to be related to the inhibition of p38MAPK activity, production of TNF-α, and the expression of S100A9 in MDSCs [30]. Furthermore, paclitaxel in ultra-low concentrations (0.5-1 nM) neither increases MDSCs apoptosis nor blocks MDSCs generation, but stimulates MDSCs differentiation towards DCs *in vitro* [29]. This mechanism is consistent with the expression of MHC II and costimulatory molecules induced by paclitaxel in preDCs and tolerogenic DCs. The above studies confirm that a low concentration of paclitaxel ensures the activation of T cells by inhibiting MDSCs and even promoting the conversion of MDSCs to mature DCs. In contrast with solvent paclitaxel, nab-paclitaxel has enhanced osmotic capacity and retention effect; it primarily accumulates in TME so as to relieve the inhibition of paclitaxel on bone marrow cells and peripheral immune cells. Moreover, nab-paclitaxel has a larger therapeutic window and linear pharmacokinetic characteristics [9], which make it more controllable to maintain a stable low concentration of paclitaxel in the blood and tumor peripheral tissues to inhibit MDSCs.

Nab-paclitaxel reverses immunosuppression by inducing apoptosis of $CD4^+25^+Foxp3^+$ Treg cells

Treg cells are a type of $CD4^+$ T cell subsets that highly express CD25 and Foxp3, accounting for about 3%-5% of $CD4^+$ T cells. They primarily maintain peripheral immune tolerance and prevent over immunity in the body [78]. Moreover, Treg cells inhibit the anti-tumor immune response by restraining tumor-specific effector T cells during tumor proliferation. This occurs through cell-to-cell contact and the production of cytokines IL-10 and TGF-β which significantly inhibit the differentiation and maturation of $CD8^+$ T cells [79]. Additionally, TGF-β promotes the growth of tumor interstitial fiber cells, which is not conducive to tumor immunotherapy [80]. An increase in the amount of Treg cells in TME promotes apparent tumor immune escape. For these reasons, the depletion of Treg cells might improve the response of the immune system on various tumors.

Paclitaxel selectively down-regulates the expression of an anti-apoptotic molecule as Bcl-2, upregulates the expression of an apoptotic molecule as Bax, then promotes the apoptosis of Treg cells. The difference in the response between Treg cells and effector T cells to paclitaxel is eliminated when blocking the Bcl-2 pathway [81]. Moreover, paclitaxel selectively reduces the size of the Treg cell population in lymphocyte subpopulations, rather than other subpopulations including effector T cells. As a result of paclitaxel treatment, the expression of cell death receptor Fas on the surface of Treg cells is upregulated, whereas the expression density of Foxp3 is significantly downregulated. This impairs the number and suppressive function of Treg cells [31]. In contrast, Th1 cells cytokines (IFN-γ and IL-2) production and the expression of the intercellular activation marker CD44 are enhanced [82, 83]. Th1 cells recruit macrophages and NK cells to the tumor site. More importantly, they significantly enhance the tumor recognition of NK cells and CTLs, and have a profound influence on the formation of $CD8^+$ memory T cells [37]. These findings strongly confirm that paclitaxel contributes to reversing the immune suppression from Treg cells, and indicate that paclitaxel plays a positive role in the proliferation and differentiation of effector T cells.

Nab-paclitaxel enhances the activity of NK cells

NK cells belong to granular lymphocytes and share common precursor cells with T/B lymphocytes [84]. They quickly dissolve tumor cells, virus-infected cells, and cells coated with antibodies, while catalyze the development of adaptive immunity (**Table 1**), thus the development of their anticancer function has been the focus of cancer research in recent years [85]. As non-specific killer cells, NK cells show a powerful killing effect on tumor cells deficient in MHC expression, compensating for the immunodeficiency of CTLs. NK cells generally identify tumor cells then secrete cytotoxic factors (perforin, granzyme, NK cytotoxic factor et al.), which induce apoptosis of susceptible targets [86]. As an effective inducer of the adaptive immune response, TNF and IFN-γ secreted from NK cells help CD4/8$^+$ T cells to obtain tumor specificity and amplifies the pro-inflammatory cytokine production of DCs [86, 87]. Paclitaxel destroys the binding of human NK cells to target cells by inhibiting the expression of adhesion molecules [88]. In patients with non-small cell lung cancer, weekly paclitaxel therapy reduces the function of NK cells, which persists during the first cycle but then gradually recovers [89]. Paclitaxel potentially inhibits the killing pathway of target cells recognized by NK cells; notably, this inhibition is dose-dependent.

Nonetheless, paclitaxel increases the cytotoxicity of human NK cells *in vitro* by initiating the production of mRNA and protein of perforin, an effector molecule of cytotoxicity mediated by NK cells. A significant correlation exists between increased perforin production and NF-κB activation in NK cells [90]. Besides, the activity of NK cells and CTLs is enhanced in patients with advanced breast cancer treated with paclitaxel or docetaxel [91]. Pretreatment of tumor targets with paclitaxel also causes increased cell lysis mediated by NK cells *in vitro*, and a non-toxic dose of paclitaxel might promote cancer cells to up-regulate the expression of MIC-A/B ligand, thereby enhancing tumor sensitivity to NK cell-mediated cytotoxicity [92, 93]. This provides potential options for combinational therapy, like the combination of F8-IL-2 and paclitaxel in the treatment of melanoma, inducing the recruitment of NK cells to tumors [94]. When paclitaxel is combined with a DNA-based cellular vaccine to treat mice, the combined treatment induces a powerful breast cancer cell immunity mediated by CD8$^+$ T cells and NK/LAK cells [95]. The activation of NK cells promotes tumor cell apoptosis and releases numerous specific antigens. At the same time, NK cells secrete perforin, TNF, and other cytokines thereby enhancing the tumor-specific killing effect of CTLs.

Nab-paclitaxel promotes activated T cells infiltration into TME

Stimulated by the signals of APCs and tumor antigen, T cells proliferate and differentiate into memory cells and effector T cells, which then migrate to and infiltrate TME [41]. TME is a type of acidic hypoxic place full of immunosuppressive factors, which cooperate with tumor and stromal cells (TAMs, MDSCs, CAFs, etc.) to promote tumor immune escape [41, 96]. The relative lack of effector T cell is largely due to the immune rejection features of TME [97]. Nab-paclitaxel targets TME via the abundant albumin receptor SPARC in tumors; it consumes the tumor stroma and softens the tumor by inhibiting the proliferation of CAFs, thereby promoting immune cells and drugs to enter into TME. In addition, paclitaxel inhibits tumor-infiltrating M2 macrophages, tolerogenic DCs, MDSCs, and other immunosuppressive cells, which secrete immunosuppressive factors including IL-10, TGF-β, and vascular endothelial growth factor (VEGF). Nab-paclitaxel might reverse the immunosuppressive trend of TME, thereby ensuring that effector T cells infiltrate the tumor and exert specific cytotoxicity (**Figure 2**).

Paclitaxel inhibits CAFs proliferation and promotes immune cells and drugs to invade tumor tissues

The CAFs located in the tumor stroma are the major non-parenchymal cells in the tumor; they are associated with excessive stroma formation and interact with cancer cells [17]. Fibroblasts are stimulated on the tumor wound to differentiate into CAFs. For instance, cancer cell-derived cytokines activate pancreatic stellate cells to form the CAFs phenotype, which cause extensive abnormal proliferation of the pancreas in pancreatic cancer [19, 98]. CAFs secrete various extracellular stroma components, cytokines, growth factors, and proteas-

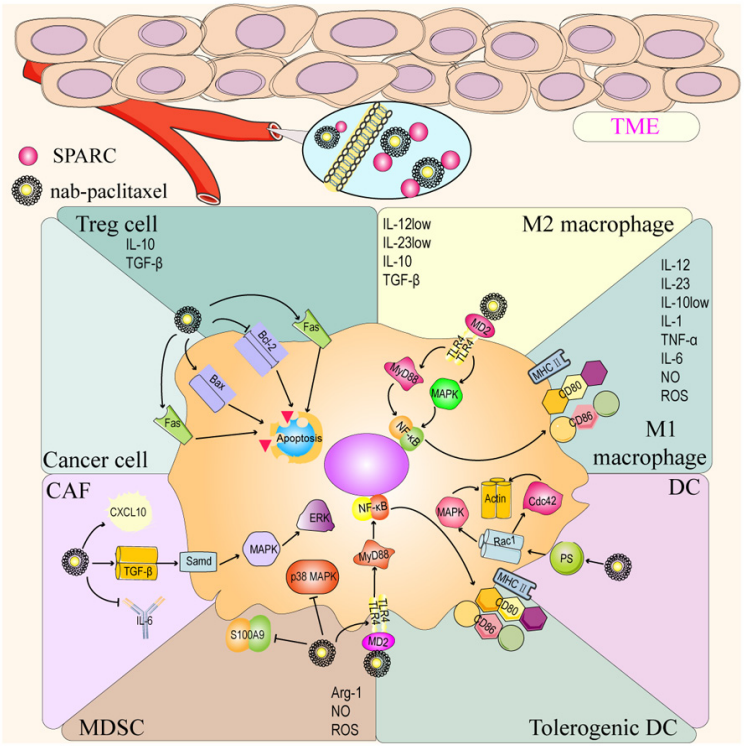

Figure 2. The regulatory mechanisms of nab-paclitaxel on the main cell types in TME. Nab-paclitaxel binds to gp60 receptor to penetrate vascular endothelial cells, and combines with SPARC expressed by tumor cells to be finally enriched in TME. Nab-paclitaxel reverses the immunosuppressive trend of TME by regulating the functions of various immune cells. The modulation of nab-paclitaxel on major immune cells and corresponding signaling pathways are described above.

and downregulated the expression of cancer cells epithelial-mesenchymal transition markers. Reports indicate that CXCL10 blocks the expression and secretion of IL-6 by CAFs. This ultimately attenuated the migration and invasion of CAFs as well as cancer cells [99]. In addition, low-dose paclitaxel relieved pulmonary fibrosis by up-regulating miR-140 to inhibit TGF-β1/Smad3/MAPK pathway [100]. Based on the depletion of CAFs by nab-paclitaxel, VEGF and TGF-β production are also reduced; this improves abnormal blood vessel development in TME [101, 102]. In summary, nab-paclitaxel effectively improves the dense immune isolation environment within the tumor and promotes T cell tumor infiltration and drug delivery by inhibiting the proliferation and function of CAFs.

Dense distribution of nab-paclitaxel reverses the immunosuppressive trend of TME

es which promote tumorigenesis, growth, and angiogenesis [18]. Furthermore, CAFs promote tumor invasion and metastasis, which are related to IL-6 secretion and the promotion of epithelial-mesenchymal transition markers in cancer cells [17, 80]. Therefore, blocking the interaction between cancer cells and CAFs is a novel target for tumor therapy.

As a first-line drug for solid tumors represented by pancreatic cancer, nab-paclitaxel acts on tumor stroma and inhibits tumor invasion and expansion. R Alvarez et al. reported the destruction of pancreatic cancer tumor stroma by nab-paclitaxel combined with gemcitabine. By measuring the collagen content, structure, and density of CAFs, nab-paclitaxel reduced collagen proliferation by changing the structure of collagen, thereby reducing the proliferation of CAFs [20]. Rui Feng et al. discovered that nab-paclitaxel upregulated the expression of C-X-C motif chemokine 10 (CXCL10) in cancer cells

The currently recognized TME cells include tumor cells, CAFs, vascular endothelial cells, and immunosuppressive cells including MDSCs, TAMs, Treg cells, and tolerogenic DCs. Immunosuppressive cells including TAMs secrete molecules including VEGF, TGF-β, and stroma metalloproteinase, which down-regulate the IL-12 expression and limit T cell tumor infiltration and cytotoxicity of CTLs [80, 96, 97]. TME is considered a complex tumor ecosystem that supports tumor growth and immune escape. Therefore, the current immunotherapy and chemotherapy need undergo extensive immunomodulation to reverses tumor immunosuppression, thereby potentiating host anti-tumor immune responses.

In the previous sections, we introduced paclitaxel regulating immunosuppressive cells including M2 macrophages, MDSCs, and tolerogenic DCs. Nevertheless, fat-soluble paclitaxel and other chemotherapeutic drugs are often

limited by administration difficulties and dose toxicity, hence unable to enrich tumor tissue for a long time. Nab-paclitaxel is known as novel solvent-free paclitaxel because it has albumin nanoparticles, a natural carrier of hydrophobic molecules. Nab-paclitaxel is cleaved *in vivo* into soluble albumin drug complex molecules and binds to gp60 receptors on endothelial cells. As a result, vesicles are formed on the target cell membrane carrying the albumin complex across the endothelial cell membrane into the surrounding tissue [9]. The binding of albumin-bound paclitaxel to the abundant SPARC in the tumor enriches paclitaxel in TME [8]. Immune cells accumulate in the periphery of the tumor and differentiate into multiple types due to the influence of tumor growth factors. Different degrees of immune response have been reported in TME; this results in various classes of tumor immune microenvironments (TIME) [103]. In terms of tumor immune escape, TIME is summarized into two main types. A major subtype is manifested by chronic inflammation, including infiltrating immune cells, extensive chemokine profiles, and type I interferon expression [16, 41]. These tumors seemingly resist immune attack via a dominant suppressive effect of an inhibitory pathway of the immune system. Another major phenotype lacks T cells and inflammatory responses, and tumors appear to resist immune attack via immune system rejection or neglect [80, 104]. Nab-paclitaxel blocks the inhibitory effect of M2 macrophages, MDSCs, and Treg cells on $CD8^+$ T cells, thereby reversing the immune suppression trend of TME. On the other hand, nab-paclitaxel inhibits the growth of CAFs and tumor cells, helping the CTLs tumor infiltration, and reduces the immune tolerance of tumors. Nab-paclitaxel effectively limits the tumor immune escape caused by these two types of TIME.

Nab-paclitaxel improves CTLs recognition and clearance of tumor cells

T cell tolerance plays an important role in tumor escape and limits efficacy of tumor immunotherapy [36]. T cell tolerance is attributed to immunosuppressive cells, for instance, MDSCs produce ROS and peroxynitrite thus inducing modification of TCR and CD8 molecule [72]. These changes cause loss in the ability of $CD8^+$ T cells to bind to the antigen-MHC complex and induce peripheral blood $CD8^+$ T cells antigen anergy [105]. Moreover, CTLs are highly differentiated cytotoxic T cells and generally cannot exist in tumor tissues for a long time. CTLs express the programmed death protein 1 (PD-1), therefore, some tumor cells express PD-L1 extracellularly causing apoptosis of CTLs [69, 106]. In addition, exposure of memory T cells are to a low antigenic TME for a long time, significantly changes the process of memory T cell differentiation (T cell failure) owing to insufficient antigen stimulation or persistent inflammation. T cell failure is manifested by several characteristics, such as gradual loss of effector functions, continuous up-regulation and co-expression of multiple inhibitory receptors, changes in expression and function of key transcription factors, metabolic disorders, and ultimately failure in conversion to CTLs [69, 106]. Therefore, use of chemotherapeutic drugs to assist $CD8^+$ T cells to obtain tumor-specific antigens and to rapidly clear tumor cells can improve anti-tumor immunity [107].

Nab-paclitaxel is a microtubule stabilizer that induces G2/M stage tumor cell cycle arrest and inhibits expression of cyclin B1, causing foration of unstable micronucleus and damage to tumor cells [108, 109]. Damaged tumor cells are gradually exposed to specific molecular patterns, such as High-mobility group box 1, calreticulin, PS, and DNA [4]. In addition, paclitaxel, a cytotoxic anticancer drug can induce tumor cell apoptosis by up-regulating expression of Fas and modulation of Bcl-2 family proteins in tumor cells [110, 111]. Apoptotic cells release high amounts of tumor-associated and specific antigens, maintain the tumor's inflammatory environment, and stimulate proliferation and differentiation of $CD8^+$ T cells. Due to the stimulation of cytokines (IFN-γ, TNF-α, etc.) and tumor antigens, CTLs cells express high levels of FasL, which bind to Fas on the surface of tumor cells to trigger programmed cell death [36]. These indicate that nab-paclitaxel promotes the proliferation and differentiation of $CD8^+$ T cells, and further cooperates with CTLs to achieve tumor clearance.

Conclusion and perspectives

Nab-paclitaxel shows the potential to promote anti-tumor immunity by regulating the immune system. High biocompatibility and immuno-susceptibility of nab-paclitaxel improves targeted delivery, therefore, tumor cells can be recognized and eliminated by induction of immune

system by nab-paclitaxel with a high degree of specificity and low toxicity. In this review, modulation of cancer-immunity cycle by nab-paclitaxel was explored under four facets including enhancing antigen presentation ability of APCs, indirectly promoting T cell activation, reversing immune suppression of TME, and cooperating with CTLs to kill tumor cells. The multi-faceted induction of nab-paclitaxel in the cancer-immunity cycle may provide new avenues for tumor treatment. Mechanisms of the cancer-immunity cycle are still being updated; it suggests nab-paclitaxel may also have novel immunomodulatory mechanism worth exploring. Further studies should explore the regulatory role of nab-paclitaxel in innate and adaptive immune activation, for instance, the stimulating effect of paclitaxel on peripheral immune cells, which helps in avoiding cancer metastasis. In addition, immune checkpoint inhibitors (such as antibodies of PD1/PD-L1 or CTLA-4) and intratumoral injection of DCs or CAR-T cells are important immunotherapy, however, single immunotherapy generally results in extreme effect, un-responsiveness or overreaction. The combination of nab-paclitaxel and immunotherapy holds great promise for cancer therapy. Studies should focus on how and what synergistic effects of nab-paclitaxel in combination with other immunotherapy and tumor vaccines may achieve anti-tumor immunity and prevent excessive immune response. Additionally, attention should be paid to the adverse reactions and drug resistance associated with nab-paclitaxel, and methods to alleviate these limitations should be developed. Antibody conjugation may be a good way to expand targeting capacity and enhance cytotoxic efficiency of nab-paclitaxel. Albumin nanoparticles have excellent modifiable properties and provide various potential options for reducing the side effects and drug resistance of nab-paclitaxel. Screening new paclitaxel-related compounds based on structure-activity relationships for the development of unique albumin-conjugated drugs can help in obtaining better safety doses and increase immunomodulatory ability, which are conducive to the personalized treatment of cancer patients.

Acknowledgements

This work was supported by the Fundamental Research Funds for the Central public welfare research institutes (ZZ11-056) and National Science and Technology Major Project (2018-ZX10101001-005-003).

Disclosure of conflict of interest

None.

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