Chapter 1
Nanotechnology-Based Bacterial Immunotherapy

Rajeshwar Kamal Kant Arya, Deepak Sati, Dheeraj Bisht, and Raj K. Keservani

Abstract Recently, the nanotechnology-based bacterial immunotherapy emerged as a new combinatory therapeutic approach for the effective treatment of cancer, which combines the bacterial immunotherapy with nanotechnology for treating cancer. Although both bacterial immunotherapy and nanotechnology are very effective and advantageous solely, single treatment system is insufficient for complete eradication of cancer. Combining nanotechnology with bacterial immunotherapy opens new avenues for treating various diseases, abates the complication of bacterial immunotherapy, and overcomes the deficiency of both systems. Nanotechnology is helpful in targeting deep into the cancer cell due to its small size, enhanced permeability and retention (EPR) effect, and immunomodulatory activity. It also plays an important role in thermal and radio immunotherapy and cancer diagnostic. In this chapter, we highlighted the role of immunity in cancer and the role of bacteria in cancerogenesis, how bacterial immunotherapy is used in combating cancer, and how nanotechnology-based bacterial immunotherapy works on cancer regression.

Keywords Nanotechnology · Immunotherapy · Cancerogenesis · Bacterial immunotherapy · Immunosuppressant

1.1 Introduction

Immunotherapy is a therapy system used to combat cancer by stimulating the inherent defense mechanism of the body. Immunostimulants are used to boost the defense system to fight with cancer cells and destroy them. Immunotherapy is a kind of natural treatment; it is comprised of white platelets, organs, and tissues of the lymph framework. Biological therapy is a sort of treatment that utilizes substances

R. K. K. Arya (✉) · D. Sati · D. Bisht
Department of Pharmaceutical Sciences, Bhimtal Campus, Kumaun University Nainital, Bhimtal, Uttarakhand, India

R. K. Keservani
Faculty of B. Pharmacy, CSM Group of Institutions, Prayagraj, Uttar Pradesh, India

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produced using living life-forms to treat malignancy. Immunotherapy therapy attracted attention as an emergent therapy system in cancer treatment due to its potential to shatter immune tolerance and induce immune effects on selected cancer cell without any side effect (Hu et al. 2015). Immunotherapy can slow down the progress of cancer or arrest the spread of cancer. Bacteria-based immunotherapy for cancer was initiated way back in the nineteenth century (McCarthy 2006). William Coley in 1983 prepared bacterial endotoxin called "Coley toxin," found its regressing effect on carcinoma, and then successfully treated various types of carcinomas (Kaimala et al. 2018). For the treatment of cancer using bacterial immunotherapy, a thorough knowledge of bacterial immunity against cancer cells is to be explored in a more aggressive way. In the last 10 years, various researches have been performed for exploring the relationship between bacteria and cancer (Linnebacher et al. 2011). The bacterial and microbial flora system maintains a balance; the imbalance between microbial floras induces the carcinogenesis by various ways, e.g., bacteria induced inflammation or immunomodulation (Song et al. 2019). Bacteria are causative agents for various communicable diseases, but they also have contribution in causing cancer, e.g., *H. pylori*-associated gastric cancer; mutagenic bacterial metabolite is also thought to be a main factor for cancer due to the inflammatory potential of the *H. pylori* that is associated with gastric cancer (Parsonnet 1995). The chronicity of the infection is directly proportional to the cancer; as long as the inflammation occurs, the chances of cancer development also enhanced (Singh et al. 2019). The intestinal microflora contains microbes that show symbiotic relationship and support the health system by enhancing the immunity against microbial pathogenesis (Zheng et al. 2020). The microflora interactions maintain the balance by protecting from detrimental microbes and provide a biological fencing on the membrane for infiltrating infectious or immunogenic molecule into the blood and also enhance forbearance toward the antigens available on the mucosa (Linnebacher et al. 2011). This process impedes pathogenic colonization, influences the mucosal barrier, and induces the immune response.

The basic concept of immunotherapy was to exploit the patient's immune system as an effective tool for cancer treatment by stimulating the immune system to attack cancer cells. Some old reports demonstrated that an inflammation-related cancer was reduced by accidental infection by erysipelas (Patyar et al. 2010). The anaerobic bacteria can grow in cancer cell, but being pathogenic, they are not good for cancer treatment. Further, studies show that obligate anaerobic bacteria, e.g., *Clostridia*, can proliferate in necrotic or anaerobic region of solid tumor and regress the cancer (Barbé et al. 2006). Various in vivo studies show that *Clostridium*, *Salmonella*, *Bifidobacterium*, and *Escherichia* can be accumulated in cancer cell than the normal cell and can germinate spores in the cancer cell (Oelschlaeger 2010).Various studies suggest that microbes influence the oncogenesis and immunotherapy; they can have the inhibitory or stimulatory effect on the initiation of cancer and progression (Inamura 2020). The bacteria generate toxin and carcinogenic substance that stimulate inflammation or immunosuppression and promote oncogenesis (Nath et al. 2010). *E. coli* causes inflammation and stays in inflammatory cells that influence the microflora composition that promote the carcinogenesis. Beside these carcinogenic

effects, several corroborations suggest bacteria can influence the chemotherapeutic and immunotherapeutic potential (Song et al. 2019).

Bacterial infection stimulates the immune system by modifying various cellular components, e.g., CD4, CD8 T cell, myeloid-derived suppressor cell (MDSC), regulator T cell (Treg), and tumor-associated macrophages (TMA). They also influence inherent immune receptors, e.g., Toll-like receptor (TLR), that are responsible for secretion of pro-inflammatory cytokines (Kaimala et al. 2018). Some bacterial exotoxins also stimulate immune response against the cancer cell and directly destroy cancer cell (Zahaf and Schmidt 2017). Immunotherapy is an impressive strategy for cancer treatment, but the effectiveness is very limited due to their inadequate assemblage at the cancer cell and various unwanted effects. Recently, nanotechnology has gained popularity for conquering these technical shortcomings due to their physicochemical property and versatility. The use of nanotechnology in bacterial immunotherapy can enhance the immunotherapy potential multifolds with their target specificity and intensify the delivery of tumor vaccine, immunomodulator checkpoint inhibitors, etc. (Gupta et al. 2010; Li et al. 2019).

1.2 Role of the Immune System in Cancer

The presence of inflammatory cell in cancer tissue has given the idea of understanding the relation between inflammation and cancer (Singh et al. 2019). Now, the capability of the immune system for initiating and arresting the cancer is established. The imbalance between intrinsic and acquired immunities show immunosuppressant action that instigates the cancerogenesis and proliferation of cancer. Intrinsic immunity generates macrophages against the infection and repairs the tissue (Gonzalez et al. 2018). Macrophages release IFN-γ which destroy the cancer cells and IL-4 and IL-13. TME induces protumorigenic tumor-associated macrophages (TAM) that stimulate angiogenesis, lymphogenesis, and cancer growth (Dhabekar 2011), releases immunosuppressant (IL-10 and TGF-β), prevents maturing of DC cell, and diminishes effector T-cell activity (Taylor et al. 2006). It releases EGF and VEGF that help in cell development, angiogenesis, and restructuring of the ECM by secreting metalloproteinases (MMPs) (Anteby et al. 2004). Damaged and inflammatory tissues contain neutrophils that release the neutrophil extracellular trap (NET), kill microbes, do phagocytosis (Riyapa et al. 2012), and also suppress inflammation. The neutrophils show both protumor and antitumor activities (Brandau et al. 2013). Cancer and stromal cells release chemokines that recruit tumor-associated neutrophils (TANs) to TME. The natural killer (NK) and effector T cells release the TNFα that stimulates the anticancer anti-metastatic hepatocyte growth factor (c-MET). TANs generate new inflammation during cancerogenesis and progression and induce immunosuppression in T cells by PD-L1 expression induced by tumor-derived granulocyte-macrophage CSF (GM-CSF) (Masucci et al. 2019). PMN-MDSCs induce the chronic inflammation and antigen-specific tolerance by T cells (Dorhoi and Du Plessis 2018). NK cells destroy infected cells and

induce the apoptosis. The antigen-presenting cells (APCs) or dendritic cells (DCs) interplay between intrinsic and acquired immunities (Steinman 2006) and present internal and foreign antigens to T cells in the context of MHC molecules (Alberts et al. 2003) and DCs found in all tissues throughout the body. During cancer progression, the DCs prime naïve, memory T cells, and antigen presentation cells develop resistance for antigens and evoke effector T-cell response. Various types of cancer cell carry the cancer-infiltrating DCs (Hubert et al. 2019). At the initiatory phase of cancer, T cell is generated, and naïve T cell invades lymph nodes, on enticement migrated to cancer cell environment, exerts immune response, and destroys cancer cells. T effector cell exhibits antigen-dependent anticancer activity. The low immunogenic cancer cells escaped from T cell, and cancer cells develop a system to prevent themselves from destruction from effector T cell (TAMs, NK cells, and TANs). The effector cells regulate immune checkpoints on CTLS and CD4+T and prevent tissue damage. The checkpoint molecules CTLA-4 and PD-1 inhibit T-cell function (Takeuchi and Saito 2017). The programmed cell death protein (PD1) and programmed cell death protein ligand (PDL-1) are expressed by immune cells and cancer cells, inhibit T-cell activity, and suppress the anticancer function such as T-cell migration, proliferation, secretion of cytotoxic mediators, and restriction of cell killing (Han et al. 2020). The cancer cell environment seize the immune checkpoints and suppress anticancer activities by recruiting regulatory $CD4^+$ T cells (Tregs) that suppress cell destroying activity of Th1 CD4 T cells, CTLs, macrophages, NK cells, and neutrophils (Lee et al. 2020). The expression of PDL-1 LAG-3, CD39/73, or PD1 regulates the Treg-derived immunosuppressive function, and LAG-3 and CD39/73 also exhibit immunosuppressant function by contact-independent mechanisms, which sequester IL-2 and produce immunosuppressants IL-10, TGF-β, prostaglandin E2, adenosine, and galectin-1 (Cai et al. 2019). Invariant NK T (iNKT) cells, subset of T cells, identify the NK cell like lipid antigen CD1d; upon activation, it secretes effector cytokines such as IFN-γ, IL-4, and IL-17 (Fujii and Shimizu 2017). B cell exhibits humoral immunity for microbes by extracting specific antibodies that converted into protein and B cells. B cell assists cancer proliferation by releasing IL-35 and enhances immunosuppressive function by secreting IL-10 and TGF-β (Klinker and Lundy 2012). B cell also induces angiogenesis and chronic inflammation by activating myeloid cells via FcRγ (Rivera and Bergers 2015).

1.3 Role of Bacteria in Immunotherapy

Bacteria functioned as a dual-edge blade for cancer. In one hand, it instigates the cancer, and in other hand, it induces the immune system and blocks cancerogenesis (Linnebacher et al. 2011). In the last 10 years, the killed or attenuated microbes have gained popularity as an important weapon for cancer immunotherapy (Kaimala et al. 2018). The explicit mechanism of bacterial immunotherapy is not so clear till date, but it is believed that weak antigenic cancer with facultative anaerobic bacteria

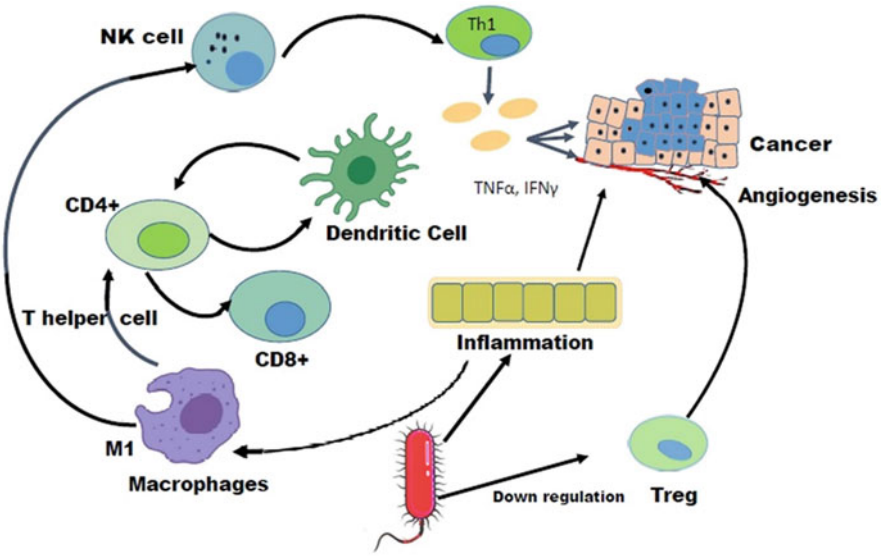

Fig. 1.1 Bacterial immunotherapy

promotes immunogenicity for cancer cell. There are several examples of bacterial immunotherapy using the underlying mechanism (Fig. 1.1). The attenuated bacterial infection harmonizes the activity of various cellular components (CD4+, CD8+ T cells, Treg) and TAM of the immune system to wield their antitumoral immunity (Nelson et al. 2015).

1.3.1 Effect of Bacteria on Myeloid Cell

1.3.1.1 Tumor-Associated Macrophages

A study on mice reveals *S. typhimurium* helps in reversing the cancer by helping in the maturation of myeloid cell (MDCs), reduces the suppressive function, and increases anticancer immune response (Kaimala et al. 2014). Another study revealed that *Salmonella* shows the immunomodulatory effect by elevating the level of TILs, CD11+myeloid cell, CD4+, and CD8 + T in mice and induces the inhibitory effect on cancer cell. The *Salmonella* treatment enhances the level of TAM which is responsible for the activation IFN-γ-dependent Sca-1 and MHC class II proteins. Leschner and colleague have shown that *S. typhimurium* SL7207 on i.v. administration *activated the* TNF-*α and* invades *the tumor cell by disrupting the vasculature* (Leschner et al. 2009). Recombinant attenuated *Salmonella enterica* serovar *typhimurium* vaccine arrests cancer development by inhibiting the HER-2/neu expression. The vaccine shows anticancer effect by diminishing the

immunosuppressive function in cancer environment, increases the level of $CD11b^{+}Gr\text{-}1^{+}$ myeloid cell MDSC and TNF-α, and modifies Treg, $CD4^{+}CD25^{+}$ $Foxp3^{+}$ Tregs (Hong et al. 2013).

1.3.1.2 Dendritic Cells and TANs

L. monocytogenes infect the melanoma cell in human or animal, convert them into antigen-presenting cell like DCs, and express CD11c, F4/80, MHCII, CD40, and CD83 similar to mature dendritic cells, and APC manages the host defense mechanism (Gorvel et al. 2014). Toll-like receptors (TLR) are responsible against microbial infection. *Salmonella* stimulates TLR signaling pathways that increase the expression of co-stimulatory molecules (e.g., CD86 and CD80) on APCs and activate the antigen-specific CD8 T cells and natural killer cells that are responsible for killing and relapse of cancer cell (Hajam et al. 2017). The TLR signal, a cytoplasmic adaptor protein (MyD88a), is responsible for inducing the TLRs and IL-1/IL-18 receptors and activation of downstream of IL-1 receptor-associated kinases (IRAKs) and NF-κB (Cohen 2014). A study show the TLR-MyD88 is necessary for bacterial immunotherapy and mice with MyD88 deficiency were found to be unable to reverse the cancer after *Salmonella* treatment (Kaimala et al. 2014). *Salmonella typhi* vaccine also activates and recruits neutrophils at cancer cell that releases the TNFα producing additive cytotoxic effect in the presence IFN-γ (Vendrell et al. 2011).

1.3.1.3 Effect of Bacteria on Tumor-Associated Lymphoid Cells

Natural Killer Cell

The cancer microenvironment nurtures the lymphoid cells, e.g., NK cells, B cells, CD4+ T cells, CD8+ T cells, and Tregs (Kaimala et al. 2018). The lymphoid cells have both inhibitory and stimulatory effects on the cancer (Wagner and Koyasu 2019). The microenvironment can affect the function of lymphoid cells in cancer cells, and the NK cells can identify and remove cancer cell without any previous antigenic exposure (Kaimala et al. 2018). S*almonella* infection *enhances* IFN-γ and NK level in mice model (Harrington et al. 2007). The cancer depresses the host immunity that disturbs the NK cell response for cancer cell (Guerrouahen et al. 2020). Recombinant *Salmonella* activates NK cell and IL-2 expression. This strain exhibits excellent anticancer activity in vivo against metastatic cancer and also enhances IFN-γ or TNF-α expression that stimulates the anticancer activity (Lin et al. 2021).

1.3.2 CD4+ and CD8+ T Cells

CD4 T cells exert anticancer activity due the helper cells (Th1, Th2, or Th17) that secrete cytokines (IFN-γ and IL-12, IL-4, IL-5 and IL-13). Th1 cell shows anticancer activity by stimulating the CD8 cell or kills the cancer cell by releasing TNF-α and/or IFN-γ (Bascuas et al. 2018). Th1 ceases the progression of cancer by allowing DCs and macrophages and enhances the antigen-presenting activity that allows TCD8+ cell for strong anticancer activity. Th2 has insignificant effect in cancer treatment, but T17 enhances the cancer progression by secreting IL-17 (Lee et al. 2019). The attenuated *Salmonella* increases the infiltration of CD4+ and CD8+ T cell about threefold into cancer cell in mice model. M1 secretes macrophage inflammatory protein 1-alpha that recruited stimulated T cell in cancer cell; *Salmonella* follows this mechanism and increases the T-cell penetration into cancer cell. *Salmonella typhi* increases the leukocyte count and decreases the cancer progression and mitotic index and increases the life span of mice (Vendrell et al. 2011).

1.3.3 Gamma-Delta (γδ) T Cells

Gamma-delta T cells belong to T-lymphocyte cell, having a surface antigen recognition complex type 2. During stimulation, it exhibits excellent anticancer potential due to the release of ample amount of IFN-γ and TNF-α (Silva-Santos et al. 2015). *Mycobacterium vaccae*, *M. obuense*, and BCG stimulate the activation of γδ T cells that result in upregulation of granzyme expression and synthesis of Th1 cytokines (IFN-γ and TNF-α) (Fowler et al. 2012).

1.3.4 Effect of Bacteria on Immunosuppressor Cells

1.3.4.1 Myeloid-Derived Suppressor Cells

Cancer or autoimmune diseases can modify the myeloid-derived suppressor cells (MDSCs), found in the bone marrow, peripheral blood, and spleen, that help cancer cell in evading from the host's immune system (Gabrilovich and Nagaraj 2009). MDSCs do nitration of T cell that represses the CD4 and CD8 T-cell-induced anticancer effect (Ostroumov et al. 2018). MDSCs can be a good target for cancer treatment. *Salmonella* triggers the differentiation of intratumoral MDSCs and improves immunomodulatory properties. *L. monocytogenes* LLO reduces the immunosuppressant activities of MDSCs and Tregs in surroundings of cancer tissues. MDSCs reduce Arg1 expression and IL-10 by Tregs. It both causes the above action (Wallecha et al. 2013) and also depresses the expression of T-cell receptor and immunosuppressive cytokines (Lindau et al. 2013).

1.4 Regulatory T Cells (Tregs)

Tregs are involved in autoimmunity and cancer diseases. Generally, $CD4^+CD25^+Foxp3^+$ has the preventive immunogenic reaction for autoantigen. These cells inhibit the tumor antigen-specific CLTs and decrease the anticancer immunity (Manzo et al. 2015). Study shows attenuated *S. typhi* reduces Tregs cells in cancer-draining lymph node that diminish lung metastasis and cancer progression, improve animal life (Vendrell et al. 2013), and also decrease the proportion of $CD25^+FoxP3^+$ cells in the spleen and CD4+ T cells in a colon cancer model. It also elicits the conversion of immunosuppressive MDSCs into TNF-α-secreting neutrophils and reduces the generation of Treg cells, especially in the presence of tumor-specific CTLs (Hong et al. 2013). *Salmonella* vaccine also acts on the downregulation of CD44, a key cell surface molecule on Tregs as well as tumor cells, and contributes to angiogenesis and proliferative potential (Liu and Chopra 2010).

1.5 Nanotechnology in Immunotherapy

The cancer immunotherapy shows promising results, but developing a low toxic dosage form with high targeting efficiency and excellent efficacy is a difficult task. Nanotechnology has the revolutionary potential to reform the cancer therapy. Nanoparticles have already established its significance in the modern drug delivery system; it ameliorates the immunostimulation, cancer cell targeting, bio-distribution, and release kinetics. All the nanoparticulated system (nanosized vesicular system, e.g., nanoparticles, liposomes, etc.) could be utilized for passive and active immunotherapy (Sharma et al. 2017; Keservani et al. 2017a, b). Nanotechnology improves the cancer immunotherapy by protecting the burden during circulation, targeting the immunotherapeutics to the cancer tissues, inducing the immune system, and modulating the immunosuppressant action for cancer cell (Qian et al. 2018; Keservani et al. 2017a, b). The nanosized immunotherapeutic system can improve the therapeutic techniques and conquer the difficulties related to the poor therapeutic responses, for example, insufficient stimulation, side effects, and suppressed pharmacological activity. Researchers are continuously evolving the nanomaterials with improved structural properties and surface modification ability that can be exploited for guiding nanotechnology-based immunotherapeutic into the vasculature, avoidance of opsonization, and assemblage of nanoparticles at the cancer cell due to enhanced permeability and retention (EPR) properties. A controlled drug release can be used to design nanoparticle systems specific for microbiome intervention in cancer. However, this represents a generally unexplored area (Song et al. 2019). The first cancer vaccine, Sipuleucel-T was approved by the US Food and Drug Administration (FDA) in the year 2010 for prostate cancer treatment (Cheever and Higano 2011).

1.5.1 Nanoparticle-Based Bacterial Immunotherapy

Recently, new combinatory therapeutic approaches emerged for the effective treatment of cancer because single-treatment method is insufficient for complete eradication of cancer. The advantages of bacterial immunotherapy and nanoparticle-based immunotherapy are combined together to overcome the deficiency of both systems and also exploit its advantages. Both systems together work effectively with enhanced cancer targeting and excellent efficacy; recently, the researcher has prepared various vaccine and outer membrane vesicles (OVM) and nanobodies for the therapeutic and diagnostic purpose as well; some example are given here. Figure 1.2 shows the nanotechnology-based immunotherapy.)

1.5.2 Nano-vaccine

Live or attenuated bacterial oral vaccine is unable to penetrate into the cancer vasculature. To enhance the penetrability, Hu et al. (2015) prepared oral nano-vaccine containing attenuated *Salmonella* bacteria; the cationic nanosized enwrapped bacterial vectors efficiently deliver the vaccine into the cancer cell. The nano-vaccine protects bacteria from phagocytosis and enhances the circulatory time and acid tolerability in GIT. The nano-vaccine stimulates the T cell, increases the

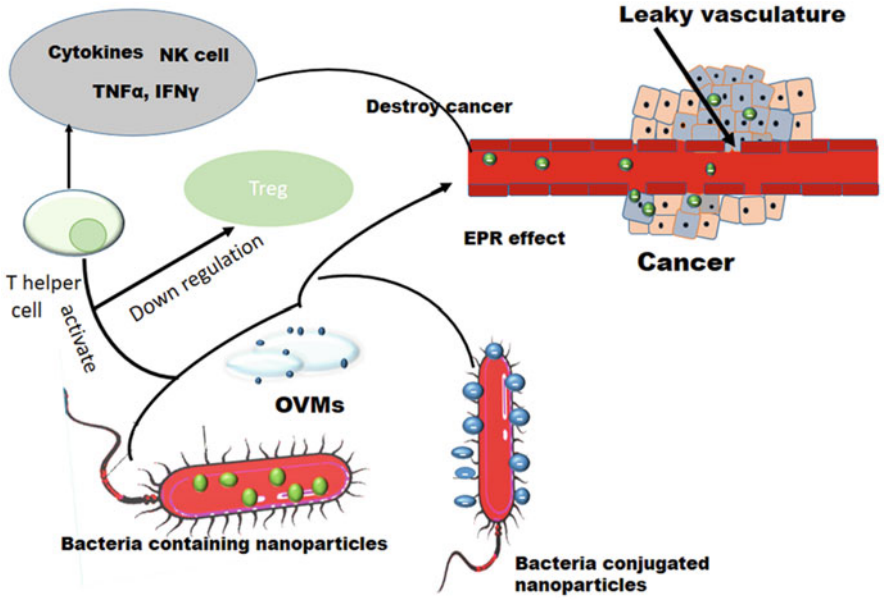

Fig. 1.2 Nanotechnology-based bacterial immunotherapy

secretion of cytokine, and inhibits the cancer growth by suppressing the angiogenesis. In 2019, Terán-Navarro and team synthesized gold nanoparticles containing a peptide (listeriolysin O) derived from bacteria for treating skin cancer. The vaccine works on dendritic cell, modifies the cancer cell sensitivity toward the immune system, and activates the cell killing function that ameliorates the cell viability. The anti-PD-1 or anti-CTLA-4 checkpoint inhibitors when incorporated into the nano-vaccine enhance the cancer eradicating function manyfold. The study shows the nano-vaccine is a safe therapeutic system and enhances the immunity against the cancer cell.

The outer membrane vesicles (OMVs) derived from Gram-negative bacteria are effective vaccine platforms with precedence for safe use in humans (Davitt et al. 2016). Unlike most subunit antigens and synthetic nanoparticles, OMVs contain endogenous immunostimulatory ligands and deliver the antigens in their native orientation. OMVs could induce antigen-presenting cells by engaging the intrinsic receptors and CMI by activating the APC, co-stimulation, and cytokine production. After the uptake, the DCs enhance the surface MHC classes I and II, CD80 and CD86 expressions. OMV-induced DCs produce the T-cell polarizing cytokines IL-1β, IL-6, IL-18, and IL-12 and their expression contributed by TLR4, whereas the NLRP3 inflammasome was required for OMV-induced production of IL-1β and IL-18. Following vaccination of mice, Th1- and Th17-type T-cell responses were observed after ex vivo restimulation with OMVs and HK bacteria. In addition, OMV vaccination produced functional $CD8^+$ T cells capable of killing bacteria-infected cells. Collectively, these results support the utility of the OMV platform as a nonliving vaccine that can lead to protective CMI against bacterial pathogens (Davitt et al. 2016).

In another study, the nanosized outer membrane vesicles (OVM) were prepared from the Gram-positive and Gram-negative (*E. coli* Δmsb B and *S. aureus*) bacteria (Kim et al. 2017). The bacterial cell allows genetic modification that can be utilized to attach targeting moieties on the surface and enhance the safety feature by eliminating the endotoxins. Bacterial cell can also load various chemotherapeutic agents. Such system can be utilized for effective anticancer immunotherapy. These extracellular vesicles induce IFN-γ generation, and the results exhibit the potential of OVM as a cancer immunotherapeutic without side effects. The nanosized OVM assemblage in the cancer cells produces the IFN-γ and cytokine CXCL10 in the cancer microenvironment and elicits the anticancer effect. This system also enhances the memory cell production for longer anticancer effects (Kim et al. 2017).

1.5.3 Nanoparticulate System for Cancer Therapy

In 2016, Lizotte and his team member prepared self-aggregating nano-based inhalant system containing cowpea mosaic virus (CPMV) for treating lung cancer; the inhalant system produces effective systemic cancer immunotherapy against the B16F10 in the skin. The nano-based inhalant system stimulates cytokines and

enhances the potency of the immune system against lung cancer, ovarian cancer, metastatic breast cancer, and colon cancer.

As we know, phagocytosis minimizes the circulation time of nanoparticles in the body. To overcome this problem, the attenuated *Salmonella* bacterial membrane vesicles were combined with nanoparticles, and the nanoparticle surface was modified with polyethylene glycol (PEG) and also attached to the Arg-Gly-Asp (RGD) peptide in which the Asp (RGD) peptide enhances the circulatory time and cancer targetability and stability (Chen et al. 2020). Tegafur is loaded to this system that removes MDSc and synergistically enhanced the anticancer effect and prevents metastasis (Chen et al. 2020). This system also induces the intrinsic immunity and accumulated into the cancer tissue due to the enhanced permeability and retention (EPR) and active targeting effects.

Probiotic bacteria were utilized to deliver the nanoparticulated checkpoint blocker to the affected area. The probiotic bacterial nanoparticulated system used a synchronized lysing integrated circuit (SLIC) using *E. coli* Nissle 1917 strain for releasing the target program cell death ligand and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) after intratumoral injection. The computerized SLIC model was used for validating the lysis mechanism for higher therapeutic efficiency. The formulation exhibited higher therapeutic efficiency over the other conventional system and suppressed the cancer in mouse by potentiating the immunity; the formulation enhanced the activation of T cells, an abscopal effect, and enhanced T memory cell in mice. The formulation enhances the cytokine granulocyte-macrophage colony-stimulating factor.

The swimming ability of *Salmonella* could be utilized for effective targeting of cancer cell by incorporating the nanomedicine; it freed the drug by degrading itself at cancer environment; for this purpose, doxorubicin incorporated nanosized liposome into bacterial cell (Zoaby et al. 2017); and the bacterial motility is pH- and glucose-dependent, which favors the cancer environment. The nanoparticles are incorporated into the bacterial cell by incubation or electroporation. Electroporation builds ephemeral aperture on the bacterial cell wall that provides entry to the nanosized liposomes inside the bacteria. The metabolic process causes the drug release from the liposome; mainly, ammonia causes the osmotic imbalance in the liposome and releases the drug that destroys the bacterial carrier and kills the cancer cell. This process enhances in situ therapeutic efficacy (Zoaby et al. 2017).

1.5.4 Thermotherapy

Several cancer therapies are available, but cancer monotherapy can result in reappearance of cancer due to partial destruction of cancer tissue. To overcome these problems, Park et al. (2020) developed nanoemulsion containing anaerobic *Clostridium novyi*-NT spores for synergistic image-guided combinational cancer therapy. The nanoemulsion also carries scintillators for guiding MRI and for an image-guided X-ray photodynamic therapy (PDT) of the normoxic peripheral

cancer. The nanoemulsion was used for the treatment of the hypoxic central tumor tissues. Photosensitizer-coated NSs (PS-NSs) and *C. novyi*-NT spores are emulsified with clinically available ethiodized oil (Lipiodol) to be the nanoemulsion and injected into the tumor with computed tomography image guidance. Following the image-guided X-ray PDT and anaerobic *C. novyi*-NT combination treatment, apoptotic cell death in cancer tissues, including both peripheral and central tumor regions, is significantly higher than in the control groups. This nanoemulsion system can overcome the limitations of conventional cancer therapy, resulting in increased cancer therapeutic efficacy.

1.5.5 Radiotherapy and Diagnostic Purpose

Nanoparticles coated with bacterial wall was prepared that also contains the immunostimulatory PC7A/CpG Polyplex core and imide groups. The bacterial nanoparticles with various attachments can ameliorate the antigen salvage, improve the affinity of antigen toward the MHC-1, and also augment the innate immunity by proliferation of the T cells. Nano-bacterial system on combining with radiotherapy arrests the neoantigens and increases their absorption in DCs and from there delivered to MHC-presenting cell and activated the effector T cell; PC7A and CpG assist in modulating the antigen uptake in DCs, raise the expression of MHC-I in TME, and increase the effector T cell and type I IFN. Treating mice with this system exhibits 100% relapse of primary cancer with high survival rate and low metastasis rate. For improving the immunotherapeutic efficiency of the nano-bacterial system, the immune checkpoint can also be combined with the nano-system that possibly impedes the immunosuppressive signals. Such nano-bacterial system can ameliorate cancer immunity and long-term immune response (Patel et al. 2019). In another study, melanin-containing OMVs were prepared from *E. coli* for therapeutic and diagnostic purpose (Gujrati et al. 2019). OMVs show good biocompatibility, biodegradability, and low-cost manufacturing. The strong acoustic waves are converted into optical image; OMVs show superiority over fluorescence method due to high resonating efficiency. OMVs can be utilized for monitoring and imaging of cancer in vivo. The nanosized bacterial cell escapes out from the phagocytosis and the EPR effect; both the two events enhance the residence time in the cancer cell. OMVs could penetrate into deep tissue due to high absorption coefficient. NIR irradiation killed the cancer cell. OMVs also induce the production of anticancer cytokines, including IFN-γ. Dosing for longer time induces IFN-γ production in a sustained manner (Gujrati et al. 2019).

1.6 Challenges and Opportunities for Nanotechnology-Based Immunotherapy

Designing the nanotechnology-based bacterial immunotherapy faces several challenges, e.g., understanding cancer site, microenvironment, microbial flora, and inflammation-derived cancerogenesis and bacterial efficacy against cancer with the toxicological effect or side effects of microflora, and their relationships with cancerogenesis, progression, and treatment of cancer (Song et al. 2019), in the last 10 years of research, several preclinical and clinical studies have been performed on nanotechnology-based immunotherapy, but just a few approaches are so far clinically approved. For example, attenuated herpes simplex virus (genetically modified)-based cancer treatment successfully passed phase III (Shukla and Steinmetz 2016). There is a lacuna between the understanding of cancerogenesis and immunology and in vivo behaviors of nanoparticles that include long-time assemblage at the nontarget site and their safety concern. They can cause toxicity, e.g., hollow silica nanoparticles induce the release of pro-inflammatory cytokines that damages the hepatic tissues. Nanoparticles can influence the constitution of microflora; a depth understanding of nanoparticles and microflora is required for developing new strategy (Song et al. 2019). The intersubject variability should also be considered. The microflora is patient-specific that changes with the food intake, drugs, and other environmental factors, and the nanoparticles can modify the composition of microflora that cannot be predicted in vivo or by clinical investigation. Other challenges may include the production, targeting efficiency, and distribution. Targeting the nanoparticles to the specific organ or tissues is affected by surface morphology, surface properties, and physicochemical properties of nanoparticles (Song et al. 2019).

1.7 Advantage and Disadvantage

The distinct characteristics of nanoparticulated system and bacterial membrane make them an excellent drug delivery system; nanoparticle-based bacterial immunotherapy has excellent immunomodulatory properties, penetrability, biocompatibility, and targeting ability to specific cells and sustained release (Li et al. 2013). The nanosized bacterial system enhances the circulatory time and targeting into deep vasculature because of EPR and also augments the efficacy of thermotherapy and radiotherapy (Park et al. 2020; Patel et al. 2019). The nanoparticle surface also allows surface modification and attachment of ligands that improve the targeting (Li et al. 2013). The nanoparticle-based bacterial system not only improves cancer targeting but also helps in cancer diagnosis. Nanoparticles that may cause immunogenic response have short residence time due to rapid blood clearance.

1.8 Conclusion

The understanding of the role of immunity in cancer pushes scientist to make new system that plays an important role in cancer. Cancer immunotherapy holds great promise in cancer therapy. Collaboration between cancer immunologists and engineers will further the understanding of the complex and underlying immunology, therefore driving technological development. With emerging nanotechnological interventions, the efficacy of many such immunotherapies could be drastically improved, and a merger of these two rapidly growing fields of science could facilitate clinical translation of many cancer immunotherapies.

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