Synthetic Biology for Breast Cancer Therapy

Wan Fatin Amira Wan Mohd Zawawi and Khairunadwa Jemon1
Department of Biosciences and Health Sciences, Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia.
1khairun_nadwa@bb.utm.my

Abstract— Breast cancer is a heterogenous disease, which can easily metastasize to other part of bodies such as bone and brain, leading to poor prognosis. In relevance to this, targeted therapy with immunotherapy and genetic engineering intervention may represent an ideal approach to destroy the cells from metastasize and prevent tumor recurrence in the future. In this review, we present the current situation of breast cancer therapy in the aspect of synthetic biology as well as its future perspectives. This review highlights the use of engineered cells or organisms as a promising strategy for breast cancer treatment. This includes the use of bacterial therapy, virotherapy and cell-based immunotherapy.

Keywords — Synthetic biology; breast cancer; immunotherapy

I. INTRODUCTION

Synthetic biology is a multidisciplinary field that makes use of simple organism or living things by manipulating their native genetic makeup and features to improve their efficacy for example in treating disease. The engineered cells will have the ability to overexpress certain desired gene products with enhanced effects, which would be very beneficial to human health and providing better quality of lives. Due to its robust application, there is increasing evidence for the use of synthetic biology approaches for the treatment of various diseases including cardiovascular diseases [1] infectious diseases [2], and cancers [3–5].

Breast cancer is the most common cancer among women worldwide, amounting to 25% of the total cancer incidence [6]. Current treatments for breast cancer rely primarily on surgical resection, chemotherapy and radiotherapy but these treatments have been reported to be associated with some limitations. Although surgical resection is effective on localized tumor but the possibility of recurrence remains as the cancer cells metastasize. Chemotherapy on the other hand was reported to be toxic to normal tissues and thus can destroy normal cells as well [7]. These limitations could be overcome using synthetic biology approaches that allow successful tumor localization and can provide efficacious treatment. In this paper, we first review the construction of modified bacteria and virus to kill the tumors. Further, we discuss the engineering of tools involved in enhancing the immune system including T cells, dendritic cells and antibodies. At the end of this review, we will discuss the future perspectives of growing applications of synthetic biology for breast cancer therapies.

II. BACTERIAL THERAPY

To date, the implementation of synthetic biology in cancer therapies is prominent and seems promising in combating the disease. Bacteria are the perfect candidates for cancer therapy as they equipped with all the important features to destroy cancerous tumors. For instance, they have the ability to specifically target the tumors and produce cytotoxic, anti-cancer proteins [8, 9]. Their cellular circuits and genetic makeup are relatively easy to rewired in order to release the cytotoxic molecules such as lipopolysaccharides [10]. Unlike other biological organisms, what makes bacteria popular in cancer therapies is due to its flagella that can penetrate the tumor tissues [11] and the chemotactic receptors that provide better sensing towards molecular signals in the tumor microenvironment [12, 13]. It was reported that bacterial strain with improper flagella fail to initiate chemotaxis within cylindroids which subsequently preventing them to accumulate and replicate in tumor tissues [12, 13]. This is done by various therapeutic approaches such as direct cell killing, bactofection and as an alternative to gene therapy. However, studies reported that Salmonella is the most intriguing bacteria used in breast cancer therapy so far.

A pre-clinical study in murine model has shown that modified auxotrophic strain of Salmonella typhimurium was able to cure breast tumors as well as prolonged the survival of the tumor-bearing mice [14]. They also reported that this reisolated leucine-arginine dependent strain known as A1-R strain target breast tumor effectively and the shrinkage of tumor was significantly observed in both in vivo and in vitro settings. These facultative anaerobic bacteria can grow and multiply in hypoxic microenvironments of tumor as well as in necrotic area indicate that they were able to work alone without any help from chemotherapeutic drugs [14]. Meanwhile, mutated strain of S. typhimurium, VNP200009 has been used
as a vector in delivery of prodrug-activating enzyme carboxypeptidase G2 (CPG2) to breast tumors [1][6]. VNP200009- S. typhimurium has also been demonstrated in phase 1 clinical trial to effectively deliver E. coli purine nucleoside phosphorylase (ePNP) gene which could convert 6-methylpurine 20-deoxyriboside (MePDr) into a toxic compound, 6-methylpurine (MeP) to solid tumors [16, 17]. Enhanced oncolytic activity in tumors was observed following the activation of pro-drug mediated by these enzyme [15]. Such activity has also been enhanced in S. typhimurium by the insertion of gene to express LIGHT cytokine, a TNF-family cytokine that could inhibit primary as well as metastatic growth of breast tumors [18].

Listeria has been extensively studied genus of bacteria that promotes direct cell killing of breast tumors and offers efficient bactofection [16]. Sun et al. demonstrated that attenuated Listeria monocytogenes (LM)-based vaccine completely eliminates the metastases and induce regression of primary tumor in 4T1 aggressive mouse breast tumor model [19]. This anti tumor activity has been thought to be mediated by two mechanisms; the release of excessive ROS level in tumor microenvironment and the generation of cytotoxic T lymphocytes response against Listeria antigens which these both subsequently promote direct cell lysis and tumor killing [19]. In another report involving in vivo breast cancer model, a well-defined attenuated Listeria construct ADXS31-164 was found to be capable in breaking immune tolerance towards the HER2/neu self-antigen, which known to be overexpressed in breast tumors [20]. Meanwhile, the affibody displayed on the surface of engineered E. coli BLD21 (DE3) resulted in the internalization of this strain into HER2/neu-positive SKBR-3 breast cancer cells [21]. This non pathogenic tumor-targeting bacteria also carried phage FX174 lysis gene E-mediated autolysis system which promotes autolysis once successfully enter the tumor cells [21]. This demonstrated the use of E. coli as an alternative gene therapy.

III. VIROThERAPY

Besides bacteria, viruses also have widely been used as a synthetic vector for DNA-delivery [22] due to their nature characteristic that can transduce cells efficiently. Several types of viruses including retrovirus, lentivirus, adenovirus, vaccinia virus, and herpes simplex virus [23–25] have been genetically modified to carry the gene of interest and selectively replicate in order to destroy cancer cells. Recombinant viruses like retroviral also involves in the gene-directed enzyme prodrug therapy (GDEPT) that release toxic metabolites from non-toxic pro-drug. For instance, Met-Xia-P450 that carry cytochrome P450 B26 to activate prodrug cyclophosphamide (CPA) in GDEPT causing regression in growth of MDS-MB-231 breast tumor in vivo [26, 27]. Report from clinical trial however revealed discouraging results as only 1% of tumor cells were transduced [28]. Nevertheless, the antitumor activity that was observed warrants further clinical trials. On the other hand, modified retroviral Rexin G equipped with collagen motif has shown its ability to target cancerous lesions [27]. This vector carries a cyclin G1 gene that cause significant reduction of tumor by inducing apoptosis as well as disrupting the proliferation of tumor vasculature [29, 30]. The safety and efficacy of this therapy have been tested in phase I/II study for patients with metastatic breast cancer, in which good prognosis were observed [30]

Virotherapy is typically known to have some limitation for malignant tumor due to the lack specificity of tumor-targeting and inadequate delivery. Interestingly, a decade ago, study by Sarkar et al. (2005) has overcome the limitation of insufficient delivery to malignant human breast cancer by dual-specific cancer targeting strategy. This was achieved by manipulating the expression of adenosine E1a protein which is vital for replication under the control of PEG-3 promoter region together with the introduction of cancer-selective cytotoxic cytokine gene, mda-7/IL-24 [31]. Administration of this adenosine CRCA (designated Ad.PEG- E1A-mda-7) into mouse model has completely eliminated the primary as well as distant breast tumors [31]. In addition to this, engineered vaccinia virus (VACV) GLV-1h164 has also shown its therapeutic potential in aggressive triple negative breast cancer that is well known to be associated with high level of vascular endothelial growth factor VEGF. Besides the oncolytic activity possessed by the VACV itself, this novel virus encodes for an anti-angiogenic scAb (GLAF-2) and had cause a significant reduction of angiogenesis and tumor sizes [32]. Synergistic oncolytic effect was also observed in GI-101A breast cancer by the combination of a β-galactosidase-activatable prodrug with VACV strain GLV-1h68 [33].

IV. CELL-BASED IMMUNOTHERAPY

Nowadays, cancer immunotherapies are gaining momentum as promising strategy to eradicate tumor cells or at least control the malignancies. The priming of T cell response to target tumor-associated antigen (TAAs) is the fundamental of this therapy. With respect to this matter, T cell itself has widely been engineered to increase its efficacy, and same goes to dendritic cells (DCs) and antibody (Abs) [34–36].

Adaptive T cell therapy involves the use of tumor infiltrating lymphocytes (TILs) particularly T cells that have been removed from cancer patient which will then undergo expansion and infusion back into the patient. In engineered receptor therapies, T cells is extracted form TILs and genetically modified ex vivo to express a cancer-targeting receptor and expanded before injected back into patient to treat cancer. There are two types of receptors involves in this approach; T cell receptor (TCR) and chimeric antigen receptor (CAR). These receptors play a significant role in targeting cancer epitopes [37] [38] and markers that expressed on the cell surface, respectively [39]. These receptors have been designed to enhance the specificity and expand the signaling pathways in controlling the therapy.

Genetically modified T cells have been tested in wide range of cancer studies. For instance, complementary CAR-engineered T cells mediated with CD28 that fused to CD3 targeting its ligand; MuC1 and ErbB2 expressed by breast cancer, respectively. Upon binding with its ligand, T cell proliferation and interleukin (IL)-2 production will be triggered, thus can kill the tumor efficiently [40]. Besides, Hereceptin-2 (HER2)-CAR-modified T cells has shown great promise in treating patients with HER2-positive tumors [41]. It is worth to note that phase I/II clinical trial is currently
ongoing to test the safety and efficacy of HER2-CAR-modified T cells for advanced HER2 positive breast cancer (www.clinicaltrials.gov; NCT02547961). Alternatively, studies reported that T cells engineered with protein TARP-specific TCR kills TARP-expressing HLA-A2+ prostate and breast cancer cells effectively, proving that TARP epitope is highly specific and relevant for T-cell therapy [42–44].

Dendritic cells (DCs) are the most potent and powerful antigen presenting cells (APCs) that permits T lymphocytes activation for host immune response initiation and regulation [45][46]. The manipulation of DCs has emerged as an attractive tool in cancer immunotherapies over the past few decades. The expression of HER-2/neu oncogene (ErbB2) has been demonstrated to be overexpressed in breast cancer and hence is an attractive target for immunotherapy. DC-based cancer vaccines in preclinical study by Sakai et al. (2004) demonstrated that truncation of neu oncogene expressed by DCs prevented the breast tumor growth in BALB-neuT transgenic mice [47]. This result to some extent contributes to the development of effective antitumor vaccine strategy. Meanwhile, in phase I clinical study, Czernecki et al. (2007) have reported that interferon- and bacterial lipopolysaccharide-activated DC that release high level of IL-12 markedly decreased the HER-2/neu expression in breast cancer patients [48]. In addition, APCs (including DC) loaded with sequence of HER-2 extracellular and intracellular domain (designated as HER500) vaccine seems to be well tolerated by patients with breast cancer and this correspond to clinical outcome reported by Peethambaram et al. [49]. DC transfected with human tumor antigen MUC1 also increase the mucin -specific interferon-gamma (IFN-γ)-secreting CD8+ T cells in advanced breast cancer patient [50], which implies the feasibility of this vaccination to advanced disease. Favorably, no toxicity and adverse effects recorded in all patients [50].

Recent advancements in antibody engineering have offered the unprecedented expansion of the usage of antibodies in cancer immunotherapy. The construction of humanized chimeric antibodies has overcome the limitation of murine antibodies for human use. They have the ability to escape the host immune response and highly specific to cell surface antigens, thus could kill the malignant cells effectively [51][52]. A lot of licensed therapeutic monoclonal antibodies (mAbs) for tumoral diseases have been developed over the last three decades [53]. These mAbs have been used in conjunction with chemotherapeutic regimens as well, in order to create a synergistic effect in killing the cancer cells. For instance, Trastuzumab or commercially known as Herceptin has been developed to particularly target HER-2 receptor and has shown significant clinical benefit in HER-2 positive breast cancer patients [54]. The combination of Trastuzumab with cisplatin, carboplatin, and emtansin have shown positive results and strongly supported the application of this neo-adjuvant chemotherapy [55–57]. Recent idea of engineering multivalent antibodies by Kang et al. (2014) to target heregulin-induced HER-3 signaling in breast cancer cells has suggested the use of bispecific anti-HER-2/HER-3 antibody (TAb6) together with lapatinib in breast cancer therapy [58]. This group also reported that tetravalent antibodies are more effective in reducing heregulin-mediated signaling and growth, but bispecific antibody has higher inhibitory activity.

On the other hand, ganglioside GD2 is highly expressed on tumor tissue and is restricted in normal tissue except for the brain, thus it has become a suitable target for mAbs in cancer immunotherapy. Moreover, recent studies have identified the presence of GD2 on breast cancer stem cells [59] and led to enhanced proliferation in triple negative breast cancer [60]. The engineered anti-GD2 antibody has been demonstrated in few clinical trials, showing its efficacy in treating neuroblastingoma [61], advanced melanoma [62] and lung cancer [63], but however, no clinical trial has been done to prove its efficiency in breast cancer treatment [59]. Nonetheless, the involvement of GD2/Met axis in ER-negative breast cancer aggressiveness firmly suggests the usefulness of anti-GD2 in therapeutic breast cancer treatment [64].

V. FUTURE DIRECTION AND CONCLUDING REMARKS

The implementation of synthetic biology in bacterial therapy, virotherapy and immunotherapies has shown excellent promise in both preclinical and clinical settings. Despite these positive early outcomes, a number of fundamental questions remain to be resolved and further improvements are required before the widespread implementation of these approaches in treating breast cancer.

There is an opportunity for synthetic biology-based approach to be combined with other antitumor therapy. Thermotherapies for instance, are more attractive to both patients and physicians and have shed promising effects in breast cancer treatment. This is because they leave native tissue unaffected and causing less disruption to the contour of the breast hence giving better cosmetic result [65, 66]. What makes this strategy is more interesting is that, in recent study, Unga and Hashida have reported that high-intensity focused ultrasound (HIFU), a type of thermo ablation, can act as immunotherapeutic agent as well due to its ability to activate immune response towards cancer [67]. This reinforces the notion that the combination of thermo therapies and engineered immunotherapeutic agent would possibly be extremely effective in improving current breast cancer therapy. This would create an excellent synergistic effect in killing the cancer, given that adaptive immune response are more potent at elevated temperature [68].

ACKNOWLEDGMENT

We are grateful for the UTM scholarship awarded to Wan Fatin Amira Wan Mohd Zawawi. We would like to thank the Ministry of Higher Education Malaysia (FRGS grant no: 4F729) for funding this research.

REFERENCES


