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Carbohydrate Based Antigens: Potential Tool for A Targeted Immunotherapeutic Approach to Treatment of Metastatic Breast Cancer

Bhagwat P*, Kale S

Sandip Foundation's Sandip Institute of Pharmaceutical Sciences, Mahiravani, Nasik- 422213, Maharashtra, India

ABSTRACT

Metastatic breast cancer (MBC) might be curable in very less percentage of cases through a multidisciplinary approach including combination chemotherapy regimens in selected patients, usually young, and with limited metastases. Glycosylation changes that occur in Metastatic breast cancer often lead to the expression of tumour-associated carbohydrate antigens. In breast cancer, these antigens are usually associated with a poor prognosis and reduced overall survival. The carbohydrate antigen Globo H commonly found on breast cancer cell is a potential target for vaccine therapy. The complex carbohydrate molecule Globo H hexasaccharide conjugated to keyhole limpet hemocynin and administered with the immunologic adjuvant QS-21 can be used as a vaccine for patients with breast cancer. Present paper discusses the therapeutic strategies attempted to target tumour-associated carbohydrate antigens in breast cancer by stressing on interconnectivity in cancer immunotherapy, tumour associated carbohydrate antigen & some problems with carbohydrate vaccine development. This treatment might emerge new hope in treatment of metastatic breast cancer.

KEYWORDS: Active immunotherapy, Tumour associated carbohydrate antigen, Globo H, Keyhole limpet hemocyanin, QS-21.

***Corresponding author-:**

Bhagwat Pranjali
Assistant Professor (Department of Pharmaceutics)
Sandip Foundation's
Sandip Institute Of Pharmaceutical Sciences, Mahiravani, Nasik- 422213, Maharashtra, India
E Mail ID: pranjalikbhagwat@gmail.com
Contact: +91-7588707393

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1. INTRODUCTION

Cancer is a generic term for a large group of diseases that can affect any part of the body. Other terms used are malignant tumours and neoplasms. One defining feature of cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs. This process is referred to as metastasis. Metastases are the major cause of death from cancer[1].

Breast cancer is a malignant tumour of breast tissue. It is the most common cancer and the second most common cause of death from cancer in women world wide[2]. Similarly breast cancer is the most common cancer in Indian women with the third most fatality rate contributing to 10.2% cancer related deaths while other fatal cancers in women being cervical (17·1%) and stomach (14·1%)[3]. According to a survey by Indian Council of Medical Research published in 2009, female breast cancer is increasing at the rate of 1-4% annually since 1995 to 2005[4]. The age at detection of breast cancer in Indian women is from early 30 to 64 while the median age of detection of breast cancer is 47 years. In breast cancer, incidence rates increases with increase in age and is highest at ages 50-64 years but peak incidence age is shifting downwards from 50-70 years to 30-50 years[5, 6].

Survival in Breast cancer varies greatly depending upon tumour biology & tumour progression in different stages of breast cancer. Survival rate of breast cancer patients is poor and disease acquired metastatic form[7]. Metastatic breast cancer is defined by tumour spread beyond breast, chest wall & lymph nodes. Breast cancer primarily metastasizes to the bone, lungs, liver and brain, with the most common site being the bone. In recent years, worldwide the early detection, and improved treatment in early stages of breast cancer with combination of local & systemic adjuvant therapy has led to increase in cure rate, however 40-45% patients metastasize during the course.

In spite of having new therapeutic regimens full of novel agents, Metastatic breast cancer has remained a big challenge. Most commonly used drug combinations for first line chemotherapy in Metastatic breast cancer are cyclophosphamide & anthracycline or taxanes (paclitaxel) (+/-) a pyrimidine analog (5-flurourasil). Second line treatment depends upon previous exposure of patients to either adrinamycin or taxol or its combination. Second line treatment may consist of single-agent vinorelbine or capecitabine & in third line treatment: single-agent capecitabine or vinorelbine (whichever was not used as second-line treatment)[8].

2. CANCER IMMUNOTHERAPY

Cancer immunotherapy uses host's immune system to reject cancer by evoking the cancer patient's immune system to attack the cancer cells that are responsible for the disease. It is based on the theory that the immune system can recognize tumour specific antigens when they are processed and presented to immune system. In cancer immunotherapy cancer patient's immune system is trained to identify tumour cells by administering a cancer vaccine or the patient's immune system is recruited to destroy tumour cells by the therapeutic antibodies. One of the modality of cancer immunotherapy involves immune cells such as the Natural killer Cells (NK cells), Lymphokine Activated killer cell(LAK), Cytotoxic T Lymphocytes(CTLs), Dendritic Cells (DC), etc., which are either activated *in vivo* by administering certain cytokines or they are isolated, enriched and transfused to the patient to fight against cancer. Such modality is called Cell based immunotherapy.

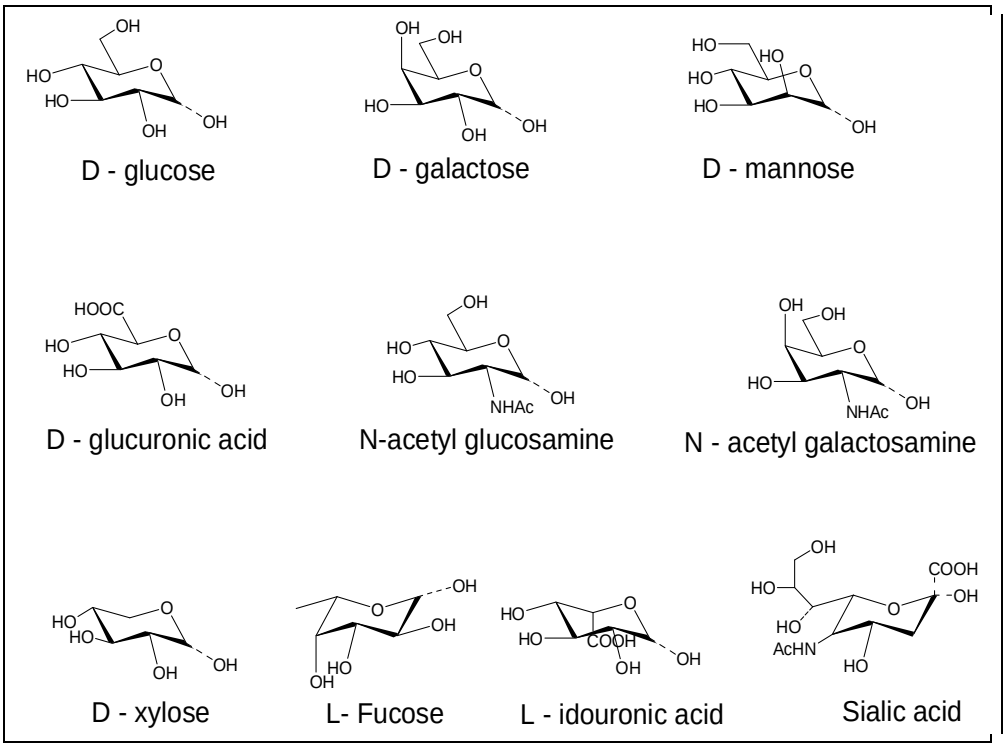
Passive immunotherapy of cancer comprised of antibodies or other immune system components that are made outside of the body (i.e. in the laboratory) and administered to patients to provide immunity against a disease, or to help them fight off an infection. In other words, passive immunotherapy does not stimulate a patient's immune system to "actively" respond to a disease in the way a vaccine does. Monoclonal antibody (mAb) therapy, the most widely used form of cancer immunotherapy today, is a

form of passive immunotherapy. In addition to the passive immunotherapy by monoclonal antibody (mAb) the active immunotherapy of human cancer is a rapidly growing concept. Active Immunotherapy consists of use of cancer vaccines, cellular therapies and adjuvants[9]. To design therapy against cancer, it is desirable to seek molecular targets of cancer that are absent from normal cells. The goal of the cancer vaccine is not to prevent cancer, but rather to stimulate an attack of the immune system on existing cancerous cells. This concept is based on peptides as well as carbohydrate antigens associated with tumour[10].

3. TUMOUR ASSOCIATED CARBOHYDRATE ANTIGEN

Carbohydrates constitute a significant class of biopolymers like DNA and proteins. Mammalian systems have only ten different monosaccharide (Fig. 1), still carbohydrates are much more structurally complex than the protein molecule[11].

Figure 1: Monosaccharides present in mammalian system



Oligosaccharides are presented on the cell surface, as glycoconjugates by covalently linking with other chemical species such as proteins, peptides and lipids forming variety of chemically unique complex

branched structures. Glycoconjugates include glycolipids, glycoproteins, and proteoglycans. Tumour associated carbohydrate antigens (TACA) are group of tumour-associated antigens that have been identified and characterized by Hakomori by their reactivity with antibodies and lectins[12, 13, 14]. The presentation of glycoconjugates on the cell surface is a dynamic system that evolves with the development and differentiation of the cell. Transformation of healthy cell to a malignant cell is related with the changes in the nature and concentration of glycoconjugate present on the cell. The structural characterization of these altered glycoconjugate has identified carbohydrate motifs associated with tumour tissue. Some antigens are exclusively tumour-specific, while others are present up to certain extent in normal tissue, but over expressed on tumour cells. Further some carbohydrates antigens are displayed during foetal development which remain inactive into adulthood and eventually arise again during conversion of healthy cell to cancer cell. Aberrant glycosylation often indicates malignant phenotype and it includes loss or over-expression of certain structures, appearance of truncated structures and the emergence of novel structures on the cell surface. [15]. It is an important criterion for determining the stage and fate of tumour progression. Tumour associated carbohydrate antigens (TACA) present on large number of tumour cell surfaces are important prophylactic treatment[16]. Numerous studies have shown that abnormal glycosylation in primary tumours is strongly associated with poor survival rates of patients. This correlation between glycosylation and tumour establishes consideration of tumour-associated oligosaccharides as components of anticancer vaccines. Breast cancer tumours are associated more frequently with TACA than oncogene products (e.g. myc, ras^{k}, HER2/neu) and their relation with tumour progression is stronger than the deletion or inactivation of tumour-suppressing genes (e.g.p53, p16). TACA are tumour markers and are part of the machinery that is crucial for inducing metastasis. This background indicates that antibodies that bind TACA with high affinity and selectivity could be valuable tools for research, diagnostics, and bio-pharmaceutics and stimulation of a strong antibody response towards these antigens could form the basis of a cancer active immunotherapy. The basic concept of Active Immunotherapy with therapeutic vaccines is based on targeting small number of cells that remain in the patient during treatment. It is the major cause of relapse in cancer[17, 18, 19]. Active Immunotherapy with therapeutic vaccines induces antibodies of sufficient titers against tumour antigens to eliminate tumour cells.

4. SOME PROBLEMS ASSOCIATED WITH CARBOHYDRATE VACCINE DEVELOPMENT

Unfortunately, standard methods used routinely to generate antibodies toward protein and small molecules do not work well in the generation of antibodies toward carbohydrates.

Four main obstacles exist as follows.

4.1 Lack of structurally well characterized carbohydrate materials: The first step in the carbohydrate vaccine development is the identification of potential carbohydrate epitope targets. Analysis and purification of such naturally occurring carbohydrate epitopes is very difficult due to the microheterogeneity of carbohydrates arising from non-template-driven biosynthesis. Further the required unseemly large amount of structurally well characterized carbohydrate materials is often difficult to obtain from natural sources.

4.2 Tumour associated carbohydrate antigens & their behaviour as auto-antigens: Though cancer cells present aberrant carbohydrate molecules on their surface, these cells are essentially originated from host cells and thus these carbohydrate antigens are tolerated to greater or lesser extent by the body. Therefore their poor immunogenicity presents a major obstacle in the development of effective carbohydrate-based vaccines[20].

4.3 Tumour associated carbohydrate and their nature as T cell independent antigen: Tumour associated oligosaccharides are a type of T-cell-independent antigens and thus cannot evoke protective antibodies. Even booster injections of them fail to produce potent immune response and promote antibody class switching.

4.4 Treg accumulation in tumour: CD4+C25+ T regulatory cells (Treg) accumulation in tumour can reduce efficiency of immunotherapy. CD4+C25+ Treg Accumulation not only suppress T cell response but also blunt innate immunity by inhibiting NK cell proliferation. In cancers inhibition of NK & T cell inhibition by CD4+C25+ Treg Accumulation is always associated with poor outcome. Therefore elimination of CD4+C25+ Treg Accumulation by low dose cyclophosphamide becomes essential part of immunotherapy[21].

Concept of Active Immunotherapy took giant step forward when some methods developed to synthesize antigens that mimic natural cancer antigens. In order to solve IInd and IIIrd problem, these synthesized antigens that mimic natural cancer antigens are conjugated to a potent immunogen which act as carrier and this conjugate is co-administered with an adjuvant which act as an immuno-stimulatory molecule. Such strategy targets the "minimal residual disease" which consists of small number of tumour cells that may be present in the patient's different organs.

5. GLOBO H AS A POTENTIAL CANCER RELATED CARBOHYDRATE ANTIGEN

Globo H is a complex glycolipid with terminal hexasaccharide and a type of carbohydrate antigen. The hexasaccharide portion of Globo H is (Fucα1–2Galβ1–3GalNAcβ1–3Galα1–4Galβ1–4Glc). Globo H was originally identified with the monoclonal antibody MBrl[22]. It is highly expressed in breast cancer, ovarian cancer and prostate cancer cells[19]. Further it is expressed by 61% breast cancer specimens and by 20% of breast cancer stem cells. Globo H is a suitable target for vaccine development because of its exceptional expression on tumour cells with only minimal level expression on normal secretary tissue. Thus immunization with Globo H would elicit an immune response to eradicate tumour cells as well as breast cancer stem cells with minimal effect on normal cells. Second reason for Globo H to act as a target is that Globo H & its pentasaccharide precursor stage specific embryonic antigen (SSEA3) are expressed in breast cancer. However, SSEA3 is highly expressed in breast cancer stem cells. In breast cancer, Globo H expression was observed in >60% of ductal, lobular, and tubular carcinoma, but not in nonepithelial breast tumours[23]. Globo H is not expressed in normal tissue except for weak expression in the apical epithelial cells at lumen borders, a site that appears to be inaccessible to the immune system[18].

5.1 Problems associated with Globo H: Globo H being a carbohydrate antigen produces a poor immunogenic response and Globo H & its pentasaccharide precursor stage specific embryonic antigen (SSEA3) are mostly present at secretary border of epithelium which is not freely accessible to immune system and thus fails to provoke immune response[24]. In order to increase the immune provoking power of the antigen, it can be combined with a carrier and an immune adjuvant[21, 25].

6. KEYHOLE LIMPET HEMOCYANIN (KLH) AS A CARRIER FOR THE ANTIGEN GLOBO H

It has been proven that the coupling of a carbohydrate antigen to a foreign protein (e.g. Keyhole Limpet Hemocyanin (KLH), detoxified tetanus toxoid) can overcome the tolerance and the T-cell independent properties. The carbohydrate-protein conjugates can activate the T-cell to produce high levels of carbohydrate specific IgG antibodies and give a booster response after reexposure[11].

Keyhole limpet hemocyanin is a large respiratory glycoprotein obtained from keyhole limpets. KLH, when used as carrier for tumour antigens, evoke long-lasting, strong, consistent and tumour specific IgM and IgG response. KLH also stimulate TH1 cells to produce INF7 and IL4. Though this is efficient way for development of potent immunogen, difficulty in formation of linkage between carbohydrate and protein poses major problem. A coupling protocol should be effective in minimizing structural changes in immunological epitope of carbohydrate antigen and carrier protein[11]. This linkage can be done by directly attaching ε- amino group of KLH to antigen or by conjugating antigen through linker group such as 4-(4-N-maleimidomethyl) cyclohexane-1- carboxyl hydrazide to thiolated ε-amino groups of KLH. Conjugation through a linker generates higher antigen to KLH ratio and better immunogenicity[26, 27].

7. QS-21 AS AN IMMUNOLOGIC ADJUVANT

Immunologic Adjuvant is a substance used in conjunction with an immunogen which enhances or modifies the immune response to the immunogen[15]. QS-21 is a triterpene glycoside saponin isolated from the bark of the South American tree *Quillaja saponaria* (Fam. Quillajaceae). The incorporation of QS-21 enhances specific immune response towards antigen specific antibody to carbohydrate tumour antigen conjugated to the carrier protein[13, 17, 24, 28, 29]. It can enhance B cell as well as T cell responses[30].

8. CONCLUSION

Worldwide breast cancer is second most common cause of death from cancer in women. Metastatic breast cancer has remained a big challenge as survival rate of metastatic breast cancer patient is poor. In order to increase progression free survival rates, introduction of new therapy is required. Cancer Active immunotherapy using Globo H + KLH + QS-21 may be the next great hope for Metastatic breast cancer treatment.

Currently, there is no any vaccine available which directly targets cancer cell. Thus development of this vaccine would become a milestone in breast cancer treatment. Globo H is also present on prostate,

gastric, pancreatic, lung, ovarian and colon cancer tissue and thus Globo H vaccine would also be useful in treatment of these cancers.

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