Cancer-associated antigens and their clinical potential for immunotherapy

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ABSTRACT

Medical and scientific advances in the diagnosis, prevention and therapeutic intervention of cancer have not been sufficient to reduce the overall burden of cancer associated deaths. The origins of cancer cell, from host provide them ultimate survival benefits as host immune system did not recognize it foreign. However, there are several unique signatures such as expression of certain mutant and oncofetal proteins, and cancer/testis (CT) antigens that are specific for cancer cells and can be exploited for the development of therapeutic interventions. In addition, known oncoviruses that participate in tumorigenesis can also be targeted. The cancer cell specific antigens can be used to develop monoclonal antibodies, inhibitors and targeted vaccines against cancer. Antigen-targeted therapy in combination with conventional therapy can offer novel interventions towards the battle against cancer.

Keywords: Cancer, oncofetal antigens, oncovirus, cancer testis antigen, immunotherapy

1. INTRODUCTION

Despite innumerable measures, cancer remains as one of the leading cause of death in men and women worldwide and is expected to surpass heart disease in the next few years [1, 2]. A normal cell transforms into a malignant cell by several means such as by deregulated signaling pathways [3], bystander signaling [4-7], activation of oncogenic pathways [8-13], and DNA damage [14, 15]. The cancer cell hijacks and corrupts the immune system of the host for its unchecked growth via secretion of several growth factors and cytokines. However, the tumor cell remains vulnerable for detection and elimination by the immune system as it produces certain unique proteins or antigens that can be recognized. These antigens produced by the tumor cells are the promising targets for present and future therapy. Cancer immunotherapy aims to trigger the adaptive arm of the host immune system to abolish tumors and prevent recurrence. The immune system harnesses the natural killer cells, macrophages, B cells and antitumor cytolytic T lymphocytes (CTL), that can recognize and kill the tumor cells. Several strategies are available for activating the anti-tumor CTL in cancer patients for the development of a strong and long-lasting antitumor response. In past few years cancer immunotherapy has produced several breakthroughs due to the development of
monoclonal antibodies and adoptive T cell therapies [16-19]. Further, understanding of tumor antigen biology have open uncharted territory for therapeutics as these antigens are specific for tumor cells and can be targeted by immune cells. In such circumstances exploiting the tumor-specific antigens for the development of targeted therapy can be revolutionary as these approaches seem to be promising with fewer chances of side effects.

Substances that are produced by tumor cells that can trigger an immune response are the tumor antigens. Tumor antigens function as biomarkers in identifying tumor cells and can potentially be used in cancer therapy [20-21]. Antigens produced by the tumor cells remain attached to the cell surface or can be released into the circulation. The host immune system detects these antigens and acts towards their eradication. However, the immune response generated against these tumor antigens varies and is often insufficient for keeping a check on the progression of the tumor. Tumor-associated/specific antigens are intracellular molecules that are expressed on the surface of tumor cells [13, 22]. Tumor antigens are generated by several mechanisms including, infection of viruses such as human papilloma virus E6 and E7 in cervical cancer [23, 24], functional changes in tumor suppressor or oncogenes by carcinogens such as p53 [25]and ras [26], mutations in oncogenes or tumor suppressor genes, abnormal expression of proteins that are expressed only during embryonic development such as prostate-specific antigens, melanoma-associated antigens, and the release of several proteins within the cell cytoplasm or its organelles where the cancer cells die [16, 27-29]. In this review, we have briefly described the different types of tumor antigens and their significance in tumor-specificity which can be exploited to target cancer. In addition, we have also discussed the opportunities and challenges posed by targeting tumor-specific antigens that can be helpful for possible future directions.

2. CATEGORIES OF CANCER ANTIGENS

Molecular characterization of antigens produced by the tumor cells has enabled a better understanding of the tumor-antigens with the possibility of targeting them. The cancer antigens are differentially expressed by the tumor cell and can be loosely categorized as oncofetal, oncoviral, cancer-testis antigen and mutated proteins. In this section we have discussed the different categories of tumor-antigens and their role in tumor progression.

2.1. Oncofetal Antigens

Oncofetal antigens are proteins that are characteristically secreted during fetal development; however aberrant expression of these proteins has been observed in certain types of cancer. In fact, measuring the level of these oncofetal proteins can used as a diagnostic as well as a prognostic marker. Meningioma is a tumor that usually arises from the membranes surrounding the brain and spinal cord [30]. Oncofetal protein IMP3 is identified as a novel marker to predict the aggressiveness of meningioma [31]. Immuno-histochemical analysis of meningioma showed an increased expression of IMP3 that correlated with increased recurrence rate and overall poor survival rate. These results suggest that IMP3 could be a potential marker for recurrent meningioma. A recent phase II trial of peptide based vaccine therapy for insulin-like growth factor II mRNA-binding protein 3 (IMP3) improved the overall survival in HNSCC patients with HLA-A*24:02 (+) compared to the HLA-A*24:02 (−) patients [32]. Alpha-fetoprotein (AFP) is an oncofetal protein that is expressed in some germ cell tumors and hepatocellular carcinoma (HCC). AFP is known to participate in the transport of fatty acids, steroids, and heavy metals. Several glycoforms of AFP has been detected in the serum of HCC patients [33]. Fucosylated variant AFP-L3 was found to be one of the key glycoform in patients with HCC and leading to a poor prognosis. In a normal individual, blood contains <5 % of the fucosylated variant, however in HCC patient’s serum >80 % level of fucosylated variant was present. Therefore, these antigens can be used for diagnosis and also as a target for vaccination in several types of cancers. However, one important disadvantage of the AFP antigen is that this type of antigen is secreted intracellularly, and has to be processed and presented by class I MHC complexes. To overcome this drawback, a recent study developed a fully human chimeric antigen receptor, ET1402L1-CAR (AFP-CAR), which is derived from AFP158-166 peptide complexed with human leukocyte antigen (HLA)-A [34]. Interestingly, the results show that liver cancer cells expressing the AFP-CAR is targeted and degranulated by the T cells both in-vitro and in-vivo. This strategy shows a promising new avenue for liver cancer immunotherapy. The fetal tight junction molecule claudin 6 (CLDN6) is an oncofetal protein that has been recently targeted for immune-cancer therapy. T-cell engineered for anti-CLDN6 specificities (6PHU3) has shown interesting
anticancer effects in ovarian cancer cells. Tumors of mice treated with 6PHU3 showed increased infiltration of activated CD4+, CD8+ T cells, and also increased the survival rate of mice [35]. CEA is well known oncofetal protein that has been targeted for cancer immunotherapy in recent years. Increased CEA has been observed in colorectal, gastric and breast cancers [36, 37]. CEA antigen mediated immunotherapy in phase I/II clinical trial treatment increased the cell-mediated immunity and overall survival rate in advanced colorectal cancer patients [38]. Similarly, dendritic cells modified for CEA antigen and MUC1 antigen showed increased survival rates in metastatic colorectal cancer patients compared to who did not receive immunotherapy [39].

5T4 is a membrane bound glycosylated oncofetal protein that is expressed in colorectal, ovarian, cervical, prostate, lung cancers, etc.[40]. 5T4 has been targeted immunologically for cancer therapy by various methods such as by using anti 5T4 vaccines or 5T4 superantigen–antibody fusion protein. Phase I and II clinical trial of 5T4 vaccine in renal [41], colorectal [42] and resistant prostate cancer [43] showed increased clinical benefits. Similarly, 5T4 superantigen–antibody fusion protein mediated immunotherapy showed significant survival in renal cancer patients [44]. ROR1 is receptor tyrosine kinase that is expressed during the development and overexpressed in certain cancers. Several studies have been carried out to target ROR1 by producing monoclonal antibodies against it. Treatment of anti-ROR1 mAbs was effective in pancreatic cancer cells and melanoma cell lines [45]. Since ROR1 is also involved in facilitating metastasis by promoting EMT, anti-ROR1 mAbs downregulated proteins involved in the metastasis of breast cancers [46].

There are also other oncofetal proteins that have been identified and used for cancer therapy, but is awaiting for clinical trials. A recent study revealed that oncofetal protein HMGA2 is overexpressed and can be targeted in many aggressive tumors. Furthermore, it was found that HMGA2 facilitates cellular transformation by trapping topoisomerase I inhibitors into the DNA which thereby blocks transcription [47]. Lin28B is an important oncofetal protein that has gained recent attention in cancer therapy. Mechanistically, it was found that Lin28B acts as a cancer stem cell marker and its overexpression correlates with the expression of important stem cell markers like OCT4, Nanog and SOX2. Further analysis revealed that expression of Lin28B correlates with HCC recurrence after hepatectomy [48].

2.2. Cancer/testis antigen

The cancer/testis antigens are found on germ cells of males and are silent in healthy somatic cells, but they are found to be expressed in a variety of tumors. There are several CT antigens that have been identified such as MAGE-1 (melanoma-associated antigen 1), BAGE and GAGE [65] which was shown to induce T cell response. MAGE-1 was the first CT antigen to be recognized 20 years ago in a melanoma patient with a favorable clinical outcome [66]. Further serological analysis of cDNA libraries (SEREX) helped in the identification of several other immunogenic CT antigens [67-71]. By now, the known CT antigen group contains more than 200 antigenic proteins [72], and the number is constantly increasing. Its specific expression in cancer cells makes CT antigens a good target for cancer immunotherapy. For example, T-cell vaccine that is modified based on the CT antigen NY-ESO-1 was injected into melanoma and ovarian cancer patients [73]. The results showed preliminary evidence of clinically meaningful benefit in these patients. Similar type of results were observed when researchers infused dendritic cell–based vaccines that is processed for human mAb specific for full-length tumor antigen NY-ESO-1 [74]. Apart from CT antigen-based vaccination, CT antigen-based adoptive T-cell therapy is also undergoing clinical trials. NY-ESO-1 CT antigens were found to be expressed in more than 80% of patients with synovial cell sarcoma and approximately 25% of patients with melanoma. Autologous T-cells that are activated against NY-ESO-1 antigen showed beneficial clinical response with complete regression of tumors in some cases. This study is the first to successfully show the effect of adoptive T-cell therapy in non-melanoma cancers [75]. Synovial sarcoma X breakpoint 2 (SSX2), is a cancer/testis antigen that is expressed in melanoma, prostate cancer, lymphoma, multiple myeloma and pancreatic cancer, among other tumors. Autologous T-cells were activated against the SSX241-49 (KASEKIFYV) peptide and tested in cancer cell lines. The results showed clinically beneficial effects suggesting CTA SSX2 as a potential target for cancer immunotherapy [76]. Similarly, MAGE-C2/CT10 is another CT antigen that is mostly overexpressed in multiple myeloma patients. T-cells were processed against different peptides of MAGE-C2/CT10 antigen; however an increased response was observed in T-cells reacting against two distinct
peptide epitopes namely MAGE-C2/CT10171-190 and MAGE-C2/CT10151–170. However, this approach has to be further studies in clinical trials [77]. Interestingly, treatment of acute myeloid leukemia (AML) and myeloma cell lines with azacitidine (AZA) and sodium valproate (VPA) results in epigenetic modification and ultimately results in the upregulation of MAGE antigens [78]. Further analysis revealed that AZA and VPA treatment activated cytotoxic T-cell against MAGE antigens and increased the recognition of myeloma cells. 8 of the 11 patients with circulating MAGE CTLs achieved a major clinical response after AZA/VPA therapy. Recently, cyclin A1, an essential protein for male gametopoiesis has been recognized as a CT antigen, because it is overexpressed in various cancers like testicular, endometrial, and epithelial ovarian cancer [79]. Immunotherapies targeting cyclin A1 CT antigens will be an interesting strategy for cancer therapy. SLLP1 is another CT antigen that has been recently identified as a potential target for T cell receptor therapy in multiple myeloma patients [80]. Newly identified CT antigen POTEE (POTE ankyrin domain family, member E) was found to be overexpressed in various cancers like colon, prostate, lung, breast, ovary, and pancreas. Further analysis revealed that the expression of POTEE level in NSCLC patients is associated with TNM stage and would be a great target for cancer immunotherapy [81]. ASC5, CTAGE1, DDX43 and IL-13RA2 are glioma associated CT antigens that have to be clinically tested [82]. Prostate-associated gene 4 (PAGE4) is a cancer/testis antigen that is typically restricted to the testicular germ cells but is aberrantly expressed in cancers and has to be validated clinically [83].

2.3. Mutated proteins or oncoproteins

Diverse proteins are involved in the maintenance of cellular homeostasis in normal conditions. However, somatic mutations in certain genes results in the translation of mutated proteins that may have relevance in cancer. These mutated proteins are highly cancer specific and are potential targets for cancer immunotherapy. Mutation-derived tumor antigens (MTAs) or tumor-specific antigens have been identified from structural rearrangements, insertions, deletions, substitutions, duplications, inversions and translocations. Furthermore, MTAs are thought to be generated from both tumor suppressor and oncogenes. Wilms tumor gene (WTG)-1 is an important MTA which is highly expressed in leukemia and in other cancers. Recently, combination therapy of WT1 peptide based vaccine and the chemotherapeutic agent gemcitabine was carried out in patients with advanced pancreatic. The results showed that this combination was well tolerated and increased the overall survival of patients [84]. Carcinoembryonic antigen (CEA) was one of the first oncoproteins that was discovered a half century ago. CEA is expressed in a subset of most epithelial cancers, including non-small-cell lung cancer, colorectal, pancreas, breast, gastric and medullary thyroid cancers. Unfortunately, CEA was not successful because of its high immune tolerance. However, recently a vaccine developed against a modified CEA peptide (CAP1-6D) was tested in patients with pancreatic adenocarcinoma. It was found that CAP1-6D vaccine bypasses the immune tolerance and elucidated a dose-dependent T cell response thus providing clinical benefits [85]. MYCN is another oncoprotein that is not expressed in normal cells but is highly increased expressed in different human malignancies such as neuroblastoma (NB), rhabdomyosarcoma, medulloblastoma, astrocytoma, Wilms’ tumor, and small cell lung cancer. Two peptides derived from MYCN have been shown to trigger T cell immune response. The first is the nine amino acid MYCN-derived S69K peptide that contains a HLA-A1 binding motif [86], and the second one is the 10 amino acid VIL peptide in MYCN sequence, predicted to bind to HLA-A2 [87]. Both peptides demonstrated that MYCN will be a useful target to elicit immune-response in MYCN overexpressing tumors. NeuVax is a vaccine developed against oncoprotein HER2 and currently under phase III trial in HER2 positive breast cancer patients with recurrence following surgery [88]. Similarly vaccines targeting human TERT [89], interleukin 12 [90], Tn carbohydrate antigen [91] and many others are currently in clinical trials.

3. OPPORTUNITIES AND CHALLENGE FOR CANCER TREATMENT

Tumor microenvironment provides survival benefits to the cancer cell by inducing immune suppressive environment. However, specific antigen produced by tumor cell can be targeted to eliminate cancer cell and provide therapeutic benefits. Several pathogens such as clostridium and staphylococcus which produces multiple lethal toxins in humans and animals have been explored for targeting cancer [92-97]. T5T4, an oncofetal protein, is a promising anti-tumor vaccine developed in combination with staphylococcus exotoxin [98]. The oncolytic efficacy of anti-S5T4 antibody in combination with the immuno-
Conjugate calicheamicin has been reported [98] and found to reduce tumor growth. In addition, anti-5T4 antibody-drug conjugates (ADC) (A1mcMMAF) targeting 5T4 expressed on tumor cell exhibited potent antitumor activity in several tumor models and induced long-term tumor regression [99]. Interestingly, A1mcMMAF treatment in a non-small cell lung cancer patient-derived xenograft model, resulted in tumor regression. These findings demonstrate the potential of ADCs in targeting cancer. Apart from oncopetals, proteins, oncoviruses have also been explored as an immunotherapeutic target. The available vaccines against HBV and HPV reiterate the importance of developing virus-specific antigens. The vaccines Gardasil and Cervarix against HPV showed significant efficacy in preventing cancer caused by HPVs, including, HPV16 and HPV18, which are known to be responsible for 70% of all cervical cancers. Similarly, cancer cell specificity and immunogenicity of CTAs have made them promising targets for immunotherapy. Therapeutic efficacy of CTAs have been evaluated in several studies [100]. Further, the MAGE-A [101] and NY-ESO-1 [102] were explored for the development of cancer vaccines. Moreover, ongoing cancer vaccination trials analyzing the effectiveness of CTAs are listed at http://www.clinicaltrials.gov. Several clinical trials have demonstrated the encouraging data and indicated the possibility of clinical translation. Recombinant fowlpox-NY-ESO-1 vaccine that promoted antibody production and CD4+ and CD8+ T-cell responses show promising results in patients with melanoma and ovarian cancer. Moreover, the CD8+ responses found to be correlated with progression-free survival [73, 103]. Further, delivery of NY-ESO-1 to dendritic cells by cell surface molecule, CD205, in combination with Toll-like receptor agonists indicated the favorable results in the patients with melanoma and ovarian cancer [74]. Combination of several antigens have shown the clinical impact of immunotherapy [104]. Thus, combination of several CTAs involved in cancer cell survival and progression may enhance the magnitude of the vaccine-induced response and may prevent the immune evasion tumor cells. MTAs are one of the promising targets for the activating T cell mediated immune responses [105]. Although, the schedule, route of delivery and dose determination is crucial for favorable effects. The ongoing clinical trials which are assessing the MTA-targeted vaccines have shown encouraging results [106]. Several peptides, cancer cell lysate, RNA and immune cell based vaccine strategies have been explored, but the vaccine adjuvant is remained to be explored to increase the efficacy of vaccine candidate. As a novel tool, several toll-like receptor ligands have also been explored to induce immune response. Though, promising adjuvants that hold the capacity to activate immune pattern recognition receptors such as nucleotide-binding oligomerization domain (NOD), C-type lectin (CLR), RIG-I–like (RLR), and stimulator of interferon genes (STING) receptor pathways, are still unstudied. Further the role of granulocyte-macrophage colony-stimulating factor (sargramostim) and stimulatory hematopoietins, such as FMS-like tyrosine kinase 3 ligand, can be considered to improve the overall outcome [107-109]. Further, targeting of MTAs through vaccine adjuvant or oncolytic virus shown to induce the immune responses [110, 111]. However, application of MTA targeted agents in combination with CTLA-4 and PD-1/programmed death ligand 1 antagonists may serve novel therapeutics against cancer [112].

4. CONCLUSION

Continuous accumulation of mutation in cancer cells makes them to acquire diverse mechanism to escape from host immune system. However, many antigen produced by cancer cells recognized as non-self by the immune system, which provides an ultimate opportunity to target this disease. Oncopetals proteins, oncovirus, CT antigens and oncoproteins offer excellent opportunity to develop targeted immunotherapy. While multiple antigens are up-regulated in various cancers, the amount of the antigen differs within individual patients. In such circumstances simultaneous targeting of multiple antigens by combination therapy can be fruitful in achieving better results. Recent advances in immunotherapy like checkpoint inhibitors have opened new avenue for scientific exploration. The conventional chemotheraphy treatment supplemented with immunotheraphy holds great promise in improving the efficacy and specificity of the treatment. Thus, targeting antigens produced by cancer cells in combination with conventional cytotoxic therapy could potentially increase the overall survival of cancer patients.

Conflicts of Interest

There are no conflicts of interest.

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