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Cancer stem cell targets – a review

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Abstract. – The varied therapeutic approaches like radiotherapy, chemotherapy, surgery, etc. primarily aimed to target cancer cells specifically. Despite these efforts, they are not completely successful in eliminating this deadly pathological state. These failures ultimately lead to cancer reoccurrence, which is again, another burning problem associated with cancer. The prime reason for the above observation has been found to be the development of resistance by cancer cells towards cancer drugs or cancer-initiating cells (cancer stem cells) remain unaffected by existing treatment procedures. Recent research has evolved two drugs, salinomycin and apoptin, that hold great potential for the future of cancer treatment not only for restricting malignancy, but also in preventing tumor recurrence.

The present review article will put light on these new upcoming cancer stem cell targeting agents.

Key Words:
Cancer stem cells, Cancer, Salinomycin, Apoptin.

Introduction

The total 12% human mortality in the world is due to cancer and environmental factors including lifestyle factors are mainly responsible for this condition[1]. Lifestyle factors comprising tobacco and alcohol use, as well as a poor diet are the main key factors responsible for developing cancer worldwide. Cancers caused by infectious agents account only for 10% of all malignancies[2]. These infectious agents included HPV (Human Papilloma Virus), EBV (Epstein-Barr Virus), and HHV (Human Herpes Virus or Kaposi Sarcoma-associated virus) responsible for causing varied forms of cancers including cancer of cervix, stomach, skin, and lymphatic system[2]. These viruses act by either promoting cancer cell proliferation, or by assisting in the development of cancer cell resistance towards anti-cancer drugs or other therapeutic approaches. Other infectious agents like bacteria and parasites have been anticipated to increase the risk of stomach cancer but are not as well studied. The infectious agents basically provided cancer cells the ability to proliferate independently and infinitely.

Current Therapeutics Overview

The prominent characteristics of cancer occurrence and progression included genetic mutations of oncogenes/tumor suppressors, acquired characteristics of cell survival, cell death resistance, ability to proliferate indefinitely and infinitely without any control or stimulus, and vascularization for spread as well as survival[3]. The therapeutic approaches are designed keeping in mind these cancer characteristics and most of the time acts by disrupting one or two of the above cancer characteristics. Furthermore, radiotherapy and chemotherapy are the most common and widely used treatment options for caner patients. Radiotherapy exploits several properties of cancer cells, such as their high proliferation rate, accumulation of DNA abnormalities, and generation of reactive oxygen species due to changes in their metabolic profile. Chemotherapy is even more varied, devising targets based on a tumor's molecular signature, or other hallmarks of cancer. The outcome of radiotherapy is largely dependent on the sensitivity of the cancer cells to radiation as well as the type of malignancy. Highly proliferating leukemias and lymphomas are more sensitive than glioblastoma (brain tumors) to radiation treatment. Moreover, the tolerance of different tissues and organs to radiation therapy varies widely and is a determining factor in the use of radiotherapy to treat malignancies. Advances in radiotherapy allow precise delivery of radioactive compounds to selectively target specific cancer cell types, causing minimal damage to the adjacent healthy tissues (e.g.: Zevalin, radioactive conjugated CD20 antibody, for the treatment of lymphomas). This has led to increased use of ra-

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diotherapy for sensitive and localized cancers. However, the use of radiotherapy in combination with chemotherapeutic drugs to hinder cell proliferation via inhibition of DNA repair or replication is still the most common due to the robust sensitization of cancer cells. Thus, the use of radiation as a combined therapy is more common than standalone therapy[4,5].

Unlike radiotherapy, chemotherapy is more dependent on cancer type. It is cell type-specific and could be used to treat a broad spectrum of cancers, depending on the mechanism of drug action and its specificity. The possibility of tailoring drugs based on the molecular profiling has resulted in customized or even personalized cancer treatment, and it has led to the generation of novel drug delivery mechanisms to efficiently target cancer cells. Drugs used for cancer therapy could broadly be classified into two groups: (1) Standard chemotherapy: including alkylation and platinum-based drugs that trigger DNA damage and interrupt the cell cycle. Other drugs that are also used in standard therapy are topoisomerase inhibitors (Topotecan, Etoposide), anthracyclines (Doxorubicin, Daunorubicin) that hamper DNA replication, and cell cycle inhibitors (Paclitaxel, Docetaxel, Vinblastine)[6]. (2) Targeted therapy employs small molecule inhibitors and monoclonal antibodies that specifically hinder the function of their respective oncoproteins, thus playing diverse roles in cell growth, proliferation, survival, and cell death, in addition to tumor angiogenesis and drug resistance[6]. Among the most prominent small molecule inhibitors are Gefitinib (EGFR) and Imatinib (ABL1). They are widely used in the treatment of breast cancer and CML patients that are positive for the Philadelphia chromosome (translocation of chromosomes 9 and 22 leading to the formation of BCR-ABL1) (for a complete list of approved drugs, their specific targets, and types of cancers, refer to www.mycancergenome.org[7]. Monoclonal antibodies like trastuzumab (Herceptin) and bevacizumab target HER2/neu, an EGFR-related kinase that is commonly expressed in breast cancers, and VEGFR, a crucial receptor required for intra-tumoral vascularization, respectively[8].

Since targeted therapy depends on the expression of the protein target, and most cancers are heterogeneous, the efficacy of small molecules and monoclonal antibodies is limited. For example, EGFR is highly expressed in most cancers, but the use of the small molecule inhibitor Gefitinib or monoclonal antibody Cetuximab is restricted to only a few cancers because EGFR is also expressed on normal cells and such use may trigger toxic effects[9]. A problem commonly encountered with the use of small molecules inhibitors (or drugs in general) is the development of drug resistance by the cancer cells. This could occur through acquiring mutations in targeted proteins or through the adaptation of alternate cancer cells survival strategies.

Cancer Stem Cells and Cancer Recurrence

The recurrence of a tumor after radiation and/or chemotherapy is often due to the presence of self-renewing cancer stem cells that are resistant to treatment[10]. Cancer cells might also acquire resistance to drug treatment by the development of alternate cancer cell survival strategies. One of the reported cell survival strategy observed in cancer cells is the expression of p-glycoprotein and multidrug-resistance-associated protein (MRP), which helps in the development of resistance[6]. Furthermore, comparative studies between cancer stem cells and normal stem cells have revealed that cancer stem cells have unique abilities to repair themselves and are able to get rid of drug easily by exploitation of active ABC drug transporters[11-13]. The hierarchical hypothesis supports the existence of a small population of cells that possess the self-renewal capacity and can generate terminally differentiated cells[14]. On the other hand, the stochastic model proposed the de-differentiation hypothesis supporting the acquisition of stem cell-like characteristics in differentiated cancer cells[15]. However, the existence of cancer stem cells is most widely accepted hypothesis[16-18]. The lack of complete set of confirmatory markers is the prime reason for this confusion among the above two hypothesis[19]. At present, the prominent confirmatory method for cancer stem cells is the flow cytometric estimation of side population (SP) cells and analyses of few cell surface protein markers like CD34, CD44, CD133, etc. are also common[20-22]. Moreover, ALDH1 is an upcoming promoter of the differentiation of cancer stem cells in some cancers and, on the other hand, its inhibition promoted stemness[23]. The identification of cancer cell-specific surface markers such as CD34+ (leukemia), CD44+ (breast cancer, prostate cancer, and head and neck cancers), and CD133+ (pancreatic, brain, liver, and lung cancers) in several cancer types supported the hierarchical model[24,25]. More recently, a mathematical model correlating the probability of developing cancer to the number

of stem cell divisions (which depends on the tissue type) showed convincing support of the stem cell origin of cancer stem cells[26]. Regardless of the complexities recurrence of cancers caused by surviving cancer stem cells is a widely accepted concept.

Targeting Cancer Stem Cells

The field of cancer research focused on cancer stem cell-targeting agents was initiated with the development of inhibitors against ABC cassette family members. Now cancer stem cell targeting is employed to treat the disease, and it is based on cell surface marker recognition through immuno-therapeutics, small molecule inhibitors against intrinsic signaling pathways like hedgehog, Wnt/β-catenin, and Notch, and tumor microenvironment targeting agents. The discovery of verapamil, a calcium channel antagonist, bolstered the search for MDR inhibitors over the last three decades. However, this search has resulted in only partial success with the development of third generation inhibitors such as tariquidar, inhibiting P-glycoprotein with least side effects[27-30]. Even though much earlier generations of MDR inhibitors showed potential *in vitro*, the clinical translation of these drugs lagged due to their toxicity towards healthy tissues. Nowadays, alternate mechanisms are being explored to target MDRs, such as the development of small peptides that have high affinity to the transmembrane domains of ABC transporters and, thereby, block their activity, transcriptional suppression of MDR genes, and the development of novel drugs that act as substrates for ATP transporters and evade efflux[27].

Xenotransplantation of tumor cells into immunocompromised mice to understand the clonogenicity or stemness properties of these cells has revealed that immune responses play a vital role in tumorigenesis and provide a counter argument for the hierarchical model of cancer stem cell origin. Differentiated oral squamous carcinoma cells are more resistant to the actions of natural killer cells compared to their stem cell counter parts that are positive for CD44 and CD133[31]. On the other hand, circulating leukemic cells and human bladder cancer initiation cells express CD47, hampering their phagocytosis by macrophages in a similar manner to normal hematopoietic stem cells before entering the blood circulation[32-34]. These studies show that cancer stem cells avoid the immune attack and that targeting the cancer stem cell microenvironment to induce directly cancer stem cell death or promote the differentiation of cancer stem cells to prevent metastatic recurrence should be further evaluated. VEGF inhibitors that block angiogenesis are shown to alter the tumor vasculature when used with chemotherapeutics that modulate the pH of the microenvironment, promoting cancer stem cell differentiation and death[35-38].

FDA recently approved vismodegib for the treatment of basal cell carcinoma; this drug targets the protein smo because these tumor cells possess an active hedgehog signaling pathway[39-42]. This has initiated interest in blocking similar signaling pathways to treat other cancers. Vismodegib's success in treating basal cell carcinoma is only partial, however, some resistant tumors develop. This drug was further tested for use in treating medulloblastoma and pancreatic tumors[41]. Similarly, inhibitors of the Wnt/β-catenin and Notch signaling pathways were also explored, however, the abysmal results obtained from treating ovarian and colorectal cancers with vismodegib made researchers cautions when using inhibitors of crucial signaling pathways that are important for normal tissue and stem cell function[41].

Salinomycin

Salinomycin was originally used as an anticoccidial drug in poultry feed and for efficient nutrient absorption in farmed pigs. Gupta et al[41] first described the preferential toxicity of salinomycin toward cancer-stem cells *in vitro*, using E-cadherin-targeted HMLER cells (HMLER-shEcad), which show increased CD44+/CD24- phenotypes with high mammosphere formation capabilities. In the same study they went on to further show that salinomycin is 300 times more effective in targeting cancer stem cell-like cells than paclitaxel and salinomycin pre-treated cells show a 100-fold decrease in seeding capacity, or the ability to form tumors upon xenotransplantation into immunocompromised mice. This study was followed by several reports confirming salinomycin's toxicity among cancer stem cells in gastrointestinal sarcoma, osteosarcoma, pancreatic, colorectal cancer, and breast cancer[43,44]. Cell death mechanisms induced by salinomycin still remain elusive even though they are thought to be largely dependent on the impairment of mitochondrial function, excessive ROS generation, and caspase-dependent or independent pathways based on cell type[45]. The Wnt/β-catenin and Akt/mTOR pathways are mostly affected path-

ways by salinomycin[46]. Moreover, salinomycin has been also reported to affect cancer stem cells[47]. Furthermore, the action of salinomycin varies with cancer type for example in non-small cell lung carcinoma and ovarian cancer stem cells it led to reduced Akt and mTOR activity[48]. On the other hand, in cancer stem cells from head and neck squamous cell carcinoma salinomycin caused elevation of Akt activity[49]. Another prominent activity of salinomycin is inhibition of MDR protein function that results in increased susceptibility of resistant cancer cells towards therapeutic treatment[50].

Salinomycin as Anti-Cancer Stem Cell Therapeutic Drug

Salinomycin is a potent cancer stem cell-targeting agent that could be developed for clinical treatment for a broad spectrum of cancers. The previous use of salinomycin in veterinary treatments provided evidence that the drug is well tolerated among mice, pigs, cats, and dogs and that only very high doses lead to the neural dysfunction that might cause paralysis[51]. So far there has been only one case of human toxicity after treatment with salinomycin where a patient reported having symptoms of weakness in the legs, tachycardia, and decreased reflexes, indicating a similar neurotoxicity as in animals[52]. Animal studies on salinomycin usage along with CGP (a benzodiazepine derivative of clonazepam), an inhibitor of NCX (sodium calcium exchanger), were shown to protect neuronal cells from the toxic effects of salinomycin without altering its anti-cancer properties[53]. This further suggests that salinomycin has great potential for clinical use. The ability of salinomycin to inhibit MDR protein activity as well as Wnt and its downstream signaling cascade, LRP6 and β-catenin, at different concentrations provides substantial support for its use as an adjuvant therapy. Much of the data promoting the use of salinomycin for clinical treatment comes from pilot studies involving four metastatic breast cancer patients, a metastatic ovarian cancer patient, and a patient with head and neck squamous cell carcinoma[51]. The patients are reported to have shown symptoms of mild acute tachycardia and tremor for about an hour, but they reported no persistent long-term side effects. The symptoms did not differ among groups of different ages (40 to 80 years of age), and all of them showed reductions in tumor volume and metastasis[51]. These promising initial successes, however, need to be further substantiated with next phase of clinical trials. Many of the recent pre-clinical studies suggested a combination of salinomycin with several commonly used chemotherapeutic drugs has a better outcome and gives the advantage of using lower doses that can be translated to even more minor side effects[54,55].

Apoptin

Apoptin is basically a chicken anemia virus (CAV)-comprised of three structural proteins of which VP3/apoptin is responsible for apoptosis[56]. Apoptin activity is regulated by different sequences like nuclear localization sequence (NLS), a putative nuclear export sequence (NES) resulting in nuclear and cytoplasmic shuffling[57,58]. Moreover, in normal cells, nuclear localization of apoptin eventually leads to senescence, whereas in cancer cells, it induces apoptosis[59]. Further, it has been confirmed in a recent work that its apoptosis mechanism is by mitochondrial death pathway[60,61]. The mechanisms of the potency of apoptin are well established, but its poor stability hampered its clinical use due to the lack of efficient tumor delivery methods[62]. This is the major negative aspect, which is preventing further development of apoptin therapeutics. So far, many studies have been focused on this aspect and aimed to develop efferent delivery methods including adenoviral, oncolytic, and bacterial systems[63]. Other non-viral, direct delivery methods using small peptide tags, such as TAT and PTD4, which assist in cellular transduction or penetration and facilitate access to the entire tumor volume, are also under consideration[64]. With the recent discovery of human gyroviral-derived apoptin showing similar function in cell death as its chicken homolog, apoptin-based therapies might be developed in the foreseeable future[65].

Conclusions

This is quite evident from the above literature that cancer stem cells targeting drugs are the future of war against cancer. The new drugs targeting cancer stem cells are being developed worldwide as they hold a marked potential to fight effectively against carcinogenesis.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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