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Cancer stem cells targeting agents – a review

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Abstract. – Major current cancer strategies like surgery, radiotherapy, and chemotherapy are compromised due to major problem of recurrence, which usually lead to mortality. The widely accepted reason for this is resistance offered by cancer cells towards cancer drugs or inability of a therapeutic procedure to target real culprits viz. cancer-initiating cells (cancer stem cells). So, there is a current need of development of new agents targeting these cancer stem cells in order to overcome resistance to therapeutic procedures.

The present review article is focused on new cancer cell targeting agents like salinomycin, apopotin etc and their mechanisms to target cancer stems cells will be discussed.

Key Words:
Cancer stem cells, Antibiotics, Resistance, Chemotherapy.

Introduction

Cancer exerts a death toll of ~8.2 million people every year, amounting to 12% of total human mortality. It is next only to cardiovascular and infectious diseases in human morbidity. The majority of cancers (~80%) are caused by environmental and lifestyle factors, while genetic predisposition constitutes the remaining 20%[1]. Lifestyle factors including tobacco and alcohol use, as well as a poor diet are the main risk factors for developing cancer worldwide. Environmental factors, including viral and bacterial infections vary geographically. Cancers caused by infectious agents account for 10% of all malignancies in technologically advanced nations. However, this number rises to 25% in tropical countries[2].

Radiotherapy and chemotherapy are widely used treatment options for the management of cancer. However, resistance offered by cancer cells is the major obstacle hampering efficiencies of these treatment modalities. 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. Cancer cells may also acquire resistance to drug treatment. The acquisition of resistance, as previously mentioned, is caused by the development of alternate cancer cell survival strategies. Cancer cells expressing glycoprotein and multidrug-resistance-associated protein (MRP) develop resistance to chemotherapy, and the identification of these resistance-mediating proteins has initiated vast interest in the field[3]. Further comparison of cancer stem cells, causing tumor recurrence, and normal stem cells that are relatively quiescent reveal that cancer stem cells possess a heightened ability to repair themselves and they express high levels of active ATP-binding cassette ABC drug transporters. These differences allude to the possible origin of drug-resistant cancer cells from normal stem cells that have been transformed (hierarchical hypothesis)[4-6]. It implies that the existence of small population of cells that possess self-renewal capacity and can generate terminally differentiated cells. The alternative stochastic model proposes the random acquisition of stem cell-like characteristics in differentiated cancer cells (the de-differentiation hypothesis)[7,8]. The authenticity of both models is widely debated, but the existence of the tumor initiating cells or cancer stem cells is more profoundly accepted[9-11]. In a recent past, a mathematical model correlating the probability of developing cancer to the number of stem cell divisions (which depends on the tissue type) shows convincing support of the stem cell origin of cancer stem cells[12]. However, because cancer cells undergo both genetic and epigenetic transformations, the de-differentiation of differentiated cancer cells to acquire stem cell-like properties (clonal evolution hypothesis) is also a possibility[13-16]. Regardless of the complexities surrounding stem cell origin, their identification, or the terminology used, metastatic recurrence of cancers caused by surviving cancer stem cells are a widely accepted mechanism.

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Cancer Stem Cells Targeting Agents

The upcoming cancer research is focused on cancer stem cell-targeting agents and was intimated by 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 signalling pathways like hedgehog, Wnt/β-catenin, and notch, and tumor microenvironment targeting agents. The unique capacity of cancer stem cells to avoid immune attack favours the strategy of targeting directly their micro-environment in order to induce cancer stem cell death or promote the differentiation of cancer stem cells to prevent metastatic recurrence.

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[17-20]. FDA recently approved vismodegib for the treatment of basal cell carcinoma; this drug targets the protein smoothened (smo) because these tumor cells possess an active hedgehog signalling pathway[21]. This has initiated interest in blocking a similar signalling 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 medullablastoma and pancreatic tumors[22]. Similarly, inhibitors of the Wnt/β-catenin and notch signalling pathways were also explored. However, the abysmal results obtained from treating ovarian and colorectal cancers with vismodegib made researchers cautious when using inhibitors of crucial signalling pathways that are important for normal tissue and stem cell function[22].

Salinomycin and its Role in Targeting Cancer Stem Cells

Salinomycin is the being explored for its anticancer stem cells properities. It was originally used as an anti-coccidial drug in poultry feed and for efficient nutrient absorption in farmed pigs. Gupta et al[23] first described the preferential toxicity of salinomycin toward cancer-stem cells *in vitro*, using E-cadherin-targeted HMLER cells (HMLERshEcad), 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. 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[24,25] confirming salinomycin's toxicity among cancer stem cells in gastrointestinal sarcoma, osteosarcoma, pancreatic, colorectal cancer, and breast cancer.

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 reactive oxygen species (ROS) generation, and caspase-dependent or independent pathways based on cell type[26]. The major pathways that are affected by salinomycin are the Wnt/β- catenin and Akt/mTOR[27-28]. Lu et al[29], showed that salinomycin targets cancer stem cells by inhibiting the Wnt/β-catenin pathway. Nanomolar concentrations are sufficient to block Wnt, whereas higher concentrations (micromolar) hamper LRP5/6 phosphorylation and β-catenin activation. Salinomycin action on Wnt signaling is further reported among cancer stem cells of chronic limphocytic leukemia (CLL) patients and gastric tumor mouse models. Salinomycin's action on Akt and mTOR is dependent on cancer cell type. While salinomycin treatment of non-small cell lung carcinoma and ovarian cancer cells led to reduced Akt and mTOR activity, cancer stem cells from head and neck squamous cell carcinoma showed elevated Akt activity[30,31]. Another vital function of salinomycin is its ability to inhibit multidrug resistance (MDR) protein function, leading to the increased susceptibility of ATP Binding Cassette (ABC) protein expressing drug resistant cells to treatment. Salinomycin inhibits the function of MDR1, ABCG2, and ABCC11 among both naturally expressing cells and cells that over express these respective proteins after drug treatment[32]. Salinomycin is also reported to be non-toxic to primary normal cells.

Apoptin in Targeting of Cancer Stem Cells

Apoptin is a chicken anemia virus (CAV)-derived nonstructural protein consisting of 121 amino acids. Of the three different proteins encoded by the virus, only VP3/apoptin is involved in induction of apoptosis by CAV. Apoptin leads to regression of tumors in chickens carrying tumors induced by the Rous sarcoma virus[33]. Apoptin consists of a bi-partial nuclear localization sequence (NLS) present at the c-terminal end along with a putative nuclear export sequence (NES) at amino acids[34]. The nuclear and cytoplasmic shuttling of apoptin is driven by these sequences. Apoptin also has hydrophobic

leucine-rich region (aa 33 to 46), which facilitates its self-association and binding with a leukemia protein and its other interacting partners[35].

As the case in cancer cells, apoptin can also translocate into the nucleus of normal cells, but it is promptly exported back to cytoplasm, owing to its nuclear export signal located close to the N-terminus. Nuclear localization of apoptin in normal cells may lead to senescence, whereas in cancer cells, nuclear-apoptin induces apoptosis (typically after 16-30 h). Apoptin triggers apoptosis through a mitochondrial death pathway. The proposed mechanism suggests that apoptin associates with and activates PI3K in the cytoplasm, which leads to translocation of Akt into the nucleus[36]. In the nucleus, Akt activates CDK2 both directly and indirectly, by degradation of p27kip1. Activated CDK2 phosphorylates apoptin at Thr-108, leading to its nuclear accumulation[37]. Apoptin in the nucleus interacts with other proteins like DEDAF, APC, PML, and Nml, and it also leads to the phosphorylation of Nur77 and its translocation to cytoplasm where it converts anti-apoptotic Bcl2 into a pro-apoptotic molecule[38]. The potency of apoptin to induce cell death among minimally transformed cells and broad range of cancer cells has been established for over a decade. However, its translation to clinic has been hampered by poor stability and due to the lack of efficient tumor delivery methods. As a chicken-infecting virus derived protein, apoptin is in itself immunogenic. Therefore, an effective mechanism to deliver apoptin to the tumor site is the main confounding factor preventing the development of apoptin-based therapies. Several delivery methods including adenoviral, oncolytic, and bacterial systems have so far been tested in preclinical studies, and the oncolytic virus newcastle disease virus (NDV) has shown promise[39]. Other non-viral 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[40]. With the recent discovery of human gyroviral-derived apoptin showing similar function in cell death as its chicken homolog, apoptin-based therapies may be developed in the foreseeable future.

Conclusions

It is clear from above literature that a lot of researches are being focused to develop new cancer stem cells targeting agents in order to overcome resistance offered by cancer cells by spherically targeting cancer stem cells. The strategy holds strong potential to become the gold standard therapeutic procedure for efficiently handling the cancer patients. Further research in the area is needed to explore more of these cancer cell targeting agents and strong initiative should be taken to encourage use of these agents in clinical settings for the help of mankind.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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