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TARGETING CANCER STEM CELLS-A NEW THERAPY TO CANCER PATIENTS: A REVIEW

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ABSTRACT

Cancer stem cells have been defined as cells within that possess the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor. It is identified in breast, brain, blood, colon, pancreatic, prostate, melanoma, ovarian, lung cancer and so on. It is considered to be associated with chemoresistance and radio-resistance that lead to failure of traditional therapies. Are directed at the fast growing tumor mass but not the slow dividing cancer stem cells.

Understanding the characteristics of cancer stem cells will help to develop novel therapies to eliminate the initiating cancer stem cell, and the relevant patents on the cancer stem cells, the root of cancer origin and recurrence, has been thought as a promising approach to improve cancer survival or cure cancer patients.

INTRODUCTION

In the world cancer remains a major cause of mortality. Despite great progresses have been in understanding the molecular basis of cancer, the progress in cancer detection and treatment, mortality is still high and there still is not a cure despite great improvements have been made in therapies. The current treatment regimens for cancer have shown limited survival benefits when used for most advanced stage cancer, because these treatments primarily target tumor bulk but not cancer stem cells. Indeed, conventional cancer therapies target neoplastic cells that are largely fast-growing, suggesting that cancer stem cells may survive due to their high resistance to drugs and slower proliferation rate.

All the traditional cancer therapies including surgery, hormonal therapy, anti-angiogenesis therapy, and immunotherapy show a lack of efficacy in terms of long-term outcome because of their failure to target cancer stem cells and toxicity due to non-specific effects on normal cells. In this review, we will focus on the following aspects: 1, Identification of cancer stem cells and therapies that were developed to target them.

In recent years, some molecules (such as CD133, CD44, ABCG2, ALDH) have been defined as the biomarkers of some kind of cancer stem cells, and the aberrant signal pathway (such as Wnt, Notch and Hedgehog signal pathway) have also been suggested as another feature of cancer stem cells. Therapeutics that based on those characters have been developed and some are on clinical trials now. 2, we also discussed the natural compound and some are on clinical trials cancer stem cells, the mesenchymal stem cell mediated gene therapy, to induce cancer stem cell differentiation and some other therapies. Current research is helping us to understand cancer stem cells and in turn will help to develop novel therapies to eliminate cancer and the initiating cancer cell.

Cancer stem cells:

Cancer stem cells are cancer cells that possess characteristics associated with normal stem cells, specifically the ability to give rise to all cell types found in a particular cancer sample. It is often considered to be associated with chemo-resistance and radio-resistance that lead to the failure of traditional therapy. There appear to be several sources from which cancer stem cells may arise. They may arise from normal ASCs (adipose-derived stromal cells), from more restricted progenitor cells or even from differentiated cells. Normal stem cells are more likely to be the targets of mutants and leading to the formation of CSCs for they already possess active self-renewal genes. However, it has been hypothesized that CSCs arising from normal stem cells are more aggressive than those from progenitor cells, though this remains to be proven.

In cancer research experiment, tumor cells are something injected into an experimental animal to establish a tumor. The efficient tumor formation required thousands of the injected cells, the CSCs, have the potential to generate a tumor. In human acute myeloid leukemia the frequency of these cells is less than 1 in 10,000. The first CSC was identified in human acute

myeloid leukemia(AML), showed that a rare malignant cell with the ability to repopulate the entire original disease over several transplantations, implying self-renewal and capacity to differentiate, was only found within the immature CD34+CD38-, but not the CD34+CD38+ subpopulation. After that, cancer stem cells were found in some solid tumors subsequently.

The first solid CSCs were identified in breast tumors in 2003 and then CSCs were isolated from brain, colon, melanoma, pancreatic, prostate, ovarian, lung and interest in the cancer field. It is believed that the creating significant excitement and interest in the cancer field. It is believed that the targeting of CSCs offers important and revolutionary advances in the targeting of cancer. Eradicating cancer stem cells, the root of cancer origin and recurrence has thought as a promising approach to improve cancer survival or even cure cancer.

In the research of killing cancer stem cells, many possible ways were developed to achieve the objective including molecular targeted therapy target molecular signaling pathway, natural compound and their potent to target CSCs, the use of mesenchymal stem cells, and differentiation therapy. Though great progresses have been made in recent year the accurate mechanism of cancer stem cells is still not clear and the really effective therapy is still not found. Here we will discuss the new therapeutic approaches to cancer based on the existence of the cancer stem cells.

Biomarkers based therapy:

Cancer stem cells have been identified in a growing number of hematopoietic cancers and solid tumors and are typically recognized by virtue of the expression of cell surface markers. These cells have been isolated from the bulk tumor population by the expression pattern of cell surface proteins (e.g., CD24, CD44, CD133) and cellular activities, such as the efflux of Hoechst dye or aldehyde dehydrogenase activity by flow cytometry and/or fluorescence activated cell sorting (FACS).

The identification markers that allow the prospective isolation of CSCs from whole tumor tissues will lead to the understanding of important biological prospective isolation properties of CSCs and protein with five transmembrane domains and two large extracellular loops. CD133+ phenotype was first used to identify and isolate brain tumor stem cells in malignant tumor and now it has recently been used to define the CSC population in lung, pancreatic, liver, prostate, gastric, colorectal, and head neck cancer.

The expression of known to play important roles in the maintenance of cancer stem cells have been investigated in putative CD133+ CSC populations of multiple tissues. These CD133+ cells undergo multi-lineage differentiation to neurons, astrocytes, and oligodendrocytes in vitro, and can recapitulate the original tumor phenotype in vivo, unlike the CD133-. Some genes associated with cancer stem cells Nestin, BMI1, Olig2, and Nanog are also found unregulated in CD133+ population of brain, lung, liver and prostate cancers [17-20].

CSCs is often associated with resistance to traditional chemotherapies CD133+ cells have had increased survival in vitro and have been enriched in vivo after treatment with cisplatin,

etoposide, doxorubicin and paclitaxel, as the expression of genes known to be markers of stemness, ABC transporters and the DNA repair pathway.

CD44 is reported as at least one characteristic of CSCs across tissues, including breast, pancreas, gastric, head and neck, ovarian and colon, whereas other markers (e.g., CD42) are not. Early result showed that invasive CD44+ prostate cells also had increased expression of Nanog, BMI1 and SHH, which is similar to CD133+ cells.

The standard CD44(CD44s)molecule is an 85- to 90-KDa transmembrane glycoprotein containing 10 standard exons and four major domains, including the hyaluronanbinding and variably spliced regions, the transmembrane sequence and the intracellular cytoskeletal-signaling domain. Interaction between CD44 and the extracellular matrix glycosaminoglycan hyaluronan (HA) are currently an exciting area of investigation.

Several studies show that the binding of CD44 with HA protein is crucial for tumor progression and also some other research that CD44variant play an important role in metastasis, especially the CD44v6 isoforms. Aldehyde dehydrogenase is a polymorphic enzyme responsible for the oxidation of aldehyde to carboxylic acids, which leave the liver and are metabolized by the body's muscle and heart. This cellular function is likely crucial for CSCs to longevity and probably a key explanation for the reported resistance of CSCs to chemotherapies ,especially those that generate toxic aldehyde intermediate .ALDH+ is also investigated as a marker and leukemia by using determined to be highly ALDH positive. ALDH activity is usually measured by using BOIDIPY amino acetaldehyde (BAAA), commonly known as Aldefuor.

In the past few years, ALDH has been used to characterize CSCs in breast, lung, head, and neck, colon and liver tumor. And several groups have found that shRNA and siRNA knockdown of ALDH1 in colorectal xenografts and lung cells respectively, sensitized ALDH+ CSCs to CPA and 4-hydroperoxycyclophosphamide treatment. Although CSCs are enriched in ALDH+ population in several tissues, it is important to acknowledge possible limitation, especially when used as a signal marker.

Target signal pathways:

Based on the research of the regulation mechanism of the cancer stem cells, cancer stem cells relied highly on the signal path way's stability if they want to maintain the ability to self – renewal and differentiate. Some researchers have suggested that signal path ways disorder or excessive activation may lead to the tumorigenicity. Understanding the mechanisms that underlie the self-renewal behavior of CSCs is of greatest important for discovery and development of anticancer drugs targeting CSCs. During those pathways Wnt Notch and hedgehog signaling pathway may play an important role in the recurrence and maintenance of cancer stem cells.

Though experimental evidence for CSC dependence on this pathway is limited it will be important to develop CSC-selective therapies that avoid potential significant side effect

caused by inhibition of normal stem cells function. Wnt is a group of secreted signaling proteins that bind receptor molecules on the surface of target cells. The strongest evidence of the importance of the Wnt pathway to CSC biology has been reported in myeloid leukemia and it is also been reported to be implicated in the maintenance of CSCs of melanoma, breast, colon, liver and lung cancer. β -Catenin the essential mediator of Wnt signaling is involved in two distinct function in the cell, depending on its cellular localization.

The membrane-localized β -Catenin is sequestered by the epithelial cell-cell adhesion protein E-cadherin to maintain cell-cell adhesion and the cytoplasmic accumulation of β -Catenin and its subsequent nuclear transition and cyclin D1. Activated Wnt / β -Catenin signaling is a key feature of epithelial cancer and is perceived as critical for metastasis and epithelial – mesenchymal transition (EMT). For tumor cells that undergo EMT share characteristics with ESC, there is no surprise that activated Wnt signaling can be linked to stemness.

Some research have shown that the necessity of β -Catenin for self-renewal both normal hematopoietic stem cells and CSCs in chronic myeloid leukemia in a mouse model, and more recently another research have shown that β -Catenin activation is necessary for myeloid precursor transformation in aHoxA9/Meis1-transduced model of AML. A Wnt/ β -Catenin pathway responses include by loss of APC which promise such agents would be therapeutically effective against colorectal cancer and other tumor. A broad spectrum of compounds seems useful to eliminate drugs-resistant CSC, which is thought to be responsible for tumor relapse and metastasis. For instance, NSAID interface with Wnt signaling by directly inhibiting the Wnt target COX2 (e.g. aspirin and sulindac) or by promoting of degradation of TCF (celecoxib). Natural compound, like vitamins A and D and their degradation compete with/ β -Catenin signals. Those drugs may also help to eliminate drugs-resistant CSC, which is thought to be responsible for tumor relapse and metastasis. For instance, NSAID interface with Wnt signaling by directly inhibiting the Wnt target COX2 (e.g. aspirin and sulindac) or by promoting derivatives, compete with β -Catenin/TCF interaction and allow Ecadherin to relocate β -Catenin to the membrane.

Furthermore newly created inhibitors of Wnt/ β -Catenin signaling have just entered preclinical trials such as monoclonal antibodies and small interfering RNAs against Wnt1/2, WIF1 and SFRPs, PRI-724 and CWP232291. The simultaneous discovery of Tankyrase (Tnks) enzymes as critical regulators of Axin and β -Catenin protein levels that can additionally be drugged has opened new opportunities for achieving this goal the success of which had depended primarily on effort to develop inhibitors of TCF/ β -Catenin interaction. The compound XAV939 antagonizes Wnt signaling via stimulation of β -Catenin degradation and stabilization of axin.

Notch signaling pathway is a highly conserved development pathway which plays a critical role in cell-fate decision tissue patterning and morphogenesis. There are four human Notch receptor that consist of an extracellular peptide containing epidermal growth factor receptor-like repeats and a transmembrane peptide. Notch 1 and Notch 2 are the most ubiquitously

distributed whereas Noth2 and Noth4 are more specifically expressed in vascular smooth muscle and endothelial cells.

Ligand binding via the jagged or delta-like family of membrane proteins leads to cleavage of the receptor by member of the A Disuniting and Metalloprotease (ADAM) and secretase families of proteases. The Notch pathway plays an important role in maintenance of the stem cell in glioblastoma, breast cancer stem cells and some other tumor stem cells. Since the activation of Notch signaling can upregulate several factors that in turn transmit signals among cancer, stoma and endothelium cells.

In a study learned about Notch signaling pathway in glioblastoma suggested Notch inhibition can lead to a decrease of cancer stem cells in glioblastoma and promotes increased responsiveness to radiation. Notch inhibition can be achieved in different level. 1, inhibition of γ -secretase mediated notch cleavage. Though most of the available γ -secretase inhibitors (GSI), including DAPT, have no preference for substrates, as is commonly observed for small molecule inhibitors, several nonspecific GSI molecules (such as MK0752 and RO4929097) are currently in clinical trials for different cancer.

Target Notch ligands such as DLL4 (Delta like ligand) or inactivate Notch receptors have also been described. The OMP21M18 has been tested in pancreatic cancer and the combination of anti-human DLL4 and antimouse DLL4 result in additive anti-tumor activity in colon tumors. 3, modulate the Notch signaling by other pathway component. Notch1 is shown to be induced by PI3K/Akt pathway in melanoma development and in human arterial endothelial cells. GSK3 α/β acted as the negative regulators of Notch1 and Notch2 was down regulated by GSK3 α/β .

Overcome the mechanisms of resistance:

Cancer stem cells is resistant to chemo and radiation therapy often lead to the failure of conventional therapy and result relapse. Frequent cancer recurrence may be due to the preferential killing of differentiated cells while leaving CSCs behind. A better understanding of the mechanisms that underlying CSCs resistance to treatment is necessary and may provide a more effective therapy to overcome the resistance. A number of genetic and cellular adaptation have been found confer resistance to classical therapeutic approaches such as relative dormancy/show cells cycle kinetics efficient DNA repair, the expression of multidrug-resistance transporters and resistance to apoptosis. One potential modulator of CSC resistance to DNA targeting agents is the family of checkpoint kinases $\frac{1}{2}$ (Chk1/2 kinases) and these kinases have higher basal and inducible activities in CSCs than in nonstem cells. CSCs may also derive resistance to chemical mutagens though the expression of drug efflux pumps for they can transport the drugs out of the cells such as the ABC family.

The activation of the Akt pathway and the over-amplification of apoptosis inhibitor proteins might also be conferred to CSCs resistance. It is first demonstrated in hepatocellular carcinoma CSCs, which were found to preferentially activate Akt/PKB and bcl-2 cell survival

pathways. An important result of the well-documented CSC resistance to radiation and chemotherapy is that these therapies lead to an increase of resistance clones within a heterogeneous CSC population. Evidence of radiation-induced enrichment has been shown in both brain and breast CSCs. CSC-specific pharmaceutical interventions are being developed that may eliminate both primary and acquired CSC chemo-resistance. This may dramatically improve the treatment of cancer by abrogating the potential for CSC-induced tumor regrowth and the systemic disease spread after initial treatment.

An experiment showing that pancreatic CSCs could survive and expand after initial treatment. An experiment showing that pancreatic CSCs could survive and expand after serial exposures to gemcitabine. This chemoresistance was overcome by the use of CD44 or ABC transporter during cancer treatment. 1, concurrent therapy. It is now well established that combination therapy helps to prevent the development of cancer resistance, but for a select group of cancer types where a single pharmaceutically correctable mutation exists. 2, surgical resection following induction: theoretically, CSC-specific induction chemotherapies should offer an immediate reduction in CSC metastatic potential and reduce any hematogenous and lymphatic CSC micrometastases that would otherwise diminish the efficacy of surgical resection.

The natural component and their ability to target cancer stem cell:

In the recent years compounds were found have the ability to kill cancer stem cells, such as salinomycin, curcumin, sulforaphane, a novel Gemini vitamin D analog (BXL0124) and so on. Salinomycin a polyether antibiotic acting as a highly selective potassium ionophore and widely used as an anticoccidial drug was recently shown to act as a specific inhibitor of cancer stem cells. Some studies showed that salinomycin acts as a potent inhibitor of multidrug P-glycoprotein (P-gp170) and the inhibitory effect of salinomycin on P-gp function is mediated by the induction of a conformation change of the ATP transporter.

In the experiment salinomycin elicited a dose-dependent inhibition of cell growth evident both in CEM and A2780 cells and caused an intracellular accumulation of doxorubicin with a dose-dependent effect in both CEM-VBL10 and CEMVBL100 MDR cells indicated that salinomycin may restore vinblastine sensitivity in vinblastine-resistant CEM-VBL100 cells. Salinomycin has also been demonstrated to significantly rupture the in vitro lung cancer tumor spheres from ALDH positive A549 lung cells with a significantly down-regulated expression of stem cell marker OCT-4, NANOG, and SOX2, which may be responsible for blocking self-renewal and proliferation.

Although the mechanisms of action of salinomycin is not yet clear it appears that it might induce terminal epithelial differentiation accompanied by cell cycle arrest rather than trigger cytotoxicity. Curcumin is a well-known dietary polyphenol present in an Indian spice, turmeric, which possesses anti-inflammatory and antioxidant activities, and has been studied as a chemoprevention agent in several cancer models.

Mesenchymal stem cell-mediated gene therapy for cancer:

Mesenchymal stem cells (MSCs) are multipoint stromal cells that can differentiate into a variety of cell types including: osteoblasts, chondrocytes, and adipocytes. They have generated a significant amount of interest as a result of their apparent ability to home to the tumor site following systemic delivery. The combination of cellular therapy and gene delivery is an attractive option for it will potentially protect the vector from immune surveillance and will supported targeted delivery of a gene or therapeutic proteins to the tumor sites.

MSCs ability for target gene delivery in the context of cancer is as exciting area of research that has gained considerable momentum in recent years. Some studies reported engineered MSCs specifically targeting multiple tumor type followed by local secretion of pigment epithelium-derived factor, therapeutic protein (IFN β ,IL2), TNF-related apoptosis including ligand(TRAIL), expression of prodrug activating suicide genes (herpes simplex virusthymidine kinase, cytosine deaminase) and delivery of replicating oncolytic viruses integration of MCSs into the tumor site is thought to be mediated by high local concentration of inflammatory chemokines and growth factors.

The most important chemokine receptor implicated in targeted homing of MCSs is CXCR4, which has potential in cell mobilization and homing. The degree of inflammation in the tumor site plays an important role in the recruitment of MSMs. In a study of MSM-IFN β -mediated therapy of pancreatic cancer, treatment with anti-inflammatory agents resulted in reduction of MSC engraftment in the tumor. Irradiation resulted in apoptosis and increased release of inflammatory signals at the site of radiation, including TNF α , as well, CLLS and CCR8. Radiotherapy is a tradition cancer therapy, however, and therefore could work in combination with MSC-based gene delivery to support improved targeting of MSCs to tumor. Along with their tumor stoma.

The integration has supported their use in delivering various biological agents, whose systemic administration is blocked due to their short half-life and toxicity at the doses required for therapy. MSCs can efficiently produce biological at the tumor sites and in a number of tumor models, MSMs expressing IFN β have been shown to result in decreased tumor burden and increased animal survival. A significant advantage of MSMs as cellular vehicles is their accessibility for genetic manipulation in vitro.

A study has engineered MSMs to express TNFrelated apoptosis-including ligand (TRAIL) which causes apoptosis and death of cancer cells, without harming normal cells, by binding to specific TRAIL receptors and leading to activation of the extrinsic apoptosis pathway. Their experiment demonstrate that TRAL-expressing MSMs are able to kill both SP and non-SP cell in squalors and adenocarcinoma lung cell lines with equal efficacy. This suggested that MSMs can be used as a cellular vehicle to delivery genes to the tumor site. There is also another group demonstrated that MSMs could inhibit the proliferation of SK-MES-1 and A549 cells and induce the apoptosis of tumor cells in vitro via some soluble factor.

In the advanced research, it is suggested that MSMs could really inhibit the lung cancer cell proliferation by the secretion of the soluble factors which could also interface in tumor angiogenesis via the down regulation of VEGF expression in tumor cells. Great progresses have been made in the research of MSC, and suggested promising potential for MSC-mediator delivery of therapeutic agents directly to tumor tissue. MSCs have plenty of advantages as cellular vehicles such as easy to isolate and expand, specifically target tumors and metastases following systemic delivery easier to be traduced with a range of vectors have immunosuppressive properties and the ability to express therapeutic protein in secretary form. The potential for MS-mediated tumor promotion must be addressed.

A better understanding of the MSCs biology and the specific combination of factors controlling their tumor-specific migration and persistence will supported translation to the clinical setting.

Differentiation therapy:

Differentiation therapy approach to the treatment of advance or aggressive malignancies so that can resume the process of maturation and differentiation into mature cells. It aims to force the cancer cell to resume the process of maturation. Differentiation therapy may use either know differentiation inducing agent and/or newly designed differentiation inducing agents. Viramin A and its analogue (retinoid) can reverse the malignant progression process though signal modulation mediated by nuclear retinoid receptors and leads to frequent remission of acute promyelocytic leukemia by inducing promyelocyte differentiation.

The new differentiation-inducing agents are represented by those ligands normally induce stem cells to undergo asymmetric mitosis. Those ligands that can deliver to the cancer stem cells to force them to switch from a symmetric to an asymmetric mitotic program. Such agents would induce gene products of Wnt, Hedgehog, TGF, and EGF. On the other hand using either inhibits asymmetric mitosis. For example the GSK-3b which normally inhibit the native Wnt pathway, could be blocked by antisense or ribozyme agent specific for the GSK-3b (Xenopus), an inhibitor of B-catenin.

Factors which block the allelic pairing /exchange such as SNRPN-inhibitors, zeste-inhibitors, and other could be inhibitors by antisense or ribozyme agents. And also antisense or ribozyme gene products that block asymmetric cell division. There are several methods that could be used to induce cancer stem cells differentiation, such as by differentiation-inducing agents, differentiation-inducing ligands to the tumor sites by activation of positive or inhibition of negative agents in the asymmetric mitotic pathway downstream of the ligands-receptor binding.

In addition there are other methods that induce a cancer stem line to activate differentiation related gene products. Therefore it has been shown the starvation can lead cells to became growth quiescent and at times differentiate or undergo apoptosis if their mitotic program is changed such as c-myc deregulation. Indeed inhibitor is Wnt signaling as ICG-001 showed

promising in vitro and in vivo efficiency without toxicity, due to its benefit differentiation of colon cancer cells.

Perspective:

Though the use of multidisciplinary approaches to cancer therapy, significant strides have been made in the treatment of cancer. There is increasing awareness that cancer stem cell represent a significant challenge to effective treatment of cancer as they are resistance to current clinical drugs. A major challenge for producing agents against CSCs is to distinguish the CSCs from the normal stem cells. Better understanding of normal stem cell biology as well as cancer stem cell biology will be essential for the identification of such target.

Though many compounds, method have been developed to target the cancer stem cell, there still have many blockades overcome. The ultimate challenge in coming years will be the understanding of the stem cell programmers particularly the control of self renewal, in an attempt to develop novel, stem cell-directed therapies. And should always remain the ultimate goal of target CSCs

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