Published in final edited form as:
J Mol Biomark Diagn. ; Suppl 8: . doi:10.4172/2155-9929.S8-003.

Targeting Cancer Stem Cells with Nanoparticle-Enabled Therapies

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Abstract

Emerging evidence suggests that multiple tumor types are sustained by a small population of transformed stem-like cells that have the ability to both self-renew and give rise to non-tumorigenic daughter cells that constitute the bulk of a tumor. These cells, which generally constitute a minority of the overall cancer cell population, are highly resistant to conventional therapies and persist following treatment, leading to disease relapse and the formation of distant metastases. Therapies that disrupt the maintenance and survival of cancer stem cells are the subject of active current investigation. This review discusses recent approaches to the application of nanomedicine to the targeting and elimination of cancer stem cells. Specifically, recent publications in the areas of nanoparticle-enabled drug and nucleic acid delivery and photothermal therapy are addressed.

Keywords

Cancer stem cells; Tumor-initiating cells; Drug delivery; Nucleic acid delivery; Targeted; Nanoparticle; Carbon nanotube; Photo thermal therapy; Hyperthermia

Introduction

The intratumoral heterogeneity of cancer cells presents a major challenge to the development of effective cancer therapies. However, a growing body of evidence suggests that tumors may be driven by a small population of transformed stem-like cells with the ability to undergo both self-renewal and differentiation into the diverse cancer cell population that constitutes the bulk of the tumor [1–4]. In 1997, Bonnet and Dick identified a single cell isolated from a bulk cancer cell population capable of initiating cancers that recapitulated the cellular heterogeneity of the parent pathology when transferred into an

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immune compromised animal model [5]. In their work, the authors demonstrated that acute myelogenous leukemias (AML) could be initiated in NOD/SCID mice through the transplantation of a rare (<0.2% of whole cell population) $CD34^{+}/CD38^{-}$ cell from human donors. Only cells displaying these markers were capable of engrafting and generating the cellular diversity evident in human AML. In 2003, Al-Hajj et al. demonstrated this cellular hierarchy extended to solid tumors by showing that a diverse set of human breast cancer specimens could be fractionated by surface markers and that only cells displaying the $CD44^{hi}/CD24^{low}/Lin^{-}$ antigen profile could form tumors in immune compromised mice [6]. Moreover, these cells displayed self-renewal and multi-lineage differentiation abilities in long-term in vitro cultures [6]. Since these seminal publications, cells displaying similar capabilities have been isolated from a range of human tumors including: brain, colon, head and neck, lung, melanoma, pancreatic, prostate and kidney [3,7–14].

Current research suggests these so-called cancer stem cells (CSCs) or tumor initiating cells (TICs) survive standard chemo and radiotherapies and persist following treatment [15,16]. As these cells are both invasive and highly tumorigenic it has been hypothesized that the inability to efficiently eliminate CSCs during conventional therapy may result in disease relapse and formation of metastases. New treatment modalities in the form of molecularly-directed nanomedicines (purpose-built constructs having principal dimensions of 1–100 nm) with the potential to deliver therapeutic payloads directly to CSCs are currently being described in the primary research literature [17,18]. Table 1 (Included as supplementary data) summarizes many of these nanomaterials and discusses their significance in greater detail. With several nanomedicines entering early stage clinical trials, it is anticipated that their ability to selectively target and kill the cellular drivers of tumor progression will fundamentally alter the clinical management of cancer. Accordingly, this review highlights recent advances in the area of nanomedicine with a specific focus on nanoparticle-mediated therapeutic delivery to CSCs and the response of those cells to such treatments.

Review of Literature

Nanoparticle platforms for CSC-targeted drug delivery

Nanoscale drug delivery technologies offer fundamental advantages over contemporary small molecule pharmaceuticals used in clinical practice. These advantages include increased bioavailibity, extended drug half-life and reduced off-target toxicities [17]. Furthermore, the new generation of therapeutic nanoparticles is inherently multifunctional: combining active drug compounds with selective targeting moieties and, in many cases, imaging agents that permit localization by standard x-ray, magnetic resonance (MR) or positron emission tomography (PET) technologies. These so called “theranostic” constructs offer the promise of diminished drug toxicity, enhanced tumor selectivity and improved disease response [18].

Chemotherapeutic resistance is a trait common to many CSCs and is mediated by diverse cellular processes such enhanced DNA damage repair or rapid drug efflux [19]. Nanoparticles (NPs) can sequester chemotherapeutic agents at a high concentration and release them within the cancer cell following uptake by CSCs, potentially overcoming such resistance mechanisms. The addition of targeting ligands to the surface of NPs may increase both target selectivity and internalization. Recently, several groups have explored the application of such NP drug delivery platforms for the selective treatment of CSCs.

In a study by Lim et al., researchers investigated the efficacy of a proprietary polymer-encapsulated curcumin NP formulation (termed NanoCurc™) for the treatment of brain tumor stem cells [20]. The NP formulation greatly increased the bioavailability of curcumin, and following treatment of four distinct brain cancer cell lines with NanoCurc™ increased

rates of cell cycle arrest, apoptosis, and dose-dependent decreases in growth and clonogenicity were observed. Critically, this treatment correlated with a >50% decrease in the $CD133^+$ stem cell population in two of the cell lines tested, suggesting that this therapy may have activity in the CSC fraction of some brain tumors [20].

Recent work by Mamaeva and coauthors describes the use of folate-conjugated mesoporous silica nanoparticles for the in vivo disruption of Notch signaling by the gamma secretase inhibitor DAPT [N-(N-((3,5-Difluorophenacetyl))-L-alanyl)-S-phenylglycerin t-butyl ester] [21]. Notch, like Wnt/β-Catenin, Hedgehog and other key developmental signaling pathways, has been implicated in the maintenance of the CSC pool of many tumors [22], and therapies that attenuate these pathways are being investigated for the treatment of several malignancies [23,24]. Initial studies characterizing the effects of the nanoparticles in breast cancer cell cultures demonstrated folate receptor mediated uptake (with high-expressing cell lines exhibiting greater uptake relative to low-expressers) along with dose-dependent inhibition of Notch intracellular domain (NICD)cleavage, a standard metric of Notch pathway activation [21]. Encouragingly, peritumoral injection of mesoporous NPs in tumor-bearing mice lead to significant tumor growth suppression, whereas free drug exhibited little effect. Moreover, it was shown that drug potency was maintained with oral dosing of the construct; an important consideration for future clinical translation.

Wang et al. described the use of a novel anti-CD44 antibody conjugated liposome to target an aggressive hepatocellular CSC with enhanced tumorigenicity and metastatic potential that over expressed both CD44 and CD90 [25]. The targeted liposomes were loaded with doxorubicin and then injected intravenously into tumor-bearing mice, resulting in a seven-fold higher drug accumulation in tumors relative to free drug, which corresponded with decreased tumor volume. Encouragingly, this effect was seen in the absence of significant changes in mouse body mass. Treatment with free drug produced similar decreases in tumor burden but with an attendant >30% loss of body mass in exposed animals. Alternatively, the authors were able to simultaneously perform tumor imaging and use gene therapy to treat the cancer by using the targeted liposome to deliver a triple fusion plasmid, consisting of gene expression cassettes for red fluorescence protein (RFP), renilla luciferase (Rluc), and a truncated herpes simplex virus thymidine kinase (HSV - TTK) gene. Treatment of tumor-bearing mice with the combination of HSV-TTK liposome and ganciclovir (a cytotoxic thymidine kinase substrate) caused a robust increase in tumor-localized apoptosis with minimal impact on normal tissues. The application of NPs for gene therapy will be discussed in more detail below.

Nanoparticle-enabled nucleic acid delivery vectors targeting CSCs

Nucleic acid-based therapies (such as RNAi) have long offered the promise of a molecularly-tailored intervention for cancer treatment, through the knockdown of vital oncogenes or disruption of tumor-essential signaling networks. Despite the theoretical potential, the clinical introduction of these therapies has been slowed by their unfavorable native pharmacokinetics and poor tumor uptake in vivo. The incorporation of therapeutic nucleic acids into NP delivery vectors is one approach being investigated to overcome these limitations.

Work by Liu et al. describes a method to overcome chemotherapy resistance in colon cancer stem cells through the siRNA-mediated knockdown of the drug efflux protein multidrug resistance 1 (MDR1), which often is over-expressed in CSCs [26]. Utilizing a moderate-throughput approach, the authors generated libraries of lipid nanocarriers composed of varying ratios of cationic polyethylenimine (PEI_{1200}), polyethylene glycol (PEG) and a biodegradable lipid crosslinker. Electrostatic complexes formed by mixing siRNA with these particles with a charge ratio of 1:16 were screened for knockdown efficiency, and

optimized nanocarrier formulations achieved >90% silencing. Treatment of colon cancer stem cells with lipid nanocarriers containing MDR1-directed siRNA led to efficient MDR1 knockdown and sensitized the cells to subsequent paclitaxel treatment [26].

MicroRNAs (miRs) have garnered interest for their ability to coordinately regulate multiple intracellular signaling networks simultaneously [27]. Two recent publications explore the efficacy of NP-delivered, tumor-suppressive miRs for the treatment of head and neck and pancreatic cancers and their constituent stem cells. In one, Piao et al. used a cationic lipid nanoparticle delivery system to express pre-miR 107 in target cells [28]. Mir-107 is a known tumor-suppressive miR capable of regulating key proliferation and survival genes such as protein kinase Cε (PKCε), cyclin-dependent kinase 6 (CDK6) and hypoxia-inducible factor 1-β (HIF1-β). Treatment of a model of head and neck squamous cell carcinoma (HNSCC) with this NP led to a reduction of cellular clonogenicity, invasion and migration [28]. Moreover, therapeutic expression of pre-miR-107 resulted in a significant down-regulation of stem cell transcription factors Nanog, Oct3/4 and Sox2 along with diminished tumor sphere forming efficiency in these same cell lines, suggesting an inhibitory effect on resident CSCs. Accordingly, systemic delivery of NP-encapsulated pre-miR-107 retarded tumor growth and significantly increased survival in HNSCC tumor-bearing mice [28].

In the second study, Pramanik et al. employed a similar cationic liposomal delivery system to investigate the therapeutic utility of forced re-expression of tumor-suppressive miRs 34a and 143/14 for the treatment of pancreatic cancer [29]. Similar to miR-107, these miRs are frequently down regulated during carcinogenesis [30,31]. Intravenous administration of miR-34a or 143/145-complexed liposomes in mice produced increased intratumoral apoptosis and growth delays in pancreatic cancer xenografts and orthotopic tumor models. Furthermore, miR-34a re-expression caused significant down-regulation of pancreatic CSC markers aldehyde dehydrogenase 1 (ALDH1) and CD44, suggesting that miR-34a therapy may be effective for the treatment of both stem and non-stem pancreatic tumor cells [32].

Nanoparticle-mediated hyperthermia for CSCs

Heat-based therapies, which involve elevating specific regions of the body to temperatures in excess of 43°C (hyperthermia) or 55°C (thermal ablation), are established therapeutic options for the treatment of refractory tumors and metastases. Raising the temperature of a tumor into a supra physiologic range enhances chemotherapeutic uptake and tumor oxygenation (a positive modifier of response to radiotherapy), as well as exerting direct cytotoxic effects [33]. While disease responses to hyperthermal therapies have been widely observed, their clinical implementation has been limited due to the nonspecific heating of normal tissues and consequent treatment-limiting toxicities. Recent evidence also suggests that CSCs are resistant to many standard thermal therapies. However, developmental advances in biocompatible near-infrared and radio frequency (RF) energy absorbing nanoparticles offer the possibility of generating tumor-specific thermal therapy in a minimally-invasive manner. In this application, nanoparticles are localized to the target lesion either by direct injection or through intravenous administration followed by either passive or targeting moiety-assisted accumulation at the tumor site. The tumor is then irradiated with either NIR or RF energy to stimulate the nanoparticles and locally generate heat within the tumor, leading to cancer cell death.

This technique was first described by Hirsch et al. using gold-coated, silica core nanoshells [34]. Similar findings have since been reported with the use of graphene [35], single [36] and multiwalled [37,38] carbon nanotubes and gold nanorods [39]. In recent work, Burke and co-authors directly investigated the response of breast cancer stem cells (BCSCs) to both conventional and nanoparticle-mediated hyperthermia (NMH) to determine the relative efficacy of each approach for the treatment of these cells [40]. They reported that BCSCs

were significantly more resistant to the cytotoxic effects of conventional hyperthermia as compared to non-stem breast cancer cells and that this resistance was mediated, in part, by high basal expression levels of heat shock protein 90 (HSP 90). Treatment of a mixed population of stem and non-stem breast cancer cells with conventional hyperthermia led to a significant enrichment of BCSCs in the surviving fraction of cells. In contrast, the researchers were able to abrogate the resistance to hyperthermia observed in BCSCs following conventional treatment through the use of NMH [40]. It this study, the researchers were able to generate precise temperature increases in target cells and tissues by exposing the cells to polyethylene glycol coated multiwalled carbon nanotubes that were then heated using a low power, 1064nm NIR laser. Treatment of the BCSCs with this form of NMH resulted in robust cell death that was proportional to laser exposure time. NMH treatments, but not conventional hyperthermia, led to rapid membrane permeabilization and necrotic death in treated cells, and were equivalently effective at treating both cancer stem cells and non-stem cancer cells. Encouragingly, use of NMH in mice bearing BCSC-driven tumors lead to complete tumor regression and 100% survival, whereas control groups exhibited >80% mortality at identical time points. Based on these findings, NMH may represent a rapid, minimally invasive approach for the simultaneous elimination of stem and non-stem cellular components of tumors [40].

NMH can also sensitize CSCs to other treatments such as ionizing radiation exposure. This type of bipartite therapeutic approach was investigated by Atkinson et al. [41]. The authors used gold nanoshells in combination with NIR laser irradiation to generate mild (≈42°C) hyperthermia in target cells and tumors and investigated the combined effects of focal hyperthermia and ionizing radiation treatment. Using two independent animal models of breast cancer they confirmed that the stem cell fraction of the tumors (identified by $CD29^+/CD24^+/Lin^-$ antigen profiles or ALDH1 enzymatic activity) was resistant to radiation monotherapy and became enriched in the population of tumor cells surviving treatment, as had been previously shown [42]. They went on to show that this effect could be prevented by the addition of hyperthermia immediately following radiotherapy, which led to a >50% reduction in the size of the CSC fraction. Moreover, cells from tumors treated with the combined therapy displayed reduced tumorigenicity and gave rise to less aggressive, more differentiated tumors (when formed) following transplantation into new hosts. These results suggested that the combination therapy durably altered the native behavior of the CSC fraction and may represent a promising approach for the treatment of CSC-harboring breast tumors. Human clinical trials using these particles under the trade name Aurolase® are currently underway.

Finally, a recent report describes a novel extension of the NMH technique to target invasive CSCs in systemic blood circulation. In a proof-of-principle study, Galanzha et al. demonstrated the use of photoacoustic (PA)/photothermal (PT) in vivo flow cytometry for the detection and elimination of circulating cancer stem cells [43]. The authors conjugated NIR-absorbing gold plated single-walled carbon nanotubes (GNTs) and spherical magnetic nanoparticles (MNPs) to folate or anti-human CD44 antibodies, and used these particles to selectively label circulating human breast cancer stem cells (which over express CD44 [6]) with nanoparticles (NPs). Cells with bound nanoparticles could then be specifically identified by detection of photoacoustic waves generated by the nanoparticle-labeled cells following excitation using a low powered laser [44,45]. Using this method, the authors demonstrated that rare $CD44^+$ circulating cancer stem cells could be detected in the vasculature of nude mice which bore human breast cancer xenografts. The authors suggested that these cells could be ablated by photothermal effect following extended irradiance with NIR. As circulating CSCs are thought to be the primary drivers of metastatic spread, this technology offers a method by which these cells may be purged from the vasculature of cancer patients to reduce the incidence of metastatic disease.

Conclusions

Cancer stem cells offer an attractive target for therapeutic intervention because therapies that ablate this critical tumor constituency offer the promise of durable disease remission and long-term survival of cancer patients. This review discussed three promising nanomedical approaches for the selective treatment of both tumors and their resident CSC populations. Each approach leverages the emergent properties of distinct nano-scale material formulations to enhance both tumor and CSC-specific drug accumulation and therapeutic effect.

It is encouraging to note that despite a challenging regulatory environment, several nanomedical technologies (including the Aurolase® nanoshell technology discussed above) are already undergoing clinical trials. While future advancements will be necessary to safely transition the investigational nanoparticles detailed in this review into the clinic, the lessons learned by pioneering treatments like Aurolase® will inform the rational design and development of future nanomedicines for targeted cancer therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by grants RO1CA12842 from the National Institutes of Health (SVT), Department of Defense Breast Cancer Research Program Predoctoral Traineeship Award W81XWH-10-1-0332 (ARB) and by a Grant-In-Aid of Research from the National Academy of Sciences, administered by Sigma Xi, the Scientific Research Society G20100315151746 (ARB). R.S. was supported in part by training grant T32CA079448 and by National Institutes of Health grant K99CA154006.

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