Nanoparticles and Cancer Stem Cells

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Cancer stem cells (CSCs) are a subpopulation of cells that can initiate, self-renew, and sustain tumor growth. CSCs are responsible for tumor metastasis, recurrence, and drug resistance in cancer therapy. CSCs reside within a niche maintained by multiple unique factors in the microenvironment. These factors include hypoxia, excessive levels of angiogenesis, a change of mitochondrial activity from aerobic aspiration to aerobic glycolysis, an upregulated expression of CSC biomarkers and stem cell signaling, and an elevated synthesis of the cytochromes P450 family of enzymes responsible for drug clearance. Antibodies and ligands targeting the unique factors that maintain the niche are utilized for the delivery of anticancer therapeutics to CSCs. In this regard, nanomaterials, specifically nanoparticles (NPs), are extremely useful as carriers for the delivery of anticancer agents to CSCs.

Keywords: targeted cancer therapy ; cancer stem cells ; nanoparticles ; polymers ; nanocarriers ; self-assembling proteins ; nanovesicles ; dual-targeted drug delivery

1. Introduction

Cancer is defined as a biological condition in which some cells in a tissue of a bodily organ undergo an uncontrolled division and growth [1]. In 1997, Bonnet and Dick realized that a small subpopulation of these abnormal cells have different properties from those of bulk tumor cells. After isolation, they demonstrated that this small population of leukemia-initiating cells have features similar to stem cells and announced the concept of cancer stem cells (CSCs) [2]. Later studies in various types of solid tumors revealed the existence of CSCs in almost all cancer types, from brain to colon and prostate. The majority of cells in bulk tumors are normal and non-tumorigenic and behave like background cells with no special privileges, compared to CSCs [3][4]. CSCs can be compared with normal stem cells in different tissues of the body. Normal stem cells, when activated, undergo an asymmetric cell division (ACD) to self-renew and give rise to a distinct population of progenitors. These progenitors then undergo a symmetric cell division (SCD) to clonally expand and replenish lost cells [5]. CSCs in some ways act like normal stem cells for the tumor tissue. Evidence shows that normal cancer cells exhibit plasticity and undergo dedifferentiation to a stem-like state, like the epithelial-to-mesenchymal transition (EMT). These dedifferentiated cells acquire properties of stemness and become more invasive and metastatic. A key characteristic of CSCs is their ability to evade the attack by immune cells, like natural killer (NK) and CD8-positive cytotoxic T cells, through the active recruitment of immune suppression cells, expression of immune suppressive factors, or induction of apoptosis in T lymphocytes [6]. Other important features of CSCs include:

- Self-renewal and DNA repair: this extraordinary property of CSCs causes tumor relapse and radiation-resistance in tumors [7].
- Differentiation into multiple cell types: the pluripotency of CSCs causes heterogeneity in solid tumors [8].
- Ionizing radiation: this feature makes CSCs resistant to radiotherapy.
- Infinite proliferative potential: unlimited cell division, which leads to rapid tumor growth.
- Dormancy state: CSCs enter dormancy to evade the attack by the immune system, awaiting new signals from the environment to re-enter the cell cycle [7].
- Changes in morphology or biological function, such as the over-expression of anti-apoptotic proteins that block the cell from entering the type I apoptosis cycle [9][7][10].
- Elevated expression of ATP-binding cassette (ABC) pumps and detoxifying enzymes to increase the drug's efflux, which is considered to be an important mechanism for multi-drug resistance (MDR). Multi-drug resistance is either intrinsic and present before the start of treatment or acquired after exposure to treatment [11].

While the exact mechanism of CSC initiation is unclear, there are two proposed theoretical models to explain their existence in tumor tissue:

- The stochastic or classical model states that any somatic cell has the intrinsic ability to undergo mutation and transform into CSCs driven by genetic instability or environmental signals, as shown in Figure 1A;
- The hierarchical or cancer stem cell model states that the initiating cancer cell self-renews in the process of cell division and forms a CSC and a normal cancer cell. The normal cancer cell divides and generates the cells in bulk tumors, as shown in Figure 1B [7][12].

2. Self-Assembling Protein NPs

Conventional approaches to cancer treatment are limited by undesired toxic side effects and a lack of control over the local drug concentration in the tumor tissue, which has led researchers to explore alternative solutions. While nanocarriers improve the drug biodistribution and passive and active targeting, reduce renal clearance, protect the drug from degradation, and enhance cell uptake, only a fraction of the administered drug reaches the tumor tissue. Further, the persistence of the carrier in the tumor and healthy tissues leads to undesired toxic effects [13][14]. NPs based on multifunctional proteins that are degraded by natural enzymatic pathways are attractive as a carrier for passive or active drug targeting to tumors [15]. CXCR4 is a viable target in cancer therapy, because it mediates cancer metastasis by inducing the migration of tumor-associated cells. A single-chain variable fragment (scFv) antibody targeting CXCR4 was fused with an RNA-binding protein peptide (RBM) and mixed with miR-127-5p, a mediator of M1 macrophage polarization, to form self-assembling RNA-protein nanoplexes [16].

These nanoplexes served as a carrier for targeting miRNA to tumor-associated cells that express CXCR4. In a 4T1 TNBC mouse model, these nanoplexes inhibited the migration of tumor-associated cells, polarized the macrophages to the M1 phenotype, and suppressed tumor growth [16]. In another study, a modular fusion protein composed of an N-terminal cationic peptide T22-targeting CXCR4 receptor on tumor cells and a C-terminal polyhistidine tag (H6) on a fluorescent GFP protein scaffold for imaging was used to form self-assembled NPs for tumor targeting [17]. The peptide T22 and polyhistidine tag H6 induced the self-assembly of the modular protein into fluorescent NPs with an average size of 12 nm. The T22 peptide facilitated the binding and internalization of the NPs in $CXCR4^+$ tumor cells for targeted intracellular drug delivery. In a recent study, the drugs, oligo-floxuridine (FdU) and monomethyl auristatine E (MMAE), were chemically coupled to exotoxin A from Pseudomonase aeruginosa and diphtheria toxin from Corynebacterium diphtheria, respectively, to form self-assembled protein NPs with an average size of 50 nm targeting $CXCR4^+$ tumor cells [14]. Based on in vitro studies, the resulting protein NPs were internalized by $CXCR4^+$ cells and inhibited the growth of tumor cells. Ribosome-inactivating proteins (RIPs) are considered potent therapeutic agents for cancer therapy, as they inactivate ribosomes in cancer cells and inhibit protein synthesis, leading to cell death. In this regard, magnetic NPs were surface modified with a fusion protein composed of the small protein, Barstar (Bs), synthesized by Bacillus amyloliquefaciens, which inhibits bacterial ribonuclease and the C-terminal part of the magnetite binding protein of magnetotactic bacteria (Mms6) [18].

These Bs-C-Mms6 magnetic NPs undergo a spontaneous self-assembly with a Barnase-containing biomolecule by a specific Barstar-Barnase interaction for targeted drug delivery. As a proof of concept, a fusion protein of Barnase and the peptide DARPin9.29 that binds to the HER2/neu receptor underwent a self-assembly with Bs-C-Mms6 NPs to target magnetic particles to HER2/neu overexpressed cells in breast cancer tissue [18]. Gelonin is a ribosome-inactivating protein (RIP) used in cancer therapy to block the growth of cancer cells. In one study, gelonin was conjugated to monocrystalline nickel-iron oxide ($NiFe_2O_4$) NPs (MIONs) using a multifunctional peptide linker for targeted delivery to tumor cells in a fibrosarcoma xenograft mouse model [19]. The multifunctional peptide consisted of a 6-mer histidine tag (6His-Tag) for attachment to the MION followed by a matrix metalloproteinase-2 (MMP-2) degradable sequence and a low-molecular-weight peptide (LMWP) for cell penetration. Following uptake, the MMP-2 degradable peptide is degraded by overexpressed MMP-2 in the tumor tissue, resulting in the release of gelonin-LMWP and endocytosis by the tumor cells, facilitated by the cell penetrating peptide. The in vivo results showed an enhanced cytotoxicity of the MIONs against the tumor cells in a fibrosarcoma xenograft mouse model [19]. These studies indicate that protein NPs, due to their biodegradability and tunable self-assembly, are especially useful for the delivery of amino acid-based bioactive agents, such as RIPs and antibodies.

Naturally occurring or synthetic amino acid sequences used in assembling protein NPs can be immunogenic. The immune response can neutralize the drug's effectiveness or cause serious side effects in therapeutic applications. In some cases, these peptides can be immunosuppressive, and their long-term administration can cause severe side effects, such as

relapsed bacterial, viral, or fungal infections [20]. The targeting agent in the delivery of cytotoxic proteins should have a high selectivity for receptors on tumor-associated cells to reduce the risk of serious side effects in healthy tissues [21].

3. Conclusions

NPs are very attractive as a carrier for targeting drugs to cancer tissue through the leaky tumor vasculature (EPR effect). The surface modification of NPs with water-soluble polymers, such as PEG, PAA, and DEX, has been used to evade the uptake of NPs by the MPS system, increase the residence time in circulation, and increase their uptake through the vasculature. Aside from surface modification, the drug-loaded NPs are targeted to CSCs within the tumor tissue by conjugation with antibodies or ligands against biomarkers, surface receptors, enzymes, and proteins associated with CSC signaling pathways. As most CSC signaling pathways and associated biomarkers are shared with normal stem cells, dual-targeting using two ligands/antibodies against those biomarkers significantly enhances CSC uptake while reducing off-target toxicity toward normal stem cells. Polymeric NPs based on PLA, PLGA, PEG, their copolymers, polylysine, lipids, hyaluronic acid, and liposomes have successfully been used as carriers for targeting therapeutic agents to CSCs.

In contrast to polymeric NPs that have a broad size distribution, the size distribution of inorganic NPs tends to be narrow, which improves their transport within the tumor tissue for targeting and uptake by CSCs. Inorganic NPs based on gold, iron oxide, and silica have been used as carriers for drug targeting to CSCs, as well as imaging. Multifunctional protein NPs, due to their degradability by natural enzymes, tunable self-assembly, and natural ability to penetrate the cell membrane, are attractive in connection with the delivery of amino-acid-based therapeutic agents, such as ribosome-inactivating proteins (RIP), to inhibit protein synthesis and cell growth in cancer cells. ADCs are highly effective in eliminating metastatic and recurrent cancers with the selection of antibodies with a high specificity against CSC surface receptors, an appropriate choice of therapeutic agents, and the proper selection of enzymatically degradable linkers for intracellular drug delivery to CSCs. Exosomes and other EVs, due to their low immunogenicity, long circulation time, and high loading capacity, are very attractive as a carrier for the delivery of functional proteins, mRNAs, miRNAs, and small DNA fragments to CSCs for reversing tumor progression, because EVs facilitate cell–cell communication by acting as antigen-presenting vesicles. Drug-loaded polymeric, inorganic or protein NPs, ADCs, and EVs that selectively interact with multiple surface receptors' tumor-associated stem cells provide the prospect of an enhanced drug bioavailability and uptake in tumor tissue, with fewer undesired side effects in healthy tissue, thus improving the quality of life of cancer patients.

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