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Review

Cancer nanotheranostics: A review of the role of conjugated ligands for overexpressed receptors

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ABSTRACT

Cancer treatment using chemotherapy has many drawbacks because of its non-specificity, in which the chemotherapeutic agent attacks both normal and cancerous cells, leading to severe damage to the normal cells, especially rapidly proliferating ones. Cancer targeting enables the drug to kill only tumor cells without adversely affecting healthy tissues, which leads to the improvement of the patient's well-being. Nanoparticles offer several advantages in drug delivery such as enhancing the solubility of hydrophobic drugs, sustaining their release and prolonging their circulation time. The ability of nanoparticles to specifically target tumor cells makes them a useful delivery system for anticancer agents. The type of the delivery system and formulation additives used can also improve the delivery of the anticancer agent. This review highlights some of the most highly sought receptors to be targeted in selective cancer treatment. It also reports some of the recent advances in cancer targeting using drug-loaded ligand-conjugated nanocarriers.

1. Introduction

Cancer is a death associated disease. The most commonly diagnosed cancer types worldwide are lung cancer followed by breast cancer then colorectal cancer (International Agency for Research on Cancer, 2013). The reason behind poor cancer prognosis and the associated death is the inability of therapeutic agents to selectively target specific diseased sites without adversely affecting normal tissues (Steichen et al., 2013). The nonsurgical methods of cancer treatment include radiation-treatment and chemotherapy, depending only on agents that cause cellular death. Despite that a degree of specificity is achieved with radiation, the radiation dose should be sufficiently high to kill cancer cells without affecting the surrounding healthy cells (Brown and Giaccia, 1998; Bucci et al., 2005). In addition, the metastatic cancer represents a challenge for radiation therapy (Wu et al., 2006).

Regarding chemotherapy, the rapid proliferation of cancer cells makes them more susceptible to the destructive action of drugs than the healthy cells (Brown and Giaccia, 1998). Chemotherapeutic agents are small drug molecules that either inhibit replication or induce apoptosis of a cell, thus causing disruption of its normal functioning (Feng and Chien, 2003; Steichen et al., 2013). The limitation of chemotherapy is its inability to selectively kill cancer tissues (Maeda, 2001), this results in detrimental effects in cells that exhibit high proliferation rate (Feng and Chien, 2003; Steichen et al., 2013). Therefore an efficient therapeutic formulation is the one that selectively targets cancer tissues, overcomes biological barriers and responds to the tumor environment by releasing the drug (Steichen et al., 2013).

Nanoparticles are colloidal entities of size that ranges from 10 nm to 1000 nm. Nanoparticles have several advantages (Singh et al., 2011) including promoting the solubility of drugs (Kang et al., 2017; Lee et al., 2015), prolonging their circulation time (Avaji et al., 2016; He et al., 2015), and achieving controlled release (Kumar et al., 2016; Ngo et al., 2016), thus reducing the frequency of drug administration (Singh et al., 2011). The slow release of active drugs from the carrier system is advantageous in the sense that it ensures high sustained levels of the drug in the tumor tissues and lower plasma drug concentrations (Arpicco et al., 2014). In order to increase nanoparticle circulation half-life, surfacely adsorbed polyethylene glycol (PEG) chains are used to create a coat which prevents the adhesion of blood serum opsonins to the particles (Owens and Peppas, 2006). Poly(carboxybetaine) surface-grafted nanoparticles are analogous to the PEG-coated nanoparticles; showing comparable *in vivo* pharmacokinetic profile to PEG-coated ones (Li et al., 2012). Moreover, compared to PEG-coated nanoparticles, hyperbranched polyglycerol-coated nanoparticles achieved longer circulation time and lower accumulation in the liver (Deng et al., 2014). Recently a study referred to a strong disopsonin effect of clusterin (apolipoprotein-J; a secreted heterodimeric glycoprotein), present in serum and plasma, which was capable of binding to the surface of

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nanoparticles, thus hindering their uptake by macrophage-like cells either in the presence of absence of PEG coating (Aoyama et al., 2016).

Nanoparticles also act as a delivery vehicle that can specifically target tumor tissues, thus preventing premature inactivation of the drug during its transport, and allow better internalization within cancer cells (Brigger et al., 2002).

2. Tumor targeting

2.1. Passive targeting

Tumor blood vessels are characterized by their leakiness. Passive targeting is best described as the movement of carriers through these blood vessels into the tumor interstitium, in which they diffuse towards the cells (Danhier et al., 2010). This leakiness associated with poor lymphatic drainage result in what is referred to as "Enhanced Permeability and Retention EPR" effect (Matsumura and Maeda, 1986). Macromolecules were reported to passively reside in tumor tissues, and the EPR effect increases their intra-tumoral concentration by about 70-fold (Duncan, 2003). Passive targeting can also be accomplished by the electrostatic interaction between positively charged nanoparticles and negatively charged tumor endothelial cells (Patra and Turner, 2014). EPR effect was also found to be enhanced by S-nitrosated form of recombinant human serum albumin dimer (Kinoshita et al., 2015). Furthermore, superparamagnetic iron oxide nanoparticles were reported to improve the accumulation of non-targeted liposomal formulation in breast tumor *via* the EPR effect (Kato et al., 2015). Pretreatment of mice bearing a large hard tumor model with combretastatin A-4 phosphate; the relatively tumor-specific vascular disrupting agent (Clémenson et al., 2013; Tozer et al., 2002) enhanced both tumor accumulation and retention of radioisotope-loaded nanoparticles. In other words, it sensitized the tumor to subsequent treatment with actively-targeted nanoparticles loaded with chemotherapeutic agent (Satterlee et al., 2017).

The EPR phenomenon was found to be the main cause of passive accumulation of nanoparticles at tumor sites. However, the *in vivo* efficacy of some nanoparticles was inadequate either due to the release of the drug before tumor deposition or due to the incapability of the EPR effect to ensure cellular internalization of the nanoparticles. Therefore, the development of drug-loaded nanoparticles which can accumulate in a tumor tissue and be uptaken by cancer cells to release their payload is essential for effective cancer therapy (Choi et al., 2012). Fig. 1 illustrates the mechanism of passive and active targeting.

2.2. Active targeting

Active targeting refers to the presence of an active moiety on the surface of the nanocarrier such as certain ligands, which are able to bind to the appropriate receptors overexpressed at the tumor tissue, thus, enhancing the internalization of drug-loaded nanoparticles (Danhier et al., 2010; Kirpotin et al., 2006). Through this approach, tumor treatment would benefit from an increased specificity and reduced side effects on normal tissues (Golla et al., 2013). Selected targeting ligands and their corresponding receptors are presented in Table 1, together with their associated drug delivery systems.

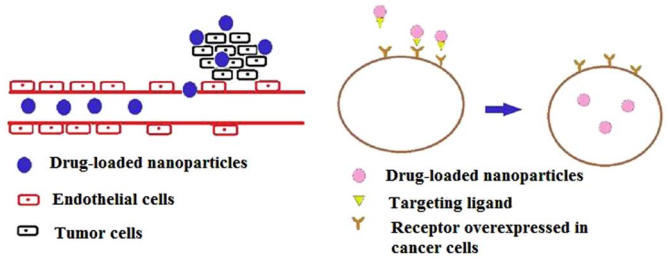

Fig. 1. Left: Passive targeting showing nanoparticles movement through leaky tumor blood vessels into the tumor interstitium. Right: Active targeting.

2.2.1. Approaches for active targeting

Active targeting involves direct and indirect approaches. Direct approach allows for tumor protein antigen targeting by either monoclonal antibodies which alter their signaling or by small drug molecules that interfere with them (Wu et al., 2006).

2.2.1.1. Monoclonal antibodies

2.2.1.1.1. Antibodies targeting epidermal growth factor receptor. Epidermal growth factor receptor (EGFR) is highly expressed in various human cancer types (Iqbal and Iqbal, 2014; Nishimura et al., 2015). Adding monoclonal antibodies against HER2 receptor (human epidermal growth factor receptor 2) such as Trastuzumab in combination with chemotherapy regimen consisting of paclitaxel (PTX), doxorubicin (DOX) and cyclophosphamide improved the survival of early-stage HER2-positive breast cancer patients (Incorvati et al., 2013; Perez et al., 2014). In a recent study, the novel combination of two chimeric monoclonal antibodies against EGFR was found to exert higher antitumor activity against the esophageal squamous cell carcinoma compared to the two marketed anti-EGFR antibodies both *in vitro* and *in vivo*. This was ascribed to the higher cellular internalization of this combination compared to the other two antibodies and their greater EGFR degradation (Fukuoka et al., 2016).

2.2.1.1.2. Antibodies against vascular endothelial growth factor receptor (VEGFR). Recently it has been found that the most promising molecules for salvage treatment are through angiogenesis signaling (Galdy et al., 2016). It was found that in early embryogenesis, the signal transduction systems responsible for oxygenation and nutrients supply develop *via* vasculogenesis and angiogenesis (Flamme et al., 1997; Shibuya and Claesson-Welsh, 2006). These systems include vascular endothelial growth factors ligands and receptors (VEGFs)/VEGFRs (Davis et al., 1996; Ferrara and Davis-Smyth, 1997). Lymphangiogenesis, develop at a later stage (Saharinen et al., 2004). VEGF-A binds and activates two types of receptors: VEGFR1 and VEGFR2 (Shibuya and Claesson-Welsh, 2006). VEGFR1 can down and up-regulate angiogenesis and vasculogenesis in embryogenesis and inflammatory conditions respectively (Shibuya and Claesson-Welsh, 2006). The placenta of preeclampsia patients revealed an overexpression of the soluble form of VEGFR1 (Helske et al., 2001). VEGFR2 was found to control endothelial cells' multiplication in angiogenesis signal transduction pathways (Shibuya and Claesson-Welsh, 2006). On the other hand, VEGFR3 expression pattern is restricted to lymphatic endothelium as a receptor for two growth factors; namely VEGF-C and VEGF-D (Alitalo and Carmeliet, 2002; Saharinen et al., 2004; Shibuya and Claesson-Welsh, 2006). Defective signaling of VEGFR3 was identified in congenital hereditary lymphedema (Irrthum et al., 2000). Besides, tumor-produced VEGF-C may stimulate the formation of tumor lymphatics resulting in enhanced spread of tumor metastases; a hypothesis which was proven in breast cancer patients (Liang and Li, 2014; Mandriota et al., 2001; Skobe et al., 2001). Soluble VEGFR-2 was found to inhibit lymphangiogenesis and lymph node metastasis in an *in vivo* model of lung cancer *via* a mechanism involving VEGF-C inhibition (Maehana et al., 2016).

A correlation was found to exist between the elevated levels of VEGFR and high tumor growth rate. In addition, it was closely linked to tumor metastatic potential and worsened patient prognosis (Galdy et al., 2016). In order to inhibit angiogenesis and suppress tumor growth, both the inhibition of VEGF activity by using antibodies (Kim et al., 1993) and the disruption of the VEGFR function (Prewett et al., 1999) are considered effective strategies (Lu et al., 2002). An example on the latter strategy is Ramucirumab which is a human immunoglobulin that binds to VEGFR-2 which prevented the attachment of VEGF ligand with subsequent receptor activation (Spratlin et al., 2010), leading to survival improvement of patients diagnosed with GIT cancer,