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Active Targeting of Breast Cancer Cells Using Nanocarriers

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Abstract

Breast cancer is the most common and lethal cancer type in women worldwide. Therapeutic strategies with better selectivity, such as active targeting by nanocarriers, are highly desirable. In this mini review, we briefly outline the evolution of nanocarriers culminating in actively targeted systems, the challenges faced when designing such carriers and, finally, the most promising breast cancer targets and possible ligands that can be coupled to the nanocarriers.

Keywords: Drug delivery systems; Active target; Breast cancer

Introduction

The treatments currently available for breast cancer treatment are allowing for the long-term survival rates to rise after a diagnosis of the disease. In 2012, around 6.2 million of women were accounted as breast cancer survivors, which represented 36.4% of the total estimated cancer survivors among women [1]. Nevertheless, the available chemotherapy schemes lead not only to severe side effects during the treatment, but also to potential long term medical issues. These challenges highlight the need of searching for new therapeutic strategies, especially ones with better selectivity [2,3]. Nanocarriers have the potential to circumvent the toxicity problems of anticancer drugs by increasing cancer cell targeting in comparison to conventional formulations. Since the approval of Doxil®, a PEGylated liposomal formulation of doxorubicin in 1995, great advances on understanding both nano systems and the molecular biology of breast cancer were made. This knowledge allow for the development of new promising nanotherapeutic strategies and devices [4].

The Evolution of Nanocarriers for Breast Cancer Treatment

The first generation of anticancer nanocarrries was planned based on the concept of passive targeting [5]. This is a size-dependent process and may occur as a result of the pathophysiological characteristics of the tumor vessels, which present a leaky vasculature and poor lymphatic drainage. These two characteristics together lead to an effect known as "enhanced permeability and retention (EPR) effect" [6]. This effect may be responsible for an increased accumulation of the nanocarrier in the tumor site compared to normal tissues, which allows higher efficacy and lower side effects [7].

Table 1: Nanocarriers currently on the market for breast cancer treatment.

Product	Nanocarrier/Drug	Composition	Year of Approval	References
Doxil®/ Caelyx®	Liposomes/Doxorubicin	HSPC/CHOL/DSPE-PEG	1995	[8]
Myocet®	Liposomes/Doxorubicin	EPC/CHOL	2000	[9]
Abraxane®	Nanoparticles/Paclitaxel	Albumin	2005	[10]
Genexol-PM®	Micelles/Paclitaxel	mPEG-PLA	2007 (Marketed in Korea)	[11]

DSPE-PEG: N-(carbonyl-methoxypolyethyleneglycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine; EPC: Egg phosphatidylcholine; HSPC: Hydrogenated soyphosphatidylcholine; mPEG-PLA: Monomethoxypoly(ethyleneglycol)-block-poly(D,L-lactide).

To better explore the passive targeting, the possibilities of enhancing the bloodstream circulation time of the nanocarriers started being explored. A successful method to obtain long-circulating nanocarriers is coating them with hydrophilic and flexible polymers such as poly(ethylene glycol) (PEG).This polymers create a sterical barrier so that interactions with blood components are reduced. Since the interaction of the nanosystems with opsonins is reduced, their uptake by the reticulo endothelial system is also decreased allowing them to achieve a longer blood circulation time [7]. All nanocarriers approved for breast cancer treatment are listed on Table 1, [8-11] and, except of Doxil®, belong to the first generation of nanosystems.

Despite of the advances concerning toxicity management, formulations with a greater impact on the overall survival rate of patients with breast cancer are still necessary. This fact encouraged the search for new strategies on nanocarriers development such as triggered drug delivery. This strategy theoretically allows the formulations to be triggered to release their contents upon the exposure to specific stimuli. These stimuli can be something such as heat, light, ultrasound, magnetic fields, low pH or enzymes [12]. ThermoDox®, a new formulation of thermo-sensitive PEGylated liposomes encapsulating doxorubicin was based on this concept. It presented encouraging efficacy results for patients with breast cancer on a Phase I clinical trial and is now undergoing a Phase III clinical trial [13]. Although they play an important role in the treatment of breast cancer, the formulations presented in Table 1 are generic anticancer products. The next step on the evolution of nanocarriers for treating breast cancer is to tailor the nanocarriers based on specific characteristics of the disease [4].

A strategy that rises from the idea of building nanocarriers to treat patients with a specific type of cancer is the active targeting. The careful selection of ligands to target surface molecules or receptors overexpressed by cancer cells leads to specific retention and uptake on tumor tissue [6]. No active targeted nanocarrier is commercially available for breast cancer treatment yet. Possible targets to be explored in breast cancer cell lines are the focus of this mini-review and will be further presented.

Passive versus active targeting

As previously mentioned, passive targeting occurs as a result of EPR effect and was the main driving force supporting the first generation of nanocarriers. However, it is known that EPR is a highly variable phenomenon and large inter- and intra-individual differences are observed. Parameters such as vascular volume, perfusion, permeability, penetration, and retention of the EPR effect differ quite significantly not only between different types of tumors, but also between different tumors within the same patient [14,15]. Active targeting, or ligand-mediated targeting, consists on binding ligands to the surface of the nanocarriers so that it can bind specifically to surface molecules or receptors overexpressed in the targeted tissue. It not only allows an accumulation of the nanocarrier at the targeted site, but also might induce receptor-mediated endocytosis, improving the intracellular delivery of the carried content [14]. A major drawback on this strategy is that the actively targeted carrier needs to be in the proximity of their target to benefit from this increased affinity. For this reason, actively targeted carriers are envisioned as a promising complementary strategy to EPR, to further augment the efficiency of the designed nanocarriers [6].

Designing actively-targeted nanocarriers

The first step when designing an actively-targeted nanocarrier is to select the target. One should consider 1) the relative degree of overexpression or selective expression on the target, 2) the ability to internalize the ligand-targeted formulation and 3) if the population that would benefit from that treatment is really significant. Then, it is necessary to think about the formulation development, considering 1) the ligand conjugation strategy to the nanocarrier (before or after nanocarrier formation, 2) the need of a linker to maximize targeting efficiency (e.g. PEG), 3) the ligand density and 4) the costs of the scaling up the formulation, as the development of targeted nanocarriers is much more expensive than that of non-targeted ones [6,14,16,17].

Table 2: Breast cancer targets, their reported prevalence of expression and possible ligands to couple to nanocarriers.

Target	Reported Prevalence of Expression in Breast Tumors	Possible Ligands
HER-2 receptor	13-20% [20]	Anti-HER2 monoclonal Antibodies [21]; Aptamers [22]
Estrogen receptor	75% [20]	Estrone [23]; Tamoxifen [24]
Folate receptor	Not well stablished. Suggested to be around 30%, but might be as high as 70–80% on triple negative breast cancers (TNBC) [25]	Folic acid [26]
Transferin receptor	74% [27]	Peptides (HAIYPRH) [28]
Integrins	Not well stablished. Highly variable between tumors [29]	Peptides (RGD) [30]
Mucin 1 (MUC1)	90% [31]	Anti-MUC1 monoclonal Antibodies [32]; Aptamers [33]
Epidermal growth factor receptor (EGFR)	15–45% of breast tumors and it is inversely related to hormone receptor expression [34]	Peptides (GE11) [35] Anti-EGFR monoclonal antibodies [36]

Active targeting to breast cancer cells

Many different receptors have been investigated for their potential to be targeted in breast cancer cells. The most relevant receptors and their reported prevalence in breast tumors are listed in Table 2. Similarly, many different nanocarriers such as liposomes, lipid nanoparticles, micelles, silica nanoparticles and gold nanorods have had their surface modified with ligands for those receptors. The ligands usually fall on one of the following categories: antibodies, peptides, aptamers or small molecules. Antibodies present high selectivity and binding affinity for the target as a direct result of the presence of two epitope binding sites in a single molecule. As limitations one can count the high cost of manufacturing, high molecular weight and batch-to-batch variation with the potential to induce an immunogenic response. Peptides have small size, low immunogenicity, easier and less expensive manufacture compared to antibodies. However, they have limitations such as a low target affinity and susceptibility to proteolytic cleavage. Aptamers have high specificity to target but might suffer from rapid clearance from the blood circulation due to nuclease degradation. Small molecules are relatively inexpensive to manufacture and versatile on structures and properties. However, they must have high specificity and affinity towards cellular receptors, what has proven to be a challenging task [18-36].

The promising *in vitro* and *in vivo* results observed to date for active-targeted nanocarriers allowed some of them to progress to clinical trials [18]. To the best of our knowledge, only one formulation designed specifically for breast cancer active targeting made its way into clinical trials. This formulation, called MM-302, is a HER2-targeted antibody-liposomal doxorubicin conjugate. In preclinical models, MM-302 had superior efficacy compared with both free doxorubicin and Doxil®. Its combination with trastuzumab also demonstrated better efficacy compared to either agent alone in HER2¬ over expressing tumor xenograft models. These findings provided support for starting the Phase Iclinical trial. This study suggested the promising efficacy of MM-302 alone or in combination with trastuzumab as well as a manageable safety profile in patients with advanced HER2-positive breast cancer. MM-302 progressed then to a Phase II study (HERmione) [37]. However, this study was halted after the observation of a shorter than expected median progression-free survival [38].

Conclusion

Active targeting is envisioned as a strategy that allows the accumulation of the nanocarrier at the targeted site and also might induce receptor-mediated endocytosis. Concerning breast cancer, some targets herein mentioned have raised special interest for active target. To date, many promising *in vitro* and *in vivo* results have been observed for nanocarriers directed to these targets. However, only one liposomal formulation designed specifically for breast cancer active targeting made its way into clinical trials. The many challenges behind this strategy might be responsible for the low translation of these formulations to the clinics. However, the need for treatments with better selectivity and efficacy should boost the research efforts based on this promising approach.

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