Theranostics

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“Theranostics,” a new concept of medical advances featuring a fusion of therapeutic and diagnostic systems, provides promising prospects in personalized medicine, especially cancer.

Keywords: theranostics ; single chain variable fragment of IgG (scFv) ; drug delivery system (DDS) ; siRNA ; PDT

1. Introduction

Theranostics is a novel term invented for drugs and mechanisms that are used for simultaneous diagnosis and treatment[1] or a simpler definition of diagnosis plus therapy[2]. The first clinical application of “theranostics” was conceptualized in 1946[3], utilizing the radioactive iodine therapy, [^{131}I] for patients with metastasized thyroid cancer. Thereafter, various radioligand-based therapies including radio-attached monoclonal antibody, small-molecule inhibitors of prostate-specific membrane antigen (PSMA), and α- and β-emitting radioisotopes have gained popularity in early phase clinical trials, particularly in PSMA expressed prostate cancer[4][5][6][7][8]. Cancer is among the top five major diseases that caused millions of deaths in the 20th century and yet remains a challenging disease to treat causing significant morbidity and/or mortality with over 10 million new cases annually[1]. Cancer therapy, for many decades, has relied on the conventional radiotherapy and chemotherapy, which have significant drawbacks and side effects where non-cancerous cells are also greatly affected by chemotherapeutic action [9]. Although recent medical advancements in the form of targeted treatments, early detection, and behavioral changes have improved cancer prognoses, many treatment options are still reported to be ineffective at preventing recurrences. Moreover, the invasive nature, drug resistance, and systemic toxicity side effects of these treatment options are highly disputed[10]. Previous literatures reported that as much as 70% of ovarian cancers and several types of pancreatic cancers have already metastasized even before diagnosis, thus imploring the need for an earlier and more precise method of diagnosis coupled with targeted treatment[11][12]. The state-of-the-art theranostics concept shifts from the conventional one-size-fits-all medicine approach to a more holistic personalized medicine approach. The goal of this therapy is to offer the right treatment, for the right patient, at the right time while providing the right dose with a more targeted and efficient pharmacotherapy profile. When developing these theranostic-based technologies for clinical translation, it is imperative to focus on adequate blood plasma circulation time, specific delivery to cancerous tissues only while successfully evading normal tissues and organs accumulations, lack of an immune response, and simultaneous treatment coupled with non-invasive monitoring for successful drug delivery. Moreover, the delivery system should also be preferably non-invasive, non-toxic, and biodegradable[13].

2. Polymeric Micelle-Type DDS Carrier as the “Core” of Theranostics Technology

Theranostic nanomedicine for medical purposes consists of colloidal nanoparticles ranging in sizes from 10 to 1,000 nm (1 μm). They include macromolecular materials/polymers in which the diagnostic and therapeutic agents are adsorbed, conjugated, entrapped, and encapsulated for diagnosis and treatment, simultaneously at both cellular and molecular level[14]. Theranostic nanomedicine is superior compared to conventional theranostics because they have advanced capabilities as an all-in-one single platform, which include targeted delivery, sustained/controlled release, higher transport efficiency via endocytosis, remotely triggered delivery, synergistic performance (e.g., siRNA co-delivery, PDT and PCI co-delivery, and chemotherapy combination therapy), and multimodality diagnosis and/or therapies and quality performances (e.g., oral delivery, evasion of multi-drug resistance (MDR) protein, and autophagy inhibition)[9]. There are several prerequisites for a successful nano-theranostics agent: 1. The nanocarrier should be easily manufactured using standard procedures in nanotechnology to provide designed functionalities and achieve specific targeting. 2. Nanomolecules must be efficient enough to improve the pharmacokinetic profile while enhancing the biodistribution of existing therapeutic moieties to the targeted site. 3. They must have promising intrinsic advantages for site specific delivery within solid tumors via the leaky vasculature[15]. 4. Nanocarriers should be non-toxic and biocompatible polymers to improve the safety index of the anticancer agent to reduce overall systemic toxicity. 5. The nano system should possess the inherent advantage of

enhancing aqueous solubility of lipophilic compounds so that they may be appropriate for parenteral administration. 6. They should have a good stability profile when loading or encapsulating therapeutic entities such as small-sized hydrophobic molecules, peptide drugs, and even oligonucleotides[16]. The nanomolecules should demonstrate little uptake into the reticuloendothelial system (RES) such as the liver and spleen. 8. The nanoparticle should also be stealthy enough to evade recognition by the immune system[17].

Among these prerequisites, stealth to evade recognition by the immune system is a major challenge for emerging nanoparticles. The first approved “PEGylated” products have been in the market for over 30 years still undergo severe interaction with the immune system when applied systemically. It was reported that the subcutaneous injection of an PEG-modified ragweed allergen in humans triggered the formation of IgM isotype antibodies to PEG and pre-existing IgG and IgM anti-PEG antibodies were identified in more than 25% of healthy donors[18][19]. The presence of these anti-PEG antibodies was strongly correlated to the rapid blood clearance of PEG conjugates, which inadvertently affects the pharmacokinetic profile of these conjugates[20]. Although considered as the gold standard in the field of polymeric drug delivery, PEG displays a vast amount of other side effects and complications such as its nonbiodegradability, unexpected changes in pharmacokinetic behavior, toxic by-products, and an antagonism arising from the easy degradation of the polymer under mechanical stress[17] There were even reports on anaphylactic shock induced by PEG[21][22][23]. Since then, a variety of promising hydrophilic polymers have emerged as synthetic alternatives to PEG. Among them is the novel amphiphilic block polymers composed of poly(L-lactic acid) (PLLA) and poly(sarcosine), molecular assemblies with various morphologies such as micelle, vesicle, and lamella were formed and collectively named as “Lactosome” particles[24].

The amphiphilic poly(sarcosine)-*b*-poly(L-lactic acid) (Lactosome) is an established polymeric micelle with an average diameter of ca. 35 nm[25][26]. Sarcosine (Sar) or N-methyl glycine is a natural amino acid and its homopolymer shows high solubility against aqueous solution like PEG [27]. Hence, polymeric micelles with their surfaces covered with poly(sarcosine) are expected to show prolonged blood circulation characteristics with negligible undesired accumulation by the RES[28]. The poly(L-lactic acid) or PLLA component, on the other hand, is one of the most commonly used biodegradable and bioinert materials[29][30] and is known to form 3_{10} helical structure[31]. In the concept of radionuclide for PET imaging, the [^{18}F]SFB labeled poly(L-lactic acid) of 30mer, consisting of the hydrophobized ^{18}F attached to the PLLA chain, was encapsulated into the core/hydrophobic region of Lactosome via hydrophobic interactions[32]. This ^{18}F labeled Lactosome particles, which was administered *in vivo* via the tail vein of the tumor transplanted mice, displayed clear PET images post 6 h of dosage. Here, Lactosome particles showed excellent blood circulation aspects owing to the surface modification with hydrophilic poly(sarcosine) chains. Hence, the signal intensity at the organs with high blood flows was high and the accumulated signal in the transplanted tumor could be detected. Since the surface of the Lactosome particles was not specifically modified by ligands in this study, Lactosome particles were considered to be passively accumulated to the tumor region by the EPR effect[32]. In the concept of therapeutic radionuclide, studies have been conducted on the *N*-succinimidyl 3-[^{131}I]iodobenzoate ([^{131}I]SIB) labeled PLLA encapsulated into the Lactosome particles as β-ray emitter by the same approach with the previous ^{18}F compound for PET imaging[33]. To the preliminary PEIT treated tumor transplanted mice, ^{131}I labeled Lactosome particles of 200 MBq/kg were injected and the time course of tumor growth was investigated. It was concluded in this study that the ^{131}I labeled Lactosome particles provided efficient delivery to the tumor region with significant tumor suppression[33]. Lactosome demonstrated excellent EPR effects in numerous cancer cells such as human pancreatic cancer, hepatocellular carcinoma, lung papillary adenocarcinoma, mouse 4T1 breast tumor, and even rat mammary adenocarcinoma[33][34][25][32]. Besides tumor imaging[25][32]and anti-tumor therapy[33], it has also been applied for tumor-selective PDT[34] through amphipathic peptide modification on Lactosome particles[35]. The most recent advancement of Lactosome mediated RNAi technology involved the successful conjugation of the ATP-binding cassette transporter G2 (ABCG2) siRNA to the L7EB1 type CPP modified Lactosome particles via disulfide bonds to improve RNA stability and transfection efficiency[36][37].

Although Lactosome particles showed promising capabilities as a nanocarrier for drug and/or imaging agent delivery, it is not without any shortcomings. For drug delivery and treatment purposes, nanocarriers are expected to show unaltered disposition upon multiple administrations. However, the production of anti-Lactosome antibody was observed not only three days after the first administration, but it remained elevated for a duration of six months[38]. As for the second dosage of Lactosome particles’ administration, it was immediately opsonized by the anti-Lactosome antibody soon after administration and subsequently entrapped by the RES. This phenomenon is commonly generalized as the accelerated blood clearance (ABC) phenomenon of Lactosome particles, which is comparable to the phenomenon sometimes observed on PEGylated materials[39][40]. Lactosome, together with the PEGylated liposomes, belong to the T cell-independent (TI) type II antigen[38][41], which are antigens that can trigger the antibody production without the help of T cells[42][43]. Previous research has reported that the peritoneal B1a cells recognize a TI type II antigen of Lactosome and hence responsible for the ABC phenomenon[44]. On the other hand, this interaction was not observed in PEGylated

liposomes where the splenic marginal zone B (MZ-B) cells[45]are responsible for anti-PEG IgM production for PEGylated liposomes[46][47]. As the antibody-secreting cells (ASCs) are found only in the spleen and bone marrow, but not in the peritoneal cavity (PerC), there is therefore a migration of Lactosome-responsive B1a cells from the PerC to the spleen upon Lactosome stimulation[48]. This difference in the trigger of B1 subsets may be related to the long-lasting memory and IgG_3 production induced by Lactosome but not by PEGylated liposomes. These reports indicate that the ABC phenomenon for different nanomolecules is driven by different immune system pathways, which should be further investigated to improve the efficacy and safety profile of potential nanotherapeutics.

Up to date, there have been extensive studies to overcome this problem. Similar to TI antigens such as PEGylated liposome, which activate B cells and induce IgM antibodies production at the early stage of administration, however, at high doses, lack the activation ability on B cells[49][50]. This phenomenon is called immune tolerance and is widely used in PEG derived therapeutic where the ABC phenomenon is suppressed by high-dose PEGylated liposomes[39][51][52]. In one study where a wide range of Lactosome dose (0–350 mg/kg) was administered in tumor transplanted mice, the production amounts of anti-Lactosome IgM were found to be inversely correlated with that of the first Lactosome dosage. The first Lactosome dose dependence of the ABC phenomenon was reported at a critical value of 50 mg/kg dose[53]. If the first administered Lactosome dose is below 50 mg/kg, the Lactosome at the second administration will be entrapped in the liver. However, when a high first Lactosome dosage was administered (>150 mg/kg), the Lactosome was found to be accumulated in the tumor region by the EPR effect indicating that the Lactosome ABC phenomenon was suppressed by the induced immunological tolerance effect[53]. However, the anti-Lactosome IgM in serum remained high irrespective of second doses indicated that the ABC phenomenon still occurred, but the anti-Lactosome IgM was saturated with excessive doses of over 50 mg/kg at the second Lactosome administration. These lead to ICG-Lactosome free from binding of anti-Lactosome IgM and to efficiently accumulate in the tumor. Overall, we interpreted that with the increment of first Lactosome doses, the anti-Lactosome IgM level decreases gradually due to partial immune tolerance. When this anti-Lactosome IgM level becomes lower than the amount required for opsonization of Lactosome dose as low as the amount of the second dose, the ABC phenomenon disappears upon the second administration of the Lactosome particles. More importantly, there was no acute toxicity induced even at such high Lactosome dosage administration[53]. Therefore, a high dose Lactosome administration approach may be adopted to evade the Lactosome ABC phenomenon, which makes it possible for Lactosome to be used for multiple imaging.

Besides using high doses of Lactosome to induce immunological tolerance, further studies were conducted on the suppression of anti-Lactosome IgM by modification of the lactosome structure itself. Several studies have reported on the improved stealth effect of Lactosome from the immune system when the nanoparticle surface is covered densely by hydrophilic polymer chains such as a high-density polymer brush structure. This dense hydrophilic shell may evade capture by B-cell receptors, thus preventing B-cell receptor recognition[54][55][56]. Spherical structures of micelles and small vesicles have a disadvantage because of the existence of their relatively looser spaces at the outer surface compared to the inner regions due to the large curvature of the molecule[54]. Hence, a sheet structure of ~20 nm was prepared from poly$(Sar)_m$-*block*-(L-Leu-Aib)$_n$, which consists of a series of amphiphilic helical peptides and a hydrophobic helical block of $(Leu\text{-}Aib)_n$ (Aib represents α-aminoisobutyric acid)[57]. This peptide nanosheet, despite having the same poly(sarcosine) chains as Lactosome, did not induce the ABC phenomenon where it completely loses any epitope activity due to the high-density polymer brush state[54]. Therefore, the formation of a high-density polymer brush on the surface of nanoparticles is one of the many strategies for nanoparticles to elude the immune system[24].

It was reported that when the surface modifying polymer chains become a high-density polymer brush state corresponding to a surface density of 0.3 chains/nm^2 or over, the surface provides a lubricating surface which also serves as an antifouling property[58][59]. Thus, a novel amphiphilic polydepsipeptides with three hydrophilic branch chains connected to one hydrophobic poly(lactic acid) chain (A_3B-type) was developed as shown in Figure 1[55]. The A_3B-type polymeric micelle size went down to ca. 22 nm from the AB-type of ca. 36 nm[60]. Notably, the A_3B-type Lactosome attenuated the ABC phenomenon compared to AB-type Lactosome as observed by the NIRF imaging and anti-Lactosome IgM production (Figure 2)[55]. The anti-lactosome IgM production was significantly lower with A_3B-type Lactosome compared to AB-type Lactosome, indicating lower immunogenicity profile of the A_3B-type Lactosome. Figure 2B shows that the AB-type Lactosome, upon first administration, spread over the whole body and gradually accumulated in the tumor region via the EPR effect. However, upon second administration (after seven days) the AB-type Lactosome immediately accumulated in the liver as previously reported (Figure 2D)[38]. On the other hand, A_3B-type Lactosome was observed to spread over the whole body to accumulate in the tumor region upon both first and second administration as shown in Figure 2C, E. There are several ABC attenuation factors regarding this: 1. By increasing the local density of the poly(sarcosine) chains on the micelle surface, which is 0.30 chain/nm^2 for the A_3B-type Lactosome, which is 4 times higher than 0.07 chain/nm^2 for the AB-type Lactosome, the higher surrounding hydrophilic polymer chains successfully prevent the interaction between the micelles and B cell receptors[55]. 2. The half-life of A_3B-type Lactosome in the

bloodstream was 4.3 hr, which is considerably shorter compared to the AB-type Lactosome of 17.2 hr. This may result in the nanoparticle having less chance to trigger the immune system. Unfortunately, a shorter lifetime in the bloodstream is also reflected in a decrease in tumor accumulation effect by the A_3B-type Lactosome particles. Therefore, the optimal chain length of poly(sarcosine) and composition of lactosome to stabilize the polymeric micelle structure were investigated[56]. Kurihara et al. reported that the optimized poly(sarcosine) chain length lies between 33mer and 55mer for ABC phenomenon suppression. There are several explanations for this: 1. These modified A_3B-type Lactosome particles showed relatively longer blood half-life, between 6.0 and 9.2 hr compared to the shorter 23mer A_3B-type Lactosome of 4.3 hr[55]. 2. After 48 hr of administration, Lactosome with longer poly(sarcosine) chain lengths above 33mer presented less than 20% dose of liver accumulation compared to 40–50% dose for Lactosome with 10mer poly(sarcosine) chain length[56]. 3. The tumor/liver accumulation ratio was double compared to Lactosome with 10mer poly(sarcosine) chain length. 4. The antigenicity of 33mer poly(sarcosine) chain length Lactosome was reduced at least 3 times compared to the AB-type Lactosome upon multiple administration (40% of anti-Lactosome IgM produced by AB-type Lactosome at first administration and about 35 to 50% of that by AB-type Lactosome on second administration). From these findings, we can confirm that it is the A_3B Lactosome structure as a whole rather than the surface density of the hydrophilic polymer chain that determines its stealth index. Hence, the optimum poly(sarcosine) chain length between 33mer and 55mer to lower the antigenicity of the nanoparticle while maintaining the *in vivo* disposition was elucidated[57][58][59][60].

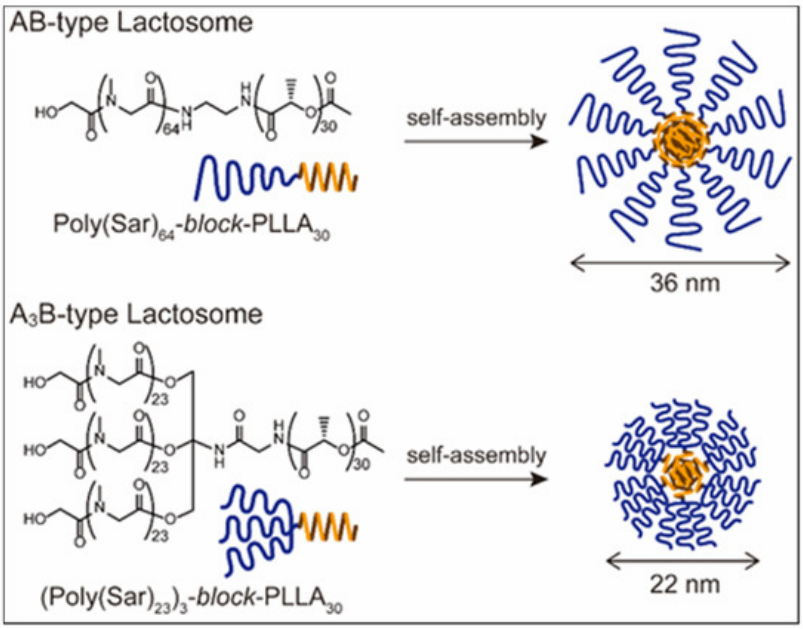

Figure 1. Illustration of the molecular structure of AB-type and A_3B-type Lactosome[55].

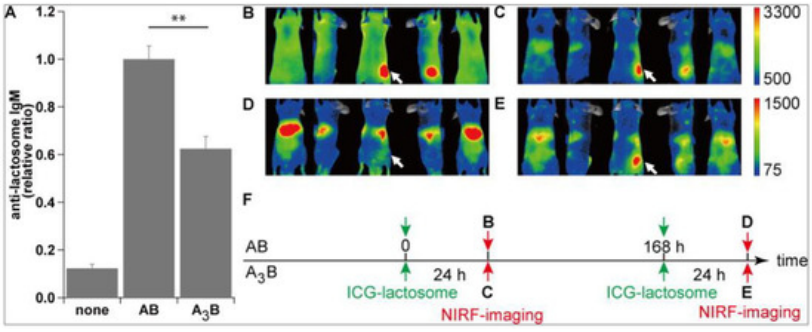

Figure 2. IgM productions at seven days after the administration of the AB- and A_3B-type Lactosomes (**A**) (*n* = 3 per group). Anti-lactosome IgM productions are normalized by taking the anti-lactosome IgM production with the AB-type Lactosome as a reference, 1.0. Pharmacokinetic changes (NIRF images) upon multiple doses of the AB- (**B,D**) and A_3B-type (**C,E**) Lactosome particles. The images D and E were taken at seven days after the first administration of the AB- and A_3B-type Lactosome particles. The time schedule is shown in panel F. Reprinted (adapted) with permission from Hara, E., et al., Factors influencing in vivo disposition of polymeric micelles on multiple administrations. ACS Med Chem Lett, 2014. 5(8): p. 873-7. *Copyright © 2014, American Chemical Society*.

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