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Personalized liposome–protein corona in the blood of breast, gastric and pancreatic cancer patients

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ABSTRACT

When nanoparticles (NPs) are dispersed in a biofluid, they are covered by a protein corona the composition of which strongly depends on the protein source. Recent studies demonstrated that the type of disease has a crucial role in the protein composition of the NP corona with relevant implications on personalized medicine. Proteomic variations frequently occur in cancer with the consequence that the bio-identity of NPs in the blood of cancer patients may differ from that acquired after administration to healthy volunteers. In this study we investigated the correlation between alterations of plasma proteins in breast, gastric and pancreatic cancer and the biological identity of clinically approved AmBisome-like liposomes as determined by a combination of dynamic light scattering, zeta potential analysis, one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (1D-SDS-PAGE) and semi-quantitative densitometry. While size of liposome–protein complexes was not significantly different between cancer groups, the hard corona from pancreatic cancer patients was significantly less negatively charged. Of note, the hard corona from pancreatic cancer patients was more enriched than those of other cancer types this enrichment being most likely due to IgA and IgG with possible correlations with the autoantibodies productions in cancer. Given the strict relationship between tumor antigen-specific autoantibodies and early cancer detection, our results could be the basis for the development of novel nanoparticle-corona-based screening tests of cancer.

1. Introduction

Due to the peculiar properties that arise when a material is reduced to the nanoscale, nanoparticles (NPs) are being utilized in almost limitless applications in biomedical research (Hafner et al., 2014). The hardest challenge occurs when NPs are dispersed in a biofluid, because of their tendency to interact with biomolecules, such as proteins, lipids and sugars driven either by a potential energy gradient, or just by diffusion. These molecules instantaneously adsorb onto the NPs' surface creating a complex biomolecular coating around the particles, referred to as the NP corona (Mahon et al., 2012; Monopoli et al., 2011a). What is clear to date is that the new identity given by the corona is the main factor controlling biodistribution, therapeutic effect and nanotoxicity of NPs in the body (Walkey and Chan, 2012). Exposure time has been identified as a key factor shaping the NP biomolecular corona (Dell'Orco et al., 2014; Tenzer et al., 2013). The biomolecular corona is highly dynamic in nature with its composition depending both on the physicochemical properties of the particles (Caracciolo et al., 2010; Docter et al., 2014; Lundqvist et al., 2008; Mahmoudi et al., 2011, 2012; Monopoli et al., 2011b; Röcker et al., 2009; Zhang et al., 2011) (e.g. material, size, surface charge, shape) and the characteristics of biological media including protein source (Caracciolo et al., 2014b), concentration (Caracciolo et al., 2011; Monopoli et al., 2011b) and temperature (Mahmoudi et al., 2013a). All the studies reported so far used prevalently plasma from healthy volunteers, while very few earlier studies have characterized the interaction

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of nanoscale particles with plasma collected from patients with different clinical manifestations (Caracciolo et al., 2014a; Hajipour et al., 2014). Recently, Hajipour et al. (2014) demonstrated that the type of disease critically affects the corona composition of commercially available silica and polystyrene NPs. This observation let the authors introduce the innovative concept of personalized protein corona (PPC). The most direct implication of the PPC is that precise corona information is needed for personalized therapeutics and diagnostic (Hamad-Schifferli, 2015). This might have obvious impact in cancer where hundreds of proteins are differentially expressed (Sallam, 2015). Proteomic variations in cancer imply that the bio-identity of NPs in the blood of cancer patients may differ from that acquired after administration to healthy volunteers. Nonetheless, since each cancer type can specifically alter the levels of relevant proteins, it is reasonable to assume that patients with specific cancers may have specific nanoparticle coronas. As such, the main goal of this investigation was to determine whether the pathologically alterations of plasma proteins in cancer influenced the protein corona and dependent NP features. To test this suggestion, we incubated liposomes with plasma from humans with histologically proven breast, gastric and pancreatic cancer with similar severity. The selected cancer types are respectively the second, fifth and twelfth most common cancers for both sexes (www.wcrf.org). Liposomes are currently tested for numerous applications in nano-biomedicine and are already used in cancer treatment (Allen and Cullis, 2013; Barenholz, 2012; Caracciolo, 2015). In particular, a lipid composition made of hydrogenated soy phosphatidylcholine (HSPC), 1,2-distearoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (DSPG), and Cholesterol (Chol) that constitutes the basis of the liposomal amphotericin B agent AmBisome (Adler-Moore and Proffitt, 2002) was employed in this study. Since AmBisome liposomes are anionic, as those used in anti-cancer therapy (Caracciolo, 2015), they are excellent model systems to study the interaction between plasma proteins and clinically approved liposomes. Basically, AmBisome is indicated in the treatment of severe systemic and/or deep mycoses and in the empirical treatment of presumed fungal infections. Anyway, patients with cancer who receive chemotherapy have an increased chance of getting fungal infections (Johansen and Gøtzsche, 2000). Antifungal drugs are therefore often given prophylactically to cancer patients, or when they have a fever. To understand whether human plasma (HP) collected from breast, gastric and plasma cancer patients could alter the corona structure and composition here we used a combination of one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (1D-SDS-PAGE), semi-quantitative densitometry, dynamic light scattering (DLS) and zeta potential analysis. Of note, exposure to plasma of pancreatic cancer significantly altered the structure and composition of the liposome–protein corona.

2. Materials and methods

2.1. Liposomes preparation

Hydrogenated soy phosphatidylcholine (HSPC), 1,2-distearoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (sodium salt) (DSPG), cholesterol (Chol.) were purchased from Avanti Polar Lipids (Alabaster, AL). AmBisome-like liposomes were synthesized according to (Marchini et al., 2011) with these molar ratios: HSPC:DSPG:cholesterol (2.5:1:1.2) (Adler-Moore and Proffitt, 2002). Lipid films were hydrated with phosphate saline buffer (PBS) 10 mmol l^{-1} (pH 7.4) to a final lipid concentration 1 mg/ml. The obtained liposome suspension was extruded 20 times through a 0.05 μm polycarbonate carbonate filter by the Avanti Mini-Extruder (Avanti Polar Lipids, Alabaster, AL).

2.2. Protein corona isolation

Milestone studies (Caracciolo et al., 2011; Cedervall et al., 2007; Lundqvist et al., 2008; Monopoli et al., 2011b) have shown that the aggregation state of NPs, thickness and composition of the protein corona can change with HP concentration. However, saturation occurs at around 40–80% plasma concentration depending on the NP's nature. According to this indication, we have previously investigated the effect of plasma concentration on the composition of the liposome–protein corona (Caracciolo et al., 2011). Saturation was found to occur at 50%, thus here we used 50% plasma concentration (i.e., 1:1 liposome:HP volume ratio). For proteomics experiments, 100 μl liposome suspension was mixed with HP (collected from both healthy and cancer patients) (1:1 v/v) and incubated for 60 min at 37 °C. After incubation, protein–liposomes corona complexes were isolated by centrifugation for 15 min at 14,000 rpm. Then pellets were washed three times with 200 μl of PBS to remove unbound proteins obtaining the "hard corona."

2.3. Human specimens

HP samples were isolated by centrifugation from heparinized venous blood of cancer patients not previously treated with radio or chemotherapy. Cancer patients included patients with breast cancer ($n = 10$), gastric cancer ($n = 10$) or pancreatic adenocarcinoma ($n = 10$) with similar severity, recruited at the Centro Oncologico Ematologico Subalpino (COES), AOU Città della Salute e della Scienza di Torino (Torino, Italy) with informed consent. Blood samples were immediately processed after drawing and plasma were stored at −80 °C until use. Human blood from healthy volunteers was obtained by venipuncture and prepared at the Department of Experimental Medicine (Sapienza University of Rome) according to the institutional bioethics code; a K2-EDTA anticoagulant and protease inhibitor cocktail were immediately added. Blood samples were immediately processed after drawing and plasma were stored at −80 °C until use. When used, aliquots were thawed at 4 °C and then let to warm at room temperature

2.4. Ethics statement

The human studies were conducted according to the Declaration of Helsinki principles. Human investigations were performed after approval of the study by the Scientific Ethics Committee of AOU Città della Salute e della Scienza di Torino, Torino, Italy (Prot. No. 0012068). Written informed consent was received from each participant prior to inclusion in the study and specimens were de-identified prior to analysis.

2.5. Size and zeta experiments

For size and zeta-potential experiments, liposome–protein complexes were prepared by incubating 10 μl of each lipid dispersion, pre-diluted with 490 μl of a PBS:H_2O solution (1:80 v/v), with 10 μl of HP pre-diluted with 490 μl of a PBS:H_2O solution (1:80 v/v). Dilution did not modify the pH of HP (pH = 7.45 ± 0.03). After 1-h incubation, samples were ready to be analyzed. Size and zeta-potential measurements were performed on a Zetasizer Nano ZS90 (Malvern, UK) at room temperature. Dynamic light scattering (DLS) allowed to retrieve normalized intensity auto-correlation functions that were analyzed by the CONTIN method, which allows one to obtain the distribution of the diffusion coefficient D of the particles. This coefficient is converted into an effective hydrodynamic radius R_H by using the Stokes–Einstein relationship $R_H = KBT/(6D)$, where KBT is the thermal energy and the solvent viscosity. Polydispersity index (PDI) of DLS measurements can be found in Table S1 of the Supplementary Material. The electrophoretic mobility