Editorial

Exploring gold nanoparticle interactions with proteins and the tumor microenvironment in biological systems

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Gold nanoparticles (AuNPs) have been widely studied as a theranostics agent, especially in the field of cancer treatment and diagnosis, due to their versatile physical and chemical properties (1). Their physical properties make them a good imaging enhancer under different modalities, including x-ray and computed tomography. Their surface plasmon resonance enables them to be combined with newer technologies, including photoacoustic imaging and thermal ablation. Their versatility in synthesis means that AuNPs can be different sizes and shapes, including colloidal gold nanoparticles, nanorods, nanostars, and nanoshells. Common surface modifications are achieved via gold-thiol bond conjugation, and this is a widely applied method of delivering anticancer drugs.

The biological effects of AuNPs, including their (cyto) toxicity and pharmacokinetics, vary according to their size, shape, and surface modifications. Most plain hydrophilic AuNPs are charged, and their charge nature depends on the capping agent. In the past, most research has ended at the verification of drug delivery or the enhancement of imaging signals at the cell or tissue level, and the anticancer effect of AuNPs has been attributed to either the drug they carry or the thermal effect they produce. Recently, research interest has expanded to understanding nanoparticles' effects on a subcellular level, including the nanoparticle/protein interaction and its effect on cellular pathways and the extracellular matrix (ECM), in the aid of further understanding the biological effect of functionalized nanoparticles. AuNPs have also served as tools to understand the nanoparticle/protein interaction and nanoparticles' behavior in the microenvironment.

Proteins are water-soluble macromolecules. There are over 37,000 kinds of proteins in the human body. The surfaces of proteins are often charged, and charge patches sometimes form on these surfaces. The net surface charge depends on the pH value of the system. The pH at which the net charge is zero is the isoelectric point (pI) of the protein. Thus, the net charge of the protein is positive when the pH is lower than the pI value and negative when the pH is higher than the pI. The protein's surface charge plays an important role in the interaction between the protein and hydrophilic polymers or polyelectrolytes. One might assume that the ion-ion interaction, hence the complexation, between a protein and a polyanion only occurs when the pH is lower than the pI. In fact, the interaction between these patches and polymers or polyelectrolytes can be so strong that "binding on the wrong side of pI" can also happen (2).

The complexation between proteins and nanoparticles is complex. In addition to the surface charges of the nanoparticle, particle size plays a role. When particles are

extremely small, their surfaces are highly curved, preventing effective complexation (3). Distortion of protein molecules may occur upon complexation, or upon the absorption of the protein by the nanoparticles. Surface modifications of the nanoparticle may affect the kinetics and extent of protein absorption but cannot eliminate protein absorption. The results of a study of bovine serum albumin (pI ~5; thus, it is negatively charged under physiological conditions) suggest that it binds to positively charged AuNPs more rapidly than to negatively charged AuNPs (4). However, it can still bind to PEGylated AuNPs after extended incubation. Thus, despite the surface charge of the nanoparticle, complexation with proteins is inevitable in biological systems.

Protein absorption on the particle surface was recently termed "protein corona" (3,5). Since the absorption is always reversible and different proteins have different binding kinetics and coefficients, proteins that have fast binding may be replaced by those with a slower binding but a higher affinity over time. Thus, the protein corona is not a solid unchanged layer but a dynamic equilibrium with exchange. Recent research suggests that this corona affects how cells "see" the particles (6), and Lynch *et al.* (6) suggested that apart from the conventional physiochemical classification, the macromolecules and nanoparticles should also be classified according to the protein corona property. Thus, it would be interesting to investigate how plain nanoparticles affect cellular behavior.

A recent original study in *ACS Nano* (7) studied the effects of plain AuNPs on pancreatic stellate cells (PSCs) and pancreatic cancer cells (PCCs), the bidirectional crosstalk between PSCs and PCCS, and on the ECM. The study used plain hydrophilic citrate-capped AuNPs of 20 nm. The AuNPs inhibited the growth of both PSCs and PCCs. They also inhibited the production of several growth factors, including EGFPs, and AuNP treatment both interrupted the crosstalk between PSCs and PCCs and affected the tumor microenvironment (TME).

The TME, which is composed of vasculature, endothelial cells, fibroblasts, inflammatory cells, immune cells, lymphocytes, chemokines, and ECM proteins, provides the scaffolding needed for a tumor to thrive (8). A disruption in any one of these components could inhibit the tumor's potential for growth and inhibit desmoplastic tissue proliferation. Because increased desmoplasia is associated with poorer patient outcomes in pancreatic ductal adenocarcinoma (PDAC), even the slightest modifications in the TME could produce a significant effect (9). Mukherjee *et al.* demonstrated that bare AuNPs disrupt tumor-stroma crosstalk in PDAC by altering the cellular secretome and subsequently halting the progression of the disease (7). This crosstalk involves PCCs activating PSCs, a type of fibroblast, to increase ECM production and proliferation. The activated PSCs then feed back onto the PCCs to allow them to evade apoptosis and migrate via epithelial-to-mesenchymal transition (10). Epithelial-to-mesenchymal transition is associated with transforming growth factor-β, which allows the tumor to evade immunological surveillance by cytotoxic and natural killer T cells.

In the TME, fibroblasts are specifically referred to as cancer-associated fibroblasts (CAFs) (8). CAFs differ from normal fibroblasts by their increased expression of alpha-smooth muscle actin (α-SMA), matrix metalloproteinases, vascular endothelial growth factors, fibroblast growth factors, platelet-derived growth factors, and other pro-angiogenic factors. They are also capable of remodeling the ECM to upregulate paracrine signals such as insulin-like growth factors 1 and 2. Together, CAFs' functions allow for tumor cell growth, invasion, and metastasis. PCC-activated PSCs take on roles similar to those of CAFs, and they are characterized by increased expression of α-SMA and diminished levels of vitamin A (11). Although it has been shown that high stromal activity and low collagen deposition contribute to poorer prognoses, the fibrotic stroma also has a surprisingly protective role in preventing the metastasis of PDAC cells (12). This finding emphasizes the notion that there is still much left to be discovered regarding the complex, multi-faceted cellular crosstalk between PCCs and PSCs, especially in the presence of AuNPs.

The hallmark function of bare AuNPs is to adsorb various growth factors, cytokines, and other secreted molecules from the PCCs and PSCs to disrupt PCC/PSC crosstalk both *in vitro* and *in vivo* (7). The downstream effects of this result in decreased activity of the mitogen-activated protein kinase signaling pathway, which tends to be upregulated in tumorigenesis. Activated PSCs treated with AuNPs also demonstrated a reinstatement of the more quiescent phenotype, as characterized by reduced α-SMA expression, decreased ECM protein production, and restored lipid synthesis. While the AuNPs were shown to reduce the expression of several key ECM proteins, such as collagens I and III, the levels of collagen IV, which is primarily found in the basal lamina, were unaffected (7). The mRNA levels of lipid metabolism genes in PSCs were increased, with the exception of those of SREBP1a and SREBP2, which were not significantly restored by

AuNPs. These curious findings further demonstrate that a more specific study of the effects of AuNPs on individual signaling cascades is necessary to elucidate their specific roles in intracellular and intercellular signaling.

Another biological mechanism of AuNP-mediated inhibition of cancer cell growth is the dose-dependent induction of ER stress through the upregulation of proteins such as IRE1α and IRE1β (7). This induces Ire1-dependent decay of mRNAs, which alters the secretome of PCCs and PSCs and disrupts the pathways involved in PDAC, including the Raf/MEK/ERK, PI3K/Pdk1/Akt, and Ral/GEF pathways (10). Furthermore, using a computational model, Mukherjee *et al*. were able to map several "hub" proteins that demonstrated widespread, maximal regulation of other key pathways (11). To validate their findings, they performed several autoregulation and heteroregulation studies that implicated transforming growth factor-β1, endostatin, thrombospondin 1, platelet-derived growth factor AA, uPA, and several other "hub" nodes as critical in PCC-PSC crosstalk. With further in-depth study, these hubs will prove invaluable in determining the exact locations of pathways in which bare AuNPs exert their effects.

Studies so far have shown that bare AuNPs are effective against PCCs and PSCs without deleteriously affecting normal human pancreatic ductal epithelial cells or other non-malignant cells (13). There has also been no demonstrated inducible systemic toxicity resulting from weeks of repeated injections of AuNPs, bolstering its promise as an effective form of localized cancer treatment. While intraperitoneal injection of these AuNPs showed tremendous efficacy in Mukherjee's study (7), different routes of administration must also be considered. In intravenous injection, which is the route most widely used by current researchers, AuNPs accumulate a hard corona composed of distinct serum proteins, such as albumin (14). Because the hard corona maintains its composition even as the AuNPs travel to different environments, AuNPs' efficacy at disrupting cellular crosstalk in malignant tumor cells may be diminished if they are delivered via intravenous injection. As a result, further study of the physicochemical properties of AuNPs and the protein interactions they encounter with different routes of administration is indicated. A keen understanding of the impact of AuNPs on biological systems is necessary for their success in various therapeutic applications.

The work by Mukherjee *et al*. (7). suggest that many different signaling pathways are at play in tumor development and that bare AuNPs have more therapeutic potential than once anticipated. Having a greater understanding of how AuNPs affect signaling in individual pathways and how the pathways interact with each other may allow researchers to develop effective new therapies that have low susceptibility to cancer cell resistance. The work may also attract more attention to further understanding the complete *in vivo* effects of these nanoparticles.

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