CLINICALLY RELEVANT

Nanomedicine: Nanotechnology, Biology, and Medicine
10 (2014) 503–514

Review Article

nanomedjournal.com

Gold nanoparticle mediated cancer immunotherapy

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Received 25 July 2013; revised 20 September 2013; accepted 24 September 2013

Abstract

Significant progress has been made in the field of cancer immunotherapy, where the goal is to activate or modulate the body's immune response against cancer. However, current immunotherapy approaches exhibit limitations of safety and efficacy due to systemic delivery. In this context, the use of nanotechnology for the delivery of cancer vaccines and immune adjuvants presents a number of advantages such as targeted delivery to immune cells, enhanced therapeutic effect, and reduced adverse outcomes. Recently, gold nanoparticles (AuNP) have been explored as immunotherapy carriers, creating new AuNP applications that merit a critical overview. This review highlights recent advances in the development of AuNP mediated immunotherapies that harness AuNP biodistribution, optical properties and their ability to deliver macromolecules such as peptides and oligonucleotides. It has been demonstrated that the use of AuNP carriers can improve the delivery and safety of immunotherapy agents, and that AuNP immunotherapies are well suited for synergistic combination therapy with existing cancer therapies like photothermal ablation.

From the Clinical Editor: Cancer immunotherapy approaches are rapidly evolving and are some of the most promising avenues to approach malignancies. This review summarizes the role of gold nanoparticles in immunotherapy agent delivery, and in the development of synergistic therapies such as photothermal ablation.

Key words: Gold nanoparticles; Immunotherapy; Biodistribution; Immune system; Cancer

Cancer immunotherapy is a promising treatment modality that is a subject of ongoing and extensive study. The goal of this treatment approach is to stimulate the host immune system to detect and eradicate cancer cells, which have developed numerous mechanisms of evading immune recognition. For example, tumor cells down-regulate expression of surface antigens and of co-stimulatory molecules, thus reducing T cell recognition and stimulation.[1,2] Tumor cells also secrete immunosuppressive cytokines such as IL-10 and TGFβ creating an environment that is not conducive to dendritic cell (DC) maturation.[1,2] In addition, they are capable of producing factors such as TRAIL and FasL, which induce apoptosis in T cells.[1,2] Finally, cancerous tissue can also attract a number of immune suppressive cell types to the tumor microenvironment. These cells include tumor associated macrophages (TAMs), regulatory T cells (T_{reg}), and myeloid derived suppressor cells (MDSCs). TAMs have been shown to promote cancer progression through the release of cytokines that induce angiogenesis, metastasis, and cell growth and can produce anti-inflammatory signals that suppress immune effectors such as natural killer cells and T cells.[3-5] T_{regs} can suppress various immune cells, including cytotoxic T cells and DCs; this suppression occurs through cell to cell contact and T_{reg} expression of inhibitory molecules such as cytotoxic lymphocyte antigen 4 (CTLA-4) and programmed cell death ligand 1 (PD-L1).[6] MDSCs originate from the bone marrow and are composed of immature myeloid cells and precursors of cells such as macrophages, granulocytes, and dendritic cells. This population is expanded in a number of tissues in tumor bearing mice, including the liver, lungs, spleen, peripheral blood, and the tumor microenvironment, and it can suppress T cell activity as well as promote the development of T_{regs}.[7-9]

Targeting these immune suppressive populations as well as stimulating immune effector cells against tumors is a major goal

Funding: This work was supported by the National Institutes of Health R01 CA172836. J. Almeida was also funded by a training fellowship from the Keck Center of the Gulf Coast Consortia, on the Nanobiology Interdisciplinary Graduate Training Program, National Institute of Biomedical Imaging and Bioengineering (NIBIB) T32EB009379, the National Science Foundation Graduate Research Fellowship #0940902, and the Howard Hughes Medical Institute Med into Grad fellowship. E. Figueroa was funded by a pre-doctoral Ford Foundation fellowship as well as a training fellowship from the Keck Center of the Gulf Coast Consortia, on the Nanobiology Interdisciplinary Graduate Training Program, National Institute of Biomedical Imaging and Bioengineering (NIBIB) T32EB009379.

The authors certify that this manuscript, or any part of it, has not been published and will not be submitted elsewhere for publication while being considered by the journal Nanomedicine: Nanotechnology, Biology, and Medicine.

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http://dx.doi.org/10.1016/j.nano.2013.09.011

of cancer immunotherapy.[10] As reviewed by Vanneman and Dranoff, combining immunotherapies with targeted molecular treatments is a particularly promising approach.[10] Agents such as sunitinib (Sutent®), a small molecule receptor tyrosine kinase inhibitor, and cetuximab (Erbitux®), a chimeric (mouse/human) monoclonal antibody that inhibits EGFR, induce immune anti-tumor responses that could be complemented with immune therapies such as cancer vaccines. For instance, cetuximab has been shown to promote dendritic cell maturation and NK cell mediated tumor killing and is currently being tested in combination with a pancreatic cancer cell vaccine.[10]

In turn, the delivery and efficacy of immunotherapeutic agents and molecular therapies can be enhanced through the use of nanotechnology. Nanoparticles are well suited for delivery of immune therapies such as vaccines or adjuvants because they preferentially accumulate within tissues and cells of the immune system.[11-14] Moon et al discuss a number of nanoparticle mediated immunotherapies that have been explored recently, demonstrating how nanoparticle delivery can improve therapeutic effect and reduce systemic toxicities.[15] Various designs have been explored, including polymeric poly(lactic-*co*-glycolic acid) (PLGA), liposomes, gelatin based nanoparticles, and AuNPs. For example, Kwong and colleagues showed that liposomes could deliver the immune stimulatory CpG oligonucleotide and anti-CD40 antibody, inducing an enhanced anti-tumor response without causing a systemic increase in inflammatory cytokines typically associated with these treatments.[16] Gelatin nanoparticles have also been used to simultaneously deliver the ovalbumin antigen and the CpG adjuvant, demonstrating enhanced effect and reduced toxicity.[17]

Recently, AuNPs have been applied in immunotherapies, including cancer antigen and immune adjuvant delivery.[18-23] AuNPs are a promising carrier for immune therapies because, like other nanoparticles, they easily accumulate in the immune system.[12,13] In addition, AuNPs are bioinert, can be functionalized with drugs and other ligands, and can also be easily tuned to a desired size or shape. Importantly, however, AuNPs, have unique optical properties that can be exploited for immune therapies, particularly their applications in photothermal ablation and light triggered drug delivery.[24-26] In this review, we discuss the most recent understanding of the biodistribution and immune interactions of AuNPs. In addition, we discuss their unique optical properties and how photothermal therapies can be used for immune applications. Finally, we review their recent use in immunotherapy, including drug and gene delivery studies. Overall, the multiple functionalities of AuNPs make them promising vehicles for immune therapies, particularly for combinatorial treatment approaches that target multiple immune pathways (Figure 1).

Biodistribution and immune response

The blood clearance and organ accumulation of AuNPs *in vivo* are affected by various factors such as particle size, shape, charge, and coating.[12,13,27,28] In general, smaller particles circulate in the blood longer and distribute more widely than larger particles, and surface coating with polyethylene glycol (PEG) can reduce opsonization and uptake by the reticuloendothelial system. For instance, Zhang and colleagues have shown that 20 nm PEGylated AuNPs accumulate in the liver and spleen to a lesser extent than 80 nm particles and that a higher percent dose of the 20 nm particles reaches the targeted tumor site.[29] The group postulates that smaller particles permit a more dense coating on the particle surface. Similarly, Perrault et al have reported that AuNP blood half-life increases with decreasing particle size and increasing PEG molecular weight.[30] Recently, however, Larson et al have shown that the PEG on the AuNP surface can be displaced with cysteine and cystine present in the blood, thereby causing protein absorption and macrophage uptake. The group improved upon the typical PEG design by adding an alkyl linker between the PEG and the thiol that binds to the gold surface, thereby reducing PEG displacement and macrophage uptake.[31]

A number of groups have investigated the mechanisms of AuNP uptake by various cell types as well as the particle characteristics affecting such uptake. Recently, Liu and colleagues characterized the effect of particle charge on AuNP uptake in both phagocytic and non-phagocytic cells.[32] Positively charged particles were taken up to a much higher extent by non-phagocytic cells than negatively charged ones. On the other hand, particle charge had little effect on uptake by phagocytic cells. The particles in non-phagocytic cells were localized to secondary lysosomes and formed small aggregates while the ones in phagocytic cells were found in phagosomes, indicating that non-phagocytic cells take up particles through clathrin-mediated endocytosis while the phagocytic ones take them up through phagocytosis.[32] Franca et al further elucidated phagocytic cell uptake by characterizing the uptake of AuNPs 30 nm and 150 nm in diameter.[33] Both particle sizes could be phagocytosed, but the group found that clathrin mediated pinocytosis could induce uptake of 30 nm particles but not 150 nm ones. Scavenger receptor mediated phagocytosis was a major factor in 150 nm AuNP uptake. Nevertheless, inhibition of clathrin and calveolin mediated pathways did not completely block AuNP uptake, indicating that there are other pathways involved, and elucidating such mechanisms merits further study.[33]

Upon uptake, studies have shown that particles can retain in the body for extended periods of time. For instance, Sadauskas et al observed gold retention within macrophage clusters in the liver over a 6 month period.[34] Balasubramanian et al, in turn, observed high gold content in the liver and spleen of rats after 2 months.[35] In general, smaller particles excrete more readily from the body than larger particles because the smaller size facilitates renal and hepato-biliary clearance.[27,35,36] Zhang et al exploited this characteristic to develop glutathione (GSH) coated gold nanoclusters that demonstrated low retention in the liver and spleen and high renal excretion.[37] As opposed to bovine serum albumin (BSA) coated nanoclusters that aggregated to a size between 40 and 80 nm and showed approximately 1% urine excretion, the GSH protected clusters remained between 5 and 30 nm in size and showed 36% urine excretion.[37]

Nevertheless, despite such design changes, AuNPs inevitably accumulate in high concentrations in the liver and spleen,