Chapter 7

Gold Nanoparticles for Multimodal Imaging in Nanomedicine

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Introduction

Due to advance developments in functionalization chemistry, novel gold nanoparticles-based nanosystems with applications in diagnosis and treatment of various human patologies have been reported. (1-5) During the last decade, gold nanoparticles (AuNPs) have been extensively explored as an x-ray contrast agents. Gold nanoparticles exhibit unique properties that makes them suitable candidates as x-ray contrast agents in clinical practice having a relatively high x- ray attenuation coefficient compared with both barium sulfate and iodine. Another major advantage of the GNPs is represented by increased vascular retention time compared with iodinated molecules. Generally, the safety/toxicity of the nanoparticles depends on shape, surface coating and their size. (6-9) This paper illustrates the latest achievements in the applications of gold nanoparticles as multimodal imaging agents for diagnosis of human diseases.

A multifunctional AS1411-functionalized fluorescent gold nanoparticle

system (named NAANPs) was synthesized and successfully applied for both targeted cancer cell imaging and efficient photodynamic therapy (PDT). The NAANPs bionanosystems were developed by functionalization of the gold nanoparticles with AS1411 aptamer and then bound with one porphyrin derivative N-methylmesoporphyrin IX (NMM). Next, using HeLa cells over expressing nucleolin as representative cancer cells, the authors proved that the formed NAANPs can target to the cell surface via the specific AS1411-nucleolin interaction. They also observed a linear correlation between fluorescence intensity of NMM and binding to AS1411 G-quadruplex. (10)

Several authors devoped polymer-coated gold nanoparticles using glycol chitosan as a reducing agent and a stabilizer. Next tomographic displays of malign lesions were successfully obtained in the tumor-xenografted animal model when the GC-AuNPs were used as a CT contrast agent. The authors showed that specific targeting of the gold nanoparticles was due to the properties of GC because GC-AuNPs were accumulated in the tumor, in contrast with control nanoparticles. The phantom images were highly contrasted with GC-AuNPs, and the X-ray absorption increased linearly according to the concentration of GC-AuNP. The in vitro behavior of GC- AuNPs was assessed on two types of cells, colon cancer cells (CT-26) and macrophage cells (RAW264.7) showing that GCAuNPs were found exclusively in CT-26 colon cancer cells, not in macrophage cells. (11)

In another paper a new platform for in vivo CT molecular imaging, using immuno-targeted gold nanoprobes that selectively and sensitively target tumor specific antigens was described. (12) The authors successfully developed gold nanoprobes that forms clusters inside cancer cells, yielding a distinguishable X-ray attenuation, feature that was not observed in normal cells or tissue. Using this technique the targeted cancer tissue was easy to be identified. In the present

research the authors synthesized 15/45 nm AuNR using the seed mediated growth method and further conjugated with UM-A9 antibody. Next UM-SCC-1 and UM-SCC-5 human head and neck cancer cell lines and the negative control samples of fibrosarcoma (UM-FS-1) and melanoma (UM-Mel-1), which are known not to express the A9 were treated with antibody- coated AuNR solution (2.5 mg/mL), and allowed to interact for 90 min at room temperature. The authors observed that the attenuation coefficient (with respect to water) of the SCC cancer cells that were targeted by the A9 antibody coated gold nanorods was more than 5 times higher than that of the non-targeted SCC cancer cells (32 and 28 HU vs. 168 and 172 HU, respectively). In contrast, the attenuation values observed for the negative control samples that were targeted with gold nanorods (larynx and oral cancer cells targeted with nanorods that were coated with non matching antibodies, and normal fibroblast and melanoma cells targeted with A9 antibodies) were significantly lower (58, 54, 50, 62 HU, respectively). This finding strongly suggested that head and neck tumors may show a likely enhancement of 3-4 times the local contrast of non- targeted tissue in vivo. (12)

In their paper Reuveni and al investigated the delivery of 30 nm GNP-EGF towards head and neck squamous cell carcinoma (SCC), since such tumors express an extremely high level of EGFR. The ability of anti-EGFR-targeted GNPs to form a concentrated assembly exclusively on the SCC cancer cells in tumor-bearing mice was investigated. The authors stated that the strong selective X-ray attenuation by gold nanobiosystems, which is distinct from the attenuation of other cell types and tissues, makes the targeted tumor highly distinguishable and easy to diagnose in CT imaging. A very important feature was also shown: active tumor targeting was more efficient than passive targeting (at specific time points) in authors opinion because of its elevated tumor uptake and retention forces. (13)

A nanoprobe (M-NPAPF-Au) co-loaded with an aggregation-induced emission (AIE) red dye and gold nanoparticles into DSPE-PEG2000 micelles for dual-modal fluorescence/CT imaging was also developed using the method of "one-pot ultrasonic emulsification". *In vivo* tests of this novel nanobiosystem demonstrated their excellent tumor-targeting ability, high fluorescence, long blood circulation half-life, superior tumor-targeting ability and CT imaging effects. (14)

Several authors reported the development of a lactobionic acid (LA)-modified dendrimer- entrapped gold nanoparticle (LA-Au DENPs) nanosystem for in vitro and in vivo targeted CT imaging of human hepatocellular carcinoma. These amine-terminated poly (amidoamine) dendrimers of generation 5 pre-modified with fluorescein isothiocyanate and poly(ethylene glycol)-linked LA showed good stability under different pH (5–8) and temperature (4–50 ℃) conditions and in different aqueous media, and showed noncytotoxic efect to normal cells but cytotoxic to the targeted hepatocarcinoma cells. In addition the authors proved that LA-Au DENPs complexes can be used as a highly effective nanoprobe for specific CT imaging of hepatocarcinoma cells in vitro and the xenoplanted tumor model in vivo even greater than clinically employed iodine-based CT contrast. (15)

Karmany et al conjugated the anti-CD105 antibody radiolabeled with 89Zr bound to gold nanoparticles (AuNPs) for further testing their biodistribution in mice with a focus on tumor targeting. Using quantitative PET imaging and ICP-MS analysis the authors assessed in vivo distribution and specific tumor targeting of these tracers showing that the tumor uptake of immunoconjugates was preserved up to 24 h after injection, with high tumor contrast and selective tumor targeting. In contrast, no major tracer accumulation was observed over time in nonspecific organs. (16)

In another study, several authors used hyperspectral imaging to assess the uptake and distribution of phosphatidylcholine-coated citrate gold nanoparticles (CGN) and silica-gold nanoshells (SGN) after tail-vein injection in mice bearing orthotopic pancreatic adenocarcinoma. For CGN, the liver and tumor showed 26.5±8.2 and 23.3±4.1 particles/100μm^2 within 10μm from the nearest source and few nanoparticles beyond 50μm, respectively. The spleen tissue had 35.5±9.3 particles/100μm^2 within 10μm with penetration also limited to 50μm. For SGN, the liver showed 31.1±4.1 particles/100μm^2 within 10μm of the nearest source with penetration hindered beyond 30μm. The spleen and tumor showed uptake of 22.1±6.2 and 15.8±6.1 particles/100μm^2 within 10μm, respectively, with penetration similarly hindered. CGH average concentration (nanoparticles/μm2) was 1.09±0.14 in the liver, 0.74±0.12 in the spleen, and 0.43±0.07 in the tumor. SGN average concentration (nanoparticles/μm^2) was 0.43±0.07 in the liver, 0.30±0.06 in the spleen, and 0.20±0.04 in the tumor. (17)

Several authors developed and tested Arg-Gly-Asp-D-Phe-Lys peptide-modified PEGylated dendrimer-entrapped gold nanoparticles for targeted CT imaging of breast carcinoma. The 2.8 nm PEGylated Au DENPs-RGD were spherical, water dispersible and biocompatible and displayed a higher x-ray attenuation intensity than Omnipaque at the same Au or I concentrations. The authors also showed that conjugated RGD ligand can specifically identify and target overexpressed integrin receptors on MDA-MB-435 cells. Following their parenteral administration, these nanoprobes accumulated in the targeted area of mice with MDA-MB-435 xenograft tumors, allowing the tumor to be detected by CT imaging. (18)

In another paper, synthesis and characterization of 1.9-4.6 nm gold nanoparticles (AuNPs) entrapped within polyethylene glycol (PEG)-modified polyethylenimine (PEI) for blood pool and tumor computed tomography (CT)

imaging was reported. Following the intravenous administration of the [(Au0) 200-PEI•NHAc-mPEG] NPs via the tail vein, the tumor-bearing mice were scanned by a clinical CT imaging system at different time points showing a better X- ray attenuation property than that of clinically used iodinated small molecular contrast agent (e.g., Omnipaque) and the prolonged half-decay time (11.2 h in rat) confirmed by pharmacokinetics studies. (19)

Chanda et al developed biocompatible gum arabic stabilized gold nanocrystals (GA-AuNPs) and further tested their role as X-ray contrast agent in tumor bearing mice and dog. Following intratumoral injections of GA-AuNP the authors obtained a X-ray contrast change of 26 HU in the tumor region after 1 hour post-injection period that reached a threshold limit within a short time period (5 h), and was retained in the tumor tissue for the rest of the period of investigation. Next, GA-AuNP was injected intratumorally in dog and a contrast enhancement of 12 HU was observed. The CT images of both mice and dog showed that GA-AuNP was effectively distributed and retained throughout the tumor site. (20)

Conclusion

We may assert that multimodal imaging using functionalized gold nanoparticles represents a feasible therapeutic method for the diagnosis of human diseases. However further studies are required to fully understand the biological interactions of nanoparticles inside biological systems.

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