Active Targeting Gold Nanoparticle for Chemotherapy Drug Delivery: A Review

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Abstract

Active targeting strategy in chemotherapy drug delivery aims to improve the therapeutic outcomes and minimize the side effects of chemotherapeutics. This review discusses utilizing ligands attached to gold nanoparticles (AuNPs) along with several specific ligands attached to AuNPs for active targeting in chemotherapy drug delivery. Antibodies, peptides, vitamins, DNA, polysaccharides, aptamers, and hormones showed active-targeting abilities as ligands attached to AuNPs. Active-targeting AuNPs enhanced cellular uptake and cytotoxicity in a specific cancer cell in vitro while reducing tumor growth in vivo by improving the photothermal, photodynamic and chemotherapy effects. Active-targeting ligands increased the internalization of AuNPs loaded onto the specific tumor site and minimized the accumulation in the normal site. AuNPs with active-targeting ligands such as antibodies, peptides, vitamins, DNA polysaccharides, aptamers, and hormones can improve the therapeutic outcomes of chemotherapeutics and can attenuate the toxicity effect in normal cells. For further research and development, researchers should be addressing AuNP characterization, drug–ligand disposition, active-targeting AuNP quantification, and target-AuNPs pertinence concerning the desired therapeutic outcomes.

Keywords:
- Active-targeting delivery
- Chemotherapy
- Conjugation
- Gold nanoparticle

Introduction

Targeted-delivery chemotherapy utilizing nanotechnology is simpler, cheaper and less time-consuming than conventional chemotherapy. Nanoparticles (NPs) optimize the therapeutic effects and minimize the adverse effects of previously approved active pharmaceutical substances, including chemotherapeutics. NPs can revamp the conventional drug delivery through passive and active targeting. Passive-targeting NPs exploit biological processes through the enhanced permeation and retention (EPR) effect that allowed small NPs and macromolecular drugs to accumulate more in tumors than in normal tissues, due to the larger pore size of neo-vasculatures and the poor lymphatic clearance of tumors. Active-targeting or ligand-mediated delivery utilizes specific ligands on the surface of NPs for the retention and uptake of chemotherapeutics into targeted cells. Ligand specific interaction with overexpressed receptors of cancer cells enhances uptake of drug delivery onto cancer cell target, increasing drug efficiency rather than passive targeting delivery and avoiding non targeted cells. McDaid et al. showed that cetuximab conjugation with camptothecin-polymeric NPs improved the NP binding and delivery of camptothecin against cetuximab-resistant cancer cell lines and reduced the tumor growth in vivo. A specific binding to an overexpressed receptor in the cancer cell leads to better uptake and internalization of the chemotherapeutic effect into the cancer cell.

AuNPs with other metal NPs such as silver, silica, platinum and iron oxide had been explored for chemotherapy drug delivery uses. Among other metal NPs, AuNPs and silver nanoparticles (AgNPs) have unique optical and surface plasmon resonance (SPR) properties. In-vitro and in-vivo studies have indicated that AuNP were non-toxic, easy to obtain with various shapes and sizes, while AgNPs were more expensive in design and had significant potential toxicity to normal cells in vitro. AuNPs’ surface modification abilities and controllable synthesis enable the AuNP surface to conjugate with another molecular compound. AuNPs have a cytotoxic effect due to the interaction of the Au atom with the intracellular protein and DNA. AuNPs also have the potential to enhance local radiation and radiation sensitivity. AuNPs exhibit photoacoustic and photothermal properties because of their ability to absorb light at a specific wavelength. These properties contribute to AuNPs’ phototherapy and radiotherapy functions. Thus, AuNPs can carry out the functions of chemotherapy, phototherapy and radiation altogether as drug delivery systems.

Bednarski et al. reported that AuNPs were absorbed only in small amounts after oral administration. After intravenous administration, AuNPs are mainly accumulated in the liver, lungs and spleen, and only...
slightly removed from the body in the urine and faeces. AuNPs physicochemical properties (shape, size and surface chemistry) may affect its pharmacokinetics and pharmacodynamics, including AuNPs toxicity. Functionalizing the AuNPs surface with ligands would help regulate and detoxify the uptake of these AuNPs.

AuNPs were produced from the reduction of Au in an oxidation state using chemical, physical and biological techniques. The most well-known chemical approach for producing AuNP was the citrate reduction method developed by Turkevich in 1951. The reaction was initiated thermally or through ultraviolet irradiation, and resulted in a wide range of sizes (9–120 nm), with the defined size distribution of AuNP. Brust-Schiffrin synthesized AuNP in 1994 using tetraoxtalamoniumbromida in toluene and natrium borohydride. This method could produce highly stable thiol-functionalized NPs and access to AuNP preparation in large amounts.

Producing AuNPs through the physical method can be achieved using laser ablation, microwave, or ultrasound. AuNP can be synthesized through the biological method using a biological membrane or a microorganism such as Enterococcus sp, Pseudomonas sp, or Streptomyces sp. The microbial synthesis of AuNPs using bacteria has already resulted in three patents. Other than conventional methods, AuNPs can also be produced through sputtering deposition in ionic liquid (IL). This novel method promises simpler and purer results because of the minimal use of chemicals and stabilizers. The formation of AuNPs through sputtering deposition involves an electrical field of IL in a vacuum chamber that produces a potential difference between cathodes and anodes.

**Gold Nanoparticle Conjugation in Chemotherapy Drug Delivery**

The biodistribution of AuNPs as drug delivery systems, like any other nanosystem, is non-specific. Therefore, to enhance their efficacy and decrease their potential toxicity, a selective moiety was preferred to attach to the AuNP surfaces. The advantages and disadvantages of active and passive targeting delivery of AuNPs were presented in Table 1.

A selective moiety assists AuNP in binding with a cancer cell overexpressed receptor and improving the therapeutic outcome. This review discusses previous studies regarding ligands attached to AuNPs as carriers of cytotoxic drugs: how the combination made, the *in-vivo* and *in-vitro* studies and the advantages and drawbacks of active-targeting AuNP chemotherapy delivery systems. The outlines of this review highlighted active targeting AuNPs based on the active-targeting ligand used: AuNPs with antibodies, AuNPs with peptides, AuNPs with vitamins and AuNPs with other ligands. Examples of active-targeting AuNPs are present in Table 2.

<table>
<thead>
<tr>
<th>AuNPs Drug Delivery Strategies</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passive Targeting</td>
<td>-Easier control of obtained size and shape of AuNPs</td>
<td>-Quantity of drug load in cell targeting not significant (less than 20%)</td>
</tr>
<tr>
<td></td>
<td>-Non-immunogenic</td>
<td></td>
</tr>
<tr>
<td>Active Targeting</td>
<td>-Enhance uptake/internalization on targeted cell</td>
<td>-Possibility of immunogenic response</td>
</tr>
<tr>
<td></td>
<td>-Reduce toxicity in normal cells</td>
<td>-Increasing in particle size</td>
</tr>
<tr>
<td></td>
<td>-Localization led to better photothermal, photodynamic, chemotherapy effects</td>
<td>-Certain ligands may carry toxicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-More complex and costly in designing the systems than passive targeting system</td>
</tr>
</tbody>
</table>

**Figure 1.** Effects of active-targeting gold nanoparticle.
Active Targeting Gold Nanoparticle for Chemotherapy

<table>
<thead>
<tr>
<th>Active-targeting drug delivery</th>
<th>Ligand</th>
<th>Chemo-therapeutic</th>
<th>Target</th>
<th>Method of evaluation</th>
<th>Mechanism of action in the targeted site</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibodies-AuNP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Enhanced proton irradiation in A431 cells$^{41}$</td>
<td></td>
</tr>
<tr>
<td>Cetuximab</td>
<td>-</td>
<td>EGFR</td>
<td></td>
<td>In vitro</td>
<td>Radio-enhancing the effect of proton therapy</td>
<td></td>
</tr>
<tr>
<td>Rituximab</td>
<td>-</td>
<td>CD20</td>
<td></td>
<td>In vitro</td>
<td>Increasing the rituximab chemotherapy effect</td>
<td></td>
</tr>
<tr>
<td>Cetuximab</td>
<td>ZnD</td>
<td>EGFR</td>
<td></td>
<td>In vitro and in vivo</td>
<td>Enhancing the chemotherapy effect of an original anticancer agent ([Zn(DION)2] Cl2)</td>
<td></td>
</tr>
<tr>
<td>Cetuximab</td>
<td>-</td>
<td>EGFR</td>
<td></td>
<td>In vitro and in vivo</td>
<td>Improving radioimaging</td>
<td></td>
</tr>
<tr>
<td>Cetuximab</td>
<td>-</td>
<td>EGFR</td>
<td></td>
<td>In vitro and in vivo</td>
<td>Enhancing the chemotherapy effect of cetuximab</td>
<td></td>
</tr>
<tr>
<td>Antibodies-AuNP</td>
<td>Trastuzumab</td>
<td>monomethyl auri-statin E (MMAE)</td>
<td>AntiHER2/ERRB2</td>
<td>In vitro</td>
<td>Increasing the cellular internalization of trastuzumab</td>
<td></td>
</tr>
<tr>
<td>Anti-PD-L1 antibody</td>
<td>Doxorubicin</td>
<td>PD-L1</td>
<td></td>
<td>In vitro</td>
<td>Increasing the photothermal (NIR irradiation) and chemotherapy effects</td>
<td></td>
</tr>
<tr>
<td>Anti-VEGF antibody</td>
<td>-</td>
<td>VEGF</td>
<td></td>
<td>In vitro and in vivo</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Rituximab</td>
<td>-</td>
<td>CD20</td>
<td></td>
<td>In vitro</td>
<td>Enhancing the photothermal effect, increasing the chemotherapy effect</td>
<td></td>
</tr>
<tr>
<td>CD133 antibody</td>
<td>Docetaxel</td>
<td>CD133</td>
<td></td>
<td>In vitro and in vivo</td>
<td>Photothermal + photodynamic + chemotherapy</td>
<td></td>
</tr>
<tr>
<td>RVG peptide &amp; Lamp2 exosome</td>
<td>-</td>
<td>αvβ3 integrins</td>
<td></td>
<td>In vitro and in vivo</td>
<td>Chemotherapy effect accumulation</td>
<td></td>
</tr>
<tr>
<td>RGD peptide</td>
<td>Bleomycin</td>
<td>αvβ3 integrins</td>
<td></td>
<td>In vitro</td>
<td>Enhancing radiation therapy + chemotherapy</td>
<td></td>
</tr>
<tr>
<td>cRGD peptide</td>
<td>-</td>
<td>αvβ3 integrins</td>
<td></td>
<td>In vitro</td>
<td>Radiotherapy, radioimaging, chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Peptide-AuNP</td>
<td>P4 peptide</td>
<td>Chlorambucil, malphalan, bendamustin</td>
<td>HL-60 and NB-4 human cells</td>
<td>In vitro</td>
<td>Enhancing the chemotherapeutic effects</td>
<td>Active targeting of P4 depending on the receptor cell surface density and the mechanism of internalization$^{38}$</td>
</tr>
<tr>
<td>CPP</td>
<td>Trastuzumab</td>
<td>Various</td>
<td></td>
<td>In vitro</td>
<td>Enhancing the chemotherapeutic effects</td>
<td>Improved the intracellular internalization in SKBR3, DLD1, MDA MB 23 and MCF-7$^{39}$</td>
</tr>
<tr>
<td>Angiopep-2 peptide</td>
<td>-</td>
<td>LRP1</td>
<td></td>
<td>In vitro and in vivo</td>
<td>Enhancing the chemotherapeutic effects</td>
<td>Crossed the blood-brain barrier and did not produce a toxic effect in the normal cells$^{36}$</td>
</tr>
</tbody>
</table>

*References:*

41. Enhanced proton irradiation in A431 cells.
42. Rituximab AuNP had better efficacy than rituximab alone.
43. Increased efficacy against DOX-resistant colorectal carcinoma; reduction of tumor growth.
44. Improved CT imaging by contrast; effect of relative binding more prominent rather than relative half of the time.
45. Suppressed cell lung cancer proliferation and accelerated apoptosis; reduced the tumor weight and volume.
46. Increased uptake in vitro for the SKBR-3 cell line and no increase in uptake for the MCF-7 and MDA-MB-231 cell lines.
47. Increased apoptosis.
49. Reduction of anti-apoptosis/BCl-2 protein; carrying and released cytotoxicity upon laser irradiation.
50. Multimodal imaging with photodynamic/photothermal chemotherapy activity.
51. Binding to the brain cell and blood-brain barrier.
52. Internalized cell, improved cytotoxicity and radiotherapy.
53. Targeting activity present when cRGD was linked to a short olygolysine spacer.
54. Active targeting of P4 depending on the receptor cell surface density and the mechanism of internalization.
55. Improved the intracellular internalization in SKBR3, DLD1, MDA MB 23 and MCF-7.
56. Crossed the blood-brain barrier and did not produce a toxic effect in the normal cells.
Table 2. Continued.

<table>
<thead>
<tr>
<th>Vitamin-AuNP</th>
<th>Folic acid</th>
<th>Methotrexate</th>
<th>Folate receptors</th>
<th>In vitro and in vivo</th>
<th>Enhancing the chemotherapeutic effects</th>
<th>Threefold-improved cytotoxicity in the brain cell; tenfold-improved cytotoxicity in the cervical HeLa cells(^{57})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin</td>
<td>Folic acid</td>
<td>Doxorubicin</td>
<td>Folate receptors</td>
<td>In vitro</td>
<td>Enhancing the chemotherapeutic and photothermal effects</td>
<td>Increased efficacy of active-targeting-involved upregulation of the pro-apoptotic protein p53 and downregulation of the anti-apoptotic protein Bcl-2(^{29})</td>
</tr>
<tr>
<td>Protein</td>
<td>Angiopep 2</td>
<td>Doxorubicin</td>
<td>LRP1</td>
<td>In vitro and in vivo</td>
<td>Enhancing the chemotherapeutic effect</td>
<td>High affinity for the HeLA, A549 and MC63 cancer cell lines; low affinity to the NIH3T3 normal cell line(^{59})</td>
</tr>
<tr>
<td>Polysaccharide</td>
<td>Hyaluronic acid</td>
<td>-</td>
<td>CD44</td>
<td>In vitro</td>
<td>Enhancing the chemotherapeutic effect</td>
<td>Distributed at a higher intensity into the glioma than the free doxorubicin or AuNP without a ligand(^{60})</td>
</tr>
<tr>
<td>Aptamer &amp; DNA</td>
<td>AS1411 aptamer</td>
<td>Ionidamine</td>
<td>Nucleolin receptor</td>
<td>In vitro and in vivo</td>
<td>Enhancing the chemotherapeutic effect</td>
<td>Enhanced the selective delivery of doxorubicin up to 2.5 times, and minimized the chemoresistance(^{61})</td>
</tr>
</tbody>
</table>

Active targeting purposes with medium-large size and minimum weight loading led to better exposure to tumor cells and reduce exposure to normal cells.\(^{63}\) Ligand structure also influences cellular uptake. The more stable (lower entropy) of ligand-AuNP would lead to better uptake.\(^{64}\) Cellular uptake of active targeting AuNP or internalization may involve receptor-mediated endocytosis or energy independent diffusion across the cell membrane through pores or opening lipid bilayer by ligand-AuNP.\(^{65}\) Modifying AuNP can affect its photothermal activity, although the effect may be from active-targeting localisation rather than the direct effect of surface modifying to AuNP's SPR properties. Increasing near-infrared (NIR) irradiation and SPR temperature of ligand-AuNP enhance localization. Localization led to accumulation onto targeted cells, increasing the AuNPs conjugate's photothermal effect and increasing the probability to destroy cancer cells. Figure 1 shows that the functionalized active-targeting ligand conjugated with AuNP for chemotherapy drug delivery had photothermal, photodynamic and chemotherapeutic effects on cancer cells.

We classify the conjugation of AuNP into two categories: that using an additional linker and that without one. The conjugation of a ligand with AuNP by a thiol-Au covalent bond was created by adding PEG-NHS, PEG-COOH or PEG-SH to AuNP. The ligand-AuNPs conjugate went through filtration, centrifugation, dialysis or chromatography to purify the conjugate from excess materials. The construction of an active-targeting AuNP was illustrated in Figure 2.

AuNPs conjugated with a linker has a stronger bond than those conjugated without a linker. This bond should cleave once it reaches its target site. Podsiadlo reported the successful conjugation of 6-mercaptopurine with AuNP by the thiol group in 6-mercaptopurine-9-β-D-ribofuranoside.\(^{66}\) The conjugate enhanced the antiproliferative effect against K-562 leukemia cells compared to 6-mercaptopurine-9-β-D-ribofuranoside in free form, and showed excellent stability over one year, without loss of inhibitory activity. The cleavage mechanism of the linker was delivered at a lower pH (4.1) by severing the S–Au bond.\(^{66,67}\) Cruz and Keyser synthesized spherical AuNPs surface-functionalized with trastuzumab and drug conjugate (ADC). The ADC was produced through the chemical attachment of monomethyl auristatin E (MMAE) to trastuzumab through a cathepsin-cleavable valine-citrulline linker and further reacted with a sulfhydryl-containing linker for surface conjugation with AuNP.\(^{68}\) Brown et al.\(^{64}\) functionalized carboxylate group-
capped-AuNPs with thiolated polyethene glycol (PEG) monolayer and active component of oxaliplatin. In vitro study of AuNP-oxaliplatin’s active-component complex in the A549 lung epithelial cancer cell line showed AuNP-oxaliplatin’s active-component complex had better cytotoxicity than oxaliplatin alone and could penetrate lung cancer cells. However, the stronger bond between an AuNP and a chemotherapeutic means that it has become more difficult to sever the bond. Ligand-AuNPs conjugation bonds lead to several questions about the nature of AuNP conjugation for drug delivery, such as the cleavage mechanism of the chemical bond and release mechanism of the chemotherapeutics from the AuNP complex related to their therapeutic effect. In vivo studies should yet be conducted regarding this mechanism.

Yahyaei and Pourali reported that one-step conjugation of biologically produced AuNP with capecitabine, tamoxifen and paclitaxel without using additional linkers could be done by directly dropping the chemotherapeutics in liquid AuNP. The conjugation was possible due to the presence of the capping protein surface of biologically produced AuNP. However, capcitabine and tamoxifen did not show a toxic effect against the MCF-7 cell lines. The difference between the average sizes of the AuNP and the conjugated AuNP probably contributed to this cytotoxicity result. AuNP had an average size of 54.27 ± 3.1 nm, capcitabine-conjugated AuNP has an average particle size of 83.51 ± 5.5 nm, and paclitaxel-conjugated AuNP has an average particle size of 61.37 ± 5.1 nm. The differences in drug loading, also could lead to a different pharmacological effect. A study by Chu et al. involving docetaxel-loaded NP in two similar average particle sizes resulted in different pharmacokinetic profiles and efficacy levels. The 9% weight loading of docetaxel in NP had a superior pharmacokinetic profile and enhanced efficacy in a murine cancer model compared to a higher docetaxel weight loading in NP (20% NP). The 9% NP docetaxel increased the plasma and tumor docetaxel exposure and reduced the liver, spleen and lung exposure compared to 20% NP docetaxel. The particle sizes of these two different docetaxel-loaded NPs were similar: 216 ± 2 nm for the 20% docetaxel-loaded NP and 216 ± 1 nm for the 9% docetaxel-loaded NP. In 2020, Mirrahimi et al. designed a nanoplatform from alginate hydrogel, cisplatin and AuNP to deliver the functions of chemotherapy, phototherapy and radiation therapy. The nanoplatform increased tumor heating and damaged tumor cells in vivo and in vitro.

Gold Nanoparticles with Antibodies as Ligands
Antibodies have chemotherapeutic properties and also have potential use for specific-targeting drug delivery. Tumor antigens such as epidermal growth factor receptor (EGFR), erythroblastic oncogene B2, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte-associated antigen-4 (CTLA4), CD20, CD30 and CD52 could exploit as antibody targets. For specific targeting, the target antigen should be abundant, accessible and preferably expressed on the surfaces of cancer cells homogeneously, consistently and exclusively. Antibodies as ligands were the most researched active-targeting ligands attached to AuNPs for chemotherapy drug delivery.

Antibodies are commonly conjugated with AuNPs using a PEG linker, although antibody–AuNP conjugation without a linker was also possible. Boca et al. synthesized...
rituximab-conjugated AuNP by mixing a rituximab solution with AuNP. The formation of an intracellular vesicle on the membrane cell surfaces accomplished the internalization of rituximab AuNP in the chronic lymphocytic leukemia cells CLL-AAT and HS 505. The AuNP surface-functionalized with trastuzumab and MMAE drug conjugate (ADC) increased uptake in vitro for the SKBR-3 cell line (HER2-targeting cell line) and did not affect the uptake for the MCF-7 and MDA-MB-231 cell lines, which are not subject to HER-2 regulation. However, the growth rate inhibition for the SKBR3 and SKOV 3 cell lines was lower in ADC than in MMAE alone. This unexpected result of ADC cytotoxicity might be due to its cleavage mechanism to release MMAE. The functionalization of the AuNP surface with the Au–S bond between AuNP and an antibody can help resolve this release issue. Pedrosa et al. showed that multifunctional AuNPs with cetuximab ligands effectively inhibited cell proliferation and triggered the death of the doxorubicin-resistant colorectal carcinoma cell. Cetuximab attached to AuNP suppressed the proliferation and migration of EGFR highly expressed in tumor cells and accelerated tumor apoptosis. The active-targeting group had greater tumor accumulation in tumor-inducing mice than the group without a targeting ligand, although the cetuximab attached to AuNP had a shorter residence time in the blood than the non-targeting AuNP and the antibody NP. The tumor accumulation might have been more affected by the relative binding affinity of ligands than by the circulation half-life of ligand attached to the AuNP.

Antibody AuNP conjugation has a role in photothermal and photodynamic therapy. The conjugation may improve the therapeutic outcome of photothermal therapy, photodynamic therapy and chemotherapy altogether. The Au nanosystem might also control the release of ligand-AuNP through irradiation. Emami et al. conjugated AuNP with doxorubicin and the anti-PD-L1 antibody. Adding PD-L1 antibody to AuNP increased the photothermal effect of AuNP exposed to NIR irradiation from room temperature (27°C) to a temperature that could destroy tumor cells (45°C). Although the photothermal conversion efficiency of AuNP has a higher value (49.6 ± 3.8%) than that of PD-L1-AuNP-DOX (45.6 ± 3.1%), PD-L1-AuNP-DOX has a successful photothermal effect. The SPR and NIR irradiation of PD-L1-AuNP-DOX increased apoptosis (66%) of the CT-26 cells. Cetuximab-conjugated AuNP, investigated by Li et al., showed enhanced irradiation in the EGFR-overexpressing A431 cell, potentiated its radiotherapy effect and showed prominent specific targeting. The multifunctional nanoplateform GNS@IR820/DTX-CD133 and AuNP coated with PEG increased the SPR temperature to 66 °C and the in-vivo NIR irradiation to 39.9 °C whereas destroying most of the cancer cells in the group of animal trials. In-vivo studies revealed no major organ damage (heart, lung, liver, kidney, spleen) after GNS@IR820/DTX-CD133 therapy; thus, the conjugate was biodistributed mainly at the tumor site. Tan et al. also reported the elevation of docetaxel release under NIR irradiation from CD133 antibody-Au nanostar loading IR820.

Specific tumor targeting in vivo, however, may bring more challenges across some biological barriers. Khongkow et al. showed that the targeting properties of the short-peptide rabies virus glycoprotein (RVG) and Lamp2 protein exosome attached to AuNP could bind AuNP to the brain cells and enhance the transport across the blood-brain barrier. RVG-Lamp2 exosome AuNPs accumulated in the mouse brain after intravenous injection. However, the conjugation of AuNP and anti-VEGF IG had not changed glioblastoma multiform tumor progression in induced mice. Another study by Betzer et al. showed that active targeting AuNP improved radiotherapy in mice with glioblastoma multiform and glucose-coated AuNP accumulated in the brain by the GLUT-1 transporter aided permeation through the blood-brain barrier. The unsuccessful uptake of AuNP alone and anti-VEGF conjugated with AuNP through GL261 brain tumor xenograft was more reasonable because the amount of AuNPs was not enough for them to be able to cross the GL261 plasma cell membrane and blood-brain barrier rather than because of the psychochemical characteristics of AuNP.

Gold Nanoparticles with Peptide as a Ligand

Peptide attached to AuNP did not manifest any drawback from antibody-AuNP conjugation, such as handling difficulty during synthesis and high study cost. A peptide can interact with a specific receptor in the cancer cell, and the peptide sequence can be modified. Modifying the peptide sequence can contribute to peptide-AuNP synthesis without other additional linkers. It had been reported that peptide-AuNP conjugates enhance the macrophage responses compared with sole peptide or AuNP.

Overexpression receptor αβ3 integrin was detected in the melanoma, glioblastoma and tumor vasculature. Domain peptide RGD binds to αβ3 integrin and can be exploited as the target domain of active-targeting nanoplatforms. Yang et al. attached bleomycin to AuNP surfaces through thiol bonding, using sequential conjugation of an RGD domain peptide (CALNN peptide). The surface of AuNP was modified with a peptide sequence (CKKKKKKKGGRDGMFG) containing the RGD domain and bleomycin without PEG as a linker. The efficacy of bleomycin-AuNP was measured by visualizing DNA breaking into the human breast cancer cells (MDA-MB-231). The 16 h irradiation treatment showed that the human breast cancer cells (MDA-MB-231) internalized with the complex had a 32 ± 9% cell survival decrease and statistically significant damage enhancement compared to the control cells (irradiated without AuNPs) after receiving a radiation dose of 2 Gy with 6 MV photons. However, an in-vitro study on the colorectal cancer cells Caco2 and SW620 showed that the cyclic RGD attached to AuNP and
linked to a PEG chain lost its targeting ability. The targeting activity of cRGD-AuNP proved to be present when cRGD was linked to PEG and a short oligosilane spacer. The fact that cRGD-AuNP was unreachable by a membrane receptor could be because the PEG layer hindered active-targeting ligands and caused loss of the active-targeting ability.\textsuperscript{54}

Kalimuthu et al.\textsuperscript{55} investigated the PEG/PDC P4 peptide and chemotherapeutic (PDC) conjugation with AuNP on A20 cells. PDC was attached to the PEG-coated AuNP and conjugated to a chemotherapeutic drug (chlorambucil, melphalan, bendamustine) to stabilize and improve the bioavailability. The A20-like leukemia cells internalize P4 peptide but not P6, a negative control. The P4 peptide was bound to the NB4 cells (10.3%) and HL-60 cells (13.8%). Meanwhile, the P6 was not bound to the NB4 cells and bound only 6.9% to the HL-60 cells. Compared to the free drugs and free PDCs, which reduced the cytotoxicity after 24 h, PDC P4-AuNP had prolonged activity 48 h after incubation. However, PDC-AuNP reduces the cytotoxicity of chlorambucil and melphalan towards the A20 cell but improves the cytotoxicity of bendamustine. This difference in cytotoxicity effect due to the active-targeting PDC-AuNP depends on the receptor cell surface density and the mechanism of internalization.

Cruz and Kayser attached trastuzumab-AuNP (ADC) to a cell-penetrating peptide to enhance intracellular delivery. The incorporation of the HIV-TAT cell-penetrating peptide (CPP) enhanced the intracellular internalization in four different cancer lines (SKBR-3, DLD-1, MDA-MB-231 and MCF-7). Uptake into cells heavily influenced by surface charge and a peptide linker had a more pronounced impact than the addition of an antibody-targeting agent for changing the surface charge.\textsuperscript{56,57} Tapia-Arellano et al.\textsuperscript{52} functionalized PEG/angiopep-2 peptide (TFFYGGSRSKRNFKTEEY) to improve the delivery of Au nanoparticles to the central nervous system. The evaluation of AuNP-PEG-ang2 showed improved delivery through BBB in zebrafish larvae by utilizing the presence of red signal fluorescence as an indicator. An in-vivo study on zebrafish larvae through immersion revealed that although the nanoplatform crossed the blood-brain barrier, it did not produce a toxic effect and was completely removed after 24 hours. Neither did the in-vitro cell viability produce a cytotoxic effect on SH-SH5Y neuroblastoma. In conclusion, even if AuNP-PEG-ang2 was able to cross the blood-brain barrier, it did not have a toxic effect on the normal cells.

**Gold Nanoparticles with Vitamins as Ligands**

Vitamins were compelling as active-targeting ligands because they had relatively small molecular sizes, are easy to handle and are cost-effective.\textsuperscript{58} Active targeting system may exploit the overexpression of the folate, biotin and vitamin B12 receptors in cancer cells. Yucel et al.\textsuperscript{51} synthesized glutathione-coated folic acid attached to AuNP and methotrexate loaded to glutathione-FA-AuNP. The in-vitro study showed that the cytotoxicity of glutathione-FA-AuNP increased threefold in the brain cells and tenfold in the cervical HeLa cells. No significant effect on lung carcinoma, prostate carcinoma and healthy kidney cells were found from in-vivo evaluation.

Folic acid-targeting also has potential use in photothermal drug delivery. Banu et al.\textsuperscript{59} study the combination of folic acid and doxorubicin AuNP for photothermal therapy in the breast cancer lines (MCF-7 cell line). The increased efficacy of this active-targeting nanoplatform was the collective effect from active targeting along with the mechanism of cell death involving the upregulation of the pro-apoptotic protein p53 and the downregulation of the anti-apoptotic protein Bcl-2. A polyethylenimine-capped AuNP-based non-viral delivery system to deliver si-RNA into prostate cancer cells using folic acid as a targeting ligand (AuNPs-PEI-FA) showed internalization of AuNPs-PEI-FA to the LNCap cells (prostate-cancer-overexpressing folate) while no internalization in the prostate cell line that was not expressing a folate substrate.\textsuperscript{53}

Biotin is essential for cancer cells for rapid proliferation. Heo et al.\textsuperscript{60} functionalized the AuNP surface with PEG, biotin, paclitaxel and β-cyclodextrin. This nanoplatform has a high affinity to the HeLa, A549 and MCG63 cancer cell lines and low affinity to the NIH3T3 normal cell. The affinity of the nanoplatform to cancer cells was threefold higher than to normal cells. The biotin-conjugated Au nanoplatform induced cytotoxicity fivefold in HeLa and below onefold in NIH3T3 compared to paclitaxel alone. The in-vitro study of the copper complex, AuNP and biotin nanoplatform against the HeLa and HaCaT cells showed no uptake and activity difference with the biotin-attached nanoplatforms and without biotin. However, an in-vivo study showed that the biotin-attached AuNP nanoplatforms reduced tumor growth 3.8-fold and the nanoplatforms without biotin reduced tumor growth 2.3-fold.\textsuperscript{62} Biotin attached nanoplatform may not improve cellular uptake of nanoplatforms in vitro but has significant targeting ability.

**Gold Nanoparticles with Proteins as Ligands**

Aside from antibodies, other proteins could act as an active targeting ligand. Ruan et al.\textsuperscript{63} loaded AuNP with doxorubicin through hydrazine and functionalized it with angiopep-2, a ligand of receptor-related lipoprotein 1 (An-PEG-DOX-AuNP) to deliver the drug to the blood-brain barrier. The study of doxorubicin release from systems showed that the doxorubicin from the An-AuNP-loaded DOX was released more quickly at a lower pH. The in-vivo study with glioma-bearing mice showed that An-PEG- AuNP-loaded DOX had higher intracellular uptake intensity and distributed at a higher intensity into glioma than the free doxorubicin or the AuNP without a ligand. In vivo study in mice found that the nanosystem-treated mice had a longer median survival time than the doxorubicin-treated mice. Combination therapy endostatin-AuNP with 5-fluorouracil is more effective than 5-fluorouracil.
Gold Nanoparticles with Polysaccharides as Ligands

Polysaccharides such as hyaluronic acid exploit as a ligand for active-targeting CD44 receptors in the cancer cell. Jacinto et al. functionalyzed Au core silica shell nanorods with hyaluronic acid and vitamin E PEG succinate (AuMSS-TPGS-HA) for cancer-targeted photothermal therapy that prolongs systemic circulation, improves cellular uptake, enhances solubility and improves the selectivity of nanorods. Functionalized Au core silica shell nanorods with hyaluronic acid and vitamin E PEG succinate did not affect the size or photothermal activity of Au core silica shell nanorods against HeLa cells but affected the surface charge.

Gold Nanoparticles with Aptamers as Ligands

Aptamers are short single-stranded DNA or RNA that selectively bind to the specific target. Ruttala attached ionidamine and aptamer AS1411 to AuNP. Conjugating AS1411 aptamer to the NP surface significantly improved the particle accumulation in cancer cells via affinity with the nucleolin receptors. An in-vitro study showed a positive effect in tumor tissues, without evident damage to the surrounding healthy tissues. The apoptosis effect was due to the increased reactive oxygen species production and the cell migration inhibition. The assembly of hybrid graphene oxide/AuNP (Au@GO NP)-based cancer-specific nucleic acid was conjugated for multimodal imaging and was combined with therapeutics, resulting in cancer detection and multimodal synergetic cancer therapy through the use of photothermal, genetic and chemotherapeutic strategies. Au@GO NP-NACs sensitively detect and effectively suppress cancer pro-survival genes.

Gold Nanoparticles with Hormones as Ligands

Hormones such as ethinylestradiol (EE) and antiandrogen hormones also had been exploited as active targeting ligands. EE was selectively bound to estrogen receptors and targeted to intracellular receptors. EE conjugated with silica-coated gold nanoparticles and loaded with anticancer such as doxorubicin and quercetin. The release capacity of EE-AuNP-quercetin was 650 times higher than quercetin alone and the release capacity of EE-AuNP- doxorubicin was 2.5 times higher than doxorubicin in vivo. EE-AuNP also accumulated in tumor tissue whereby health cells were unaffected. Antiandrogen ligands (α-Bicalutamide and β-Bicalutamide) conjugated with AuNP via PEG -SH showed a reduction in cancer growth and progression. Antiandrogen conjugated AuNP selectively stimulated G-protein coupled receptor (GPRC6A) multivalent affinity and accumulated in hormone-insensitive and chemotherapy-resistant prostate cancer cells.

Discussion and Future Recommendations

Active targeting AuNPs utilize a ligand attached to the AuNPs to improve the chemotherapeutic delivery. The attached ligand objective was to ‘direct’ the platform to the target cell. The term directing involves specific binding to an overexpressed receptor in the cancer cell that leads to better uptake and internalization of chemotherapeutic agents into the cancer cell, thus improving photothermal, photodynamic and chemotherapy effects of the platform towards the targeted cell while reducing effect towards normal cells.

Macromolecules and small molecules as active targeting ligands in AuNP conjugates exploit overexpressed receptors of the cancer cell. Thus, molecule selection as active targeting ligands must consider the specific overexpressed receptors of the cell target. Ligands-targeted receptor-bound would carry the desired targeting activity. Some targeted receptors showed in Table 3.

Macromolecules were better researched as active targeting ligands of AuNPs rather than small molecules. Small molecules such as vitamins are cost-effective and easy to handle but do not carry affinity and specificity as highly as macromolecules as ligands. Protein such as antibodies are the most well researched as ligand; they carry their cytotoxicity effect but are also immunogenic and complex in handling. Peptide sequences can modify ligand-AuNPs conjugate. Using peptides also overcomes the ligand antibody disadvantage. Proteins, including antibodies, can
Table 3. The targeted receptor of ligand AuNP.

<table>
<thead>
<tr>
<th>Overexpressed receptor on cancer cell</th>
<th>Ligands</th>
<th>Type of Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>Cetuximab (Antibody)</td>
<td>Small Cell Lung cancer, Prostate cancer</td>
</tr>
<tr>
<td>CD 20</td>
<td>Rituximab (Antibody)</td>
<td>Lymphomas, colorectal cancer, thyroid cancer</td>
</tr>
<tr>
<td>ERRB2</td>
<td>Trastuzumab (Antibody)</td>
<td>Breast cancer, pancreatic cancer</td>
</tr>
<tr>
<td>VEGF</td>
<td>Anti-VEGF (Antibody)</td>
<td>Thyroid cancer, ovarian cancer, breast cancer</td>
</tr>
<tr>
<td>CD13</td>
<td>antiCD13 (Antibody)</td>
<td>Colon cancer, breast cancer</td>
</tr>
<tr>
<td>Avβ3 Integrin</td>
<td>PVC peptide, RGDpeptide</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td>LRP1</td>
<td>Angiopep 2 (Protein)</td>
<td>Colon cancer, breast cancer</td>
</tr>
<tr>
<td>Carbonic anhydrase IX</td>
<td>Endostatin (Protein)</td>
<td>Lung cancer, leukemia</td>
</tr>
<tr>
<td>PD-L1</td>
<td>AntiPD-L1 (Antibody)</td>
<td>Stem cancer, breast cancer</td>
</tr>
<tr>
<td>CD44</td>
<td>Hyaluronic Acid (Polysaccharide)</td>
<td>Epithelial cancer (breast cancer, ovarian cancer, cervix cancer)</td>
</tr>
<tr>
<td>Folate Receptor</td>
<td>Folic Acid (vitamin)</td>
<td></td>
</tr>
<tr>
<td>Transferrin Receptor</td>
<td>Transferrin (Protein)</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>Estrogen Receptor</td>
<td>Ethinylestradiol/Estrogen (Hormones)</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>Androgen Receptor</td>
<td>Antiandrogen (Hormones)</td>
<td>Prostate cancer</td>
</tr>
</tbody>
</table>

attach to AuNPs via polyvalent interactions, whereas small molecules such as vitamins could not obtain this property. The attachment of active-targeting ligands could change the properties of an individual component, resulting in different pharmacokinetic and pharmacodynamic profiles. The previous in-vivo studies on active-targeting AuNP did not always support the active-targeting purposes, especially when it needed to attach to a more complex biological membrane (e.g. blood-brain barrier). The active-ligands attached to AuNPs nanoplatorms may contribute to the character of the AuNPs nanoplatorms, macrophage-induced response, the ligand-AuNP mechanism of cellular or intracellular attachment and internalization, thus affecting the delivery to the target. The inability to ensure the consistency of the quantity of substance attached to the AuNP surface, whether active ligands or chemotherapeutic agents, may result in varied pharmacokinetic and pharmacodynamic properties. The non-optimal drug release mechanism, e.g. because of the bond between ligand and AuNPs platform, can contribute to unfavored active targeting. Another major concern was the lack of comparison between ligand-AuNPs interaction toward a similar target.

Current in vivo research of active targeting AuNP did not conduct on specific cancer organs. Most in vivo studies involve general tumor bearing mice when it was also necessary to study the effect of active targeting AuNP onto different and specific cancer cells in vivo. The affinity and binding capacity of ligand-AuNP in the same receptor/same cell target were not comparable between one another. Although some commercial Au, such as Aurimune™, auroshell® already in the clinical trial phase, ligand conjugated AuNPs were still yet to begin the clinical trial to support its commercial uses.

During AuNPs conjugate design, researchers should carefully address the correlation of characterization properties such as particle size, surface charge, loading efficiency and drug release mechanism with the therapeutic outcome. Further research and development should also consider drug disposition/internalization in vivo and
quantification of the active-targeting AuNP delivery system to achieve the desired therapeutic outcome. Artificial intelligence may play a bigger role in active targeting AuNPs conjugate design for future development considering the vast amount of study. The optimized development of active-targeting chemotherapeutic AuNP is illustrated in Figure 3.

**Conclusion**

Active targeting AuNPs showed great potential in improving therapeutics outcomes and reducing toxicity in normal cells better than AuNP or conventional chemotherapeutics alone. Active targeting ligands such as antibodies, peptides, vitamins and small molecules, DNA with AuNPs enhance the photothermal, photodynamic and chemotherapy effects of AuNPs systems. More studies should be conducted to quantify the attached ligand to AuNPs nanoplatform and the relation between the quantity of attached ligand and its pharmaceutical effect. We also suggested conducting comparative studies between ligand-AuNPs interaction toward a similar target to optimize the active targeting AuNPs towards a specific target.

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**Author Contributions**

We declare that this work was done by the author(s) named in this article and that all the liabilities from claims relating to the contents of this article will be borne by the authors. This study was written by AG, with input and correction from SS.

**Conflict of Interest**

The authors report no conflicts of interest.

**References**


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