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Cancer Nanodiagnostics and Nanotherapeutics through the Folate-Conjugated Nanoparticles

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Introduction

According to the US National Cancer Institute [1] "Nanotechnology will change the very foundations of cancer diagnosis, treatment, and prevention…. To help meet the goal of eliminating death and suffering from cancer by 2015, the NCI is engaged in efforts to harness the power of nanotechnology to radically change the way we diagnose, image, and treat cancer". We have already seen how nanotechnology, an extremely wide and versatile field, can affect many of its composing disciplines in amazingly innovative and unpredictable ways [2]. In fact, nanotechnology and the ideas and methods that it encompasses can be applied to almost any problem that leading researchers face today. Even the most seemingly impossible problems like cancer [3] and Alzheimer's disease [4,5] become only obstacles in the path to solutions, if we take an imaginative approach.

The development of specialized nanoparticles for use in the detection and treatment of cancer is increasing. Methods are being proposed and tested that could target treatments more directly to cancer cells, which could lead to higher efficacy and reduced toxicity, possibly even eliminating the adverse effects of damage to the immune system and the loss of quickly replicating cells [3,6,7]. In this short report we focus on recent studies that employ folatenanoconjugates to target the folate-receptor. Folate-receptors are highly overexpressed on the surface of many tumor cell types. This expression can be exploited to target both imaging molecules and therapeutic compounds directly to cancerous tissues. We specifically report the details of advances made in attachment of gold nanoparticles to folic acid and it's its in vitro internalization into cancerous cells [7-13].

Folate and Folate-Receptor

The folate-receptor, a glycosylphosphatidylinositol anchored cell surface receptor, is overexpressed on the vast majority of cancer tissues, while its expression is limited in healthy tissues and organs. Folate-receptors are highly expressed in epithelial, ovarian, cervical, breast, lung, kidney, colorectal, and brain tumors. When expressed in normal tissue, folate-receptors are restricted to the lungs, kidneys, placenta, and choroid plexus. In these tissues, the receptors are limited to the apical surface of polarized epithelia [14-17].

Folate, the folic acid (Figure 1) salt, also known as pteroylglutamate, is a non-immunogenic water-soluble B vitamin that is critical to DNA synthesis, methylation, and repair.

Folic acid is small (441 Da), stable over a broad range of temperatures and pH values, inexpensive, and non-immunogenic, and it retains its ability to bind to the folate-receptor after conjugation with drugs or diagnostic markers [18]. After folate attaches to the receptors located within caveolae, it is internalized through the endocytic pathway (Figure 2). As the pH of the endosome approaches five, the folate dissociates from the receptor and the drug is released.

Folate conjugates for cancer detection and treatment

The folic acid/folate-receptor interaction can be targeted for imaging cancer cells by the attachment of imaging probe molecules to folate. Folic acid conjugated functionalized carbon nanotubes, gold nanoparticles (AuNPs), magnetic nanoparticles, liposomes loaded with quantum-dots, and photon emission tomography (SPECT and PET) tracers are studied for use as imaging probes in various imaging methods [18-25]. Gold nanorods strongly absorb and scatter light in the visible and NIR region, and have been tested as novel MRI contrast agents [24,26-29].

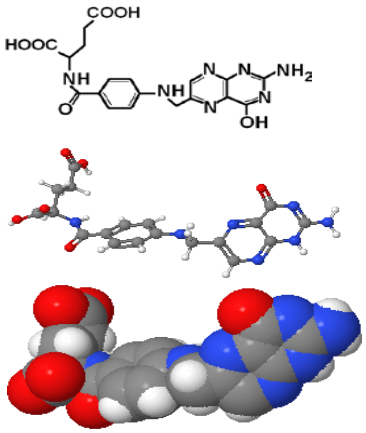

Figure 1: The molecular structure of folic acid [8,9,12].

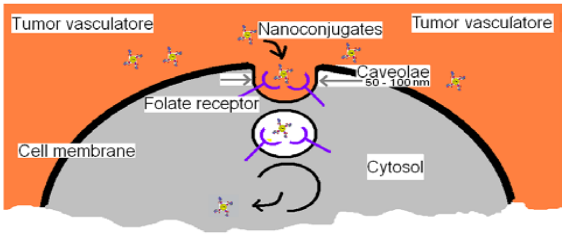

Figure 2: Attachment of nanoconjugate of gold nanoparticle with folate (AuNP-Linker-Folate) to cell's folate-receptor and its internalization through the endocytic pathway [13].

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In a 2004 study the efficacy of nanoscale-sized folate-receptor-targeted doxorubicin aggregates were tested for the treatment of cancer [15]. Doxorubicin-polyethylene glycol-folate (DOX-PEG-FOL) conjugate micelles produced were 200 nm in average diameter. The polymeric micelles exhibited enhanced and selective targeting to folate-receptor-positive cancer cells in vitro. More DOX-PEG-FOL nanoaggregates accumulated in folate-receptor-positive human epidermal carcinoma KB cells than in folate-receptor-negative A549 cells. When including unconjugated folate along with the nanoaggregates, the folate competitively inhibited binding of the DOX-PEG-FOL nanoaggregrates to the folate-receptor-positive cells. During in vivo animal experiments, the nanoaggregates caused significant tumor suppression. In human tumor xenograft nude mice, DOX-PEG-FOL nanoaggregates had a superior antitumor effect compared to other doxorubicin aggregates and free doxorubicin. In the mice treated with DOX-PEG-FOL nanoaggregrates, tumor volumes decreased by approximately 40% more than in mice treated with free doxorubicin. The enhanced antitumor effect of the nanoaggregrates was attributed to passive targeting through leaky vasculature in addition to active targeting of the nanoaggregates to folate-receptors. Furthermore, the DOX-PEG-FOL nanoaggregates exhibited a sustained release effect because of prolonged circulation time in the bloodstream. Overall, the aggregates exhibited enhanced cellular uptake, increased targeting capacity, and increased cytotoxicity of folate-receptor-positive cells.

In a 2005 study targeting of folate-linked methotrexate dendrimers was tested in immunodeficientathymic nude female mice and Fox Chase severe combined immunodeficient female mice [14]. Mice were first injected with KB folate-receptor-positive human cell lines. Tumors were allowed to grow for 2 weeks and reached a volume of 0.9 cm^3. Then the mice were injected with the nanoconjugates twice a week via a lateral tail vein. Folic acid conjugates were delivered at an equimolar concentration with methotrexate, based on the number of methotrexate molecules present in each type of nanoparticle. The results from the study showed that conjugated methotrexate in dendrimers significantly lowered toxicity and resulted in a 10-fold higher efficacy compared to free methotrexate at an equal cumulative dose. Because of the ability to deliver a higher dose of methotrexate as the conjugate compared to the free drug, mice survived longer. However, the optimal dose of the targeted drug was not definitively established because no toxic dose of the drug conjugate could be determined from either gross clinical evaluation or histopathology.

Brandenburg et al. [30] compared the original design of a cancer nanotechnology process involving folate-conjugated nanoparticles in 2005 and they reported their design results in early 2006. Simultaneously, Mansoori developed a biosynthesis method for industrial-scale production of metallic nanoparticles [31]. As a result of these two initial findings we undertook a comprehensive in vitro project on cancer nanotechnology treatment designing various folate-conjugated gold-nanoparticles [8,9,12] as shown in Figure 3. Meanwhile two related papers by other groups [32,33] have reported of other folate-AuNPnanoconjugates.

In a recent publication Mansoori et al. [13] made a detailed comparison of the efficacy of two folates conjugated gold nanoparticles which were designed for cancer treatment. Our group actively targeted a gold nanosphere for use in the heat ablation of folate-receptor-positive cancer cells [8,9,12]. A combination of gold nanoparticles and an intense pulsed light, along with an incubation time, resulted in the significant death of cells with a high level of folate-receptor expression

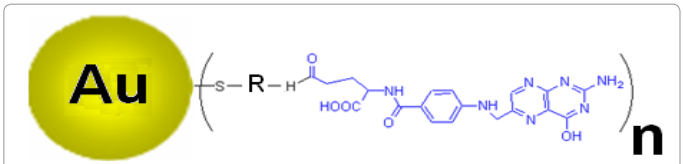

Figure 3: Schematics of nanoconjugate of gold nanoparticle with folate (AuNP-Linker-Folate) [8,9,12].

and no significant cell death in cells with a low level of folate-receptor expression. The two conjugates which were designed during our studies included folate-4-aminothiophenol-gold nanoparticles (FOL4Atp-AuNP) and folate-6-mercapto-1-hexanol-gold nanoparticles (FOL-MH-AuNP). Both conjugates have an absorption peak at a wavelength of ~560 nm. Twenty pulses (3 ms) of intense pulsed light, with a wavelength of 560 nm, were used to heat the gold nanoparticles that were taken up by the cells that expressed a high level of folate-receptors. During testing we found that using up to 20 pulses of intense pulsed light had no harmful effects, and that nanoconjugate concentrations used in the study showed no toxicity. Treatments were evaluated at multiple time durations after heating. Results from the study indicated that a longer treatment time is favorable over increased concentrations of the nanoconjugate. The highest level of cell death was observed after 4 hours of incubation and 5 mg/mL of either nano-conjugate. The FOL-4ATP-AuNP was slightly more effective than the FOL-MH-AuNP at lower concentrations. Our in vitro experimental results show that a combination of gold nanoparticles and 20 pulses of intense ultraviolet (UV) light resulted in approximately 98% lethality of the cells expressing high level of folate-receptors and only approximately 9% lethality of cells expressing a low level of folate-receptors. For in vivo applications, IR and/or NIR lights might be more effective than UV light as they penetrate deeper into tissues. Replacing the gold nanosphere moiety with nanoshells and nanorods, which absorb light more efficiently near IR wavelengths, could also be used for in vivo testing in the future. In addition, fiber optics might serve as an in vivo method for the deeper penetration of the light into the tissue.

Recently several groups have used mesoporous particles as targeted delivery agents [34,35]. In 2010, researchers found that mesoporous particles are well tolerated by mice, with a maximum dose of 100 mg/kg [34]. In a 2012 study, the cytotoxicity of folate targeted mesoporous silicon doxorubicin drug conjugates was tested [36]. It was found that the mesoporous drug conjugates exhibited a substantially higher toxicity for tumor cells compared to free doxorubicin [36]. Using folate as a targeting agent was clearly shown to enhance the toxicity of functionalized mesoporous silicon drug conjugates [36]. The ability of CNTs to be easily functionalized makes them a promising candidate for cancer treatment. However, there are two major barriers to their use as cancer therapeutics. These include non-specificity and low potency [36]. In 2010, Li et al. tested folate and iron difunctionalized MWCNTs for the delivery of doxorubicin into HeLa cells. The efficiencies of the drug conjugates were tested on HeLa cells in 96-well assays [37]. The MWCNTs were shown to have sufficient load capacity and controlled release by near IT radiation [37]. Results from this study demonstrated a six-fold increase in doxorubicin delivery compared to free doxorubicin alone [37].

More recently, in 2012, publications have appeared in the literature using folate-receptor-directed dendrimers for the delivery of methotrexate to cancerous cells [38-40]. One study cited a 4,300-fold higher affinity for folate-receptor-mediated methotrexate dendrimers

than free drug alone [39]. Dendrimers were used to deliver siRNA in order to improve its specificity and transfer activity [40]. Results from the study indicated no inflammatory or interferon response, common non-specific effects of siRNA, suggesting future use as a potential cell-selective delivery method.

Conclusions

Overall, folate-conjugated nanoparticles have great potential for cancer detection and treatment. Methods are being proposed and tested that could make diagnosis and treatment of cancer non-invasive, targeting tumors directly through their overexpressed folate-receptors. Folate-receptors are highly overexpressed on the surface of many tumor cell types. This expression can be exploited to target therapeutic compounds directly to cancerous tissues using many avenues. While these studies prove to be promising, the use of folate directed cancer treatments in human subjects still needs further development and testing. Nevertheless, the successful use of folate conjugates indicates that receptor targeted nanoparticle treatments are a likely candidate for managing cancer.

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