Folate-Conjugated Gold Nanoparticles for Cancer Nanotechnology Applications

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ABSTRACT

A need exists to target cancer treatments specifically to the tumor site, without damaging healthy tissue. Folate is an essential nutrient required for DNA replication and is brought into a cell via a folate-receptor, making it an ideal cell surface receptor to specifically target fast growing cancer. A Folate-nanoconjugate was tested for cytotoxicity against two types of cancers cells: HeLa and MCF7. The nanoconjugate itself was not cytotoxic to either cell line. The greatest cell lethality upon stimulation with intense pulsed light was observed in the HeLa cells. The lowest concentration of the nanoconjugate and incubation time that caused the greatest cell death (~98%) was 5 µg/ml concentration and 4 hours incubation in HeLa cells. The same conditions only caused a cell lethality rate of ~9% in MCF7 cells. Thus we deduced that the difference in cell lethality would be the greater association and increased internalization of the nanoconjugate in HeLa cells over MCF7 cells.

Keywords: folate, folate-receptor, gold nanoparticle, cancer nanotechnology, photothermal treatment

1 BACKGROUND AND INTRODUCTION

A need exists to specifically target sites of cancer to limit the damage on healthy tissues. Nanotechnology offers one solution to achieving this goal [1]. The pathology of malignant cancers is typically associated with vaculature which contains defects or pores which allows appropriate sized particles to deposit within the tumor. If the particles are small enough (i.e. within the 1-100 nanometer size) they can freely diffuse into tumors. This process is called passive targeting because any site in the body that has damaged or defective vasculature can have deposition of the nanoparticles into it [2-5]. However, this diffusion occurs both into and out of the cancer site, therefore there is an additional need to increase the residence time of the particles within tumors [6]. Active targeting is considered selectively targeting cancer cells through specific binding interactions on the cell surface, such as a receptor.

Folic acid/Folate (Mw = 441.4 Da) [Figure 1] is a water soluble molecule that is internalized by both cancerous and non-cancerous cells via the Folate-receptor. Folate is required for the production of thymine by dihydrofolate reductase, required for DNA synthesis. Therefore, the cell's function and requirement for DNA syntheis has a direct impact on the need for Folate internalization. Cancer cells typically have a greater requirement for Folic acid than non-cancerous cells, and tend to overexpress the Folate-receptor on their surface [7]. In addition to greater expression of this receptor in cancerous tissue, the ability to conjugate it with many different nanotechnology platforms has increased interest in this molecule as a targeting moiety. This report will focus on the rationale of using Folate in acitve targeting as well as our own examination of the use of Folic Acid to deliver gold nanoparticles into cancer cells *in vitro* for subsequent thermal destruction of the cells.

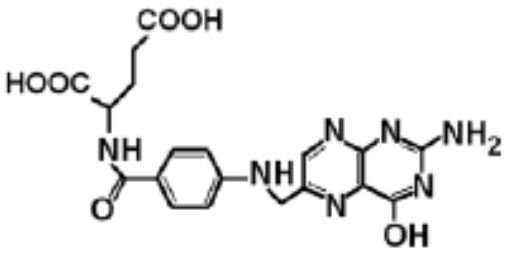

Figure 1: The molecular structure of Folic Acid.

1.1 Folate-receptor Tissue Distribution

The Folate-receptor is a glycosyl-phosphatidylinositol linked membrane protein. This receptor has bee shown to be present in many healthy tissues and organs, such as: ovaries, kidneys, lungs, and choroid plexus [8]. Since, the Folate-receptor is commonly expressed in healthy tissues why should it be a target for anti-cancer treatments? The reason why it can be used as a targeting moiety is entirely based on the membrane localization of the receptor in healthy tissues versus cancerous tissues.

In healthy tissues, epithelial cells have two distinct membrane forms. The apical membrane is the side which is located on the outside of the body or interior cavities, such as the lungs or gastrointestinal tract. In contrast, the basal membrane is located on the side of epithelial cells that are exposed to the blood stream or other tissues. In healthy tissues the Folate-receptor are primarily localized to this apical memrbane, meaning that they are exposed to the air (lungs), urine (kidneys), or cerebrospinal fluid (choroid plexus) and not the blood stream [9].

The defects in the vasculature of tumors allow nanoparticles to accumulate within the tumor. The size of

the defects in tumor vasculature are typically hundreds of nanometers in diameter compared to the pores in healthy tissue vasculature of only a few nanometers in diameter. By designing the nanocarrier to have a larger diameter than 10 nm, it can be prevented from entering into healthy tissues, and thus passively target tumor sites. Since, a Folate targeted anti-cancer platform would be exposed to the interior of the tumor it can freely intereact with the Folate-receptors on the cancer cells. Upon binding to it receptor the Folate targeted nanoconjugate can be brought into the cell via caveolae mediated endocytosis.

1.2 Folate-Receptor Endocytosis

As reported by Hong *et al.* [10] Folate has a strong binding affinity for its receptor having an association constant (K_A):

$$K_A = \left[\frac{Folate-receptor \bullet Folate\ Aggregate}{[Folate-receptor][Folate]} \right] = 2\times10^7[s]. \quad (1)$$

The Folate-receptor is located in caveolae on the surface of the cell and typically between 50 – 100 nm in diameter. It is estimated that each caveolae has approximately 750 Folate-receptors in it. Upon binding to it receptor, the Folate targeted nanoparticles will begin to transition across the cell membrane via endocytosis. It is important to note that this pathway does not enter the clathrin coated pit pathway, thereby allowing release of the Folate directly into the cytosol. Upon migration of the caveolae across the membrane, the interior becomes slightly more acidic (pH ~5) which causes disassociation of Folate from its receptor. The Folate targeted nanoparticles are thus released into the cytosol of the cancer cell. Figure 2 is a diagram of the binding of the Folate targeted nanoconjugate binding to the Folate-receptor and migration across the membrane.

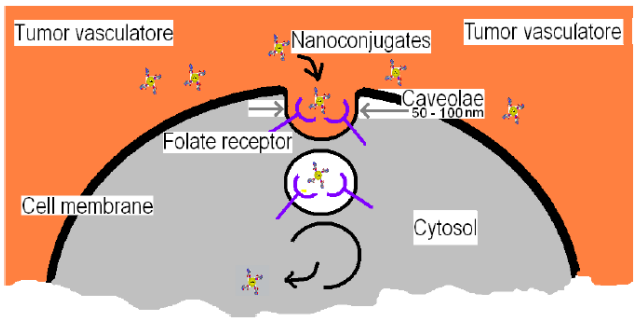

Figure 2: Diagram of uptake and transfer of Folate-targeted nanoconjugate into cancer cells.

1.3 Folate Targeted Nanoconjugates

The original design of this technology was created in 2005 and reported in early 2006 [11]. All together 6 projects have been reported by our research group [12-17] based on Folate targeted gold nanoparticles. In this paper we will focus on the *in vitro* anticancer activity of gold nanoparticles conjugated with Folate with 4-aminothiophenol [Figure 3], hereafter named Folate-4Atp-AuNP and the TEM of the resulting nanoparticles is shown in Figure 4.

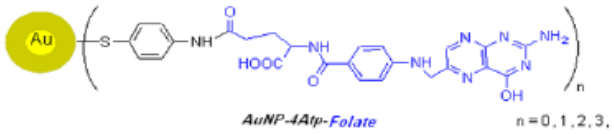

Figure 3: Schematic of Folate-4Atp-AuNP.

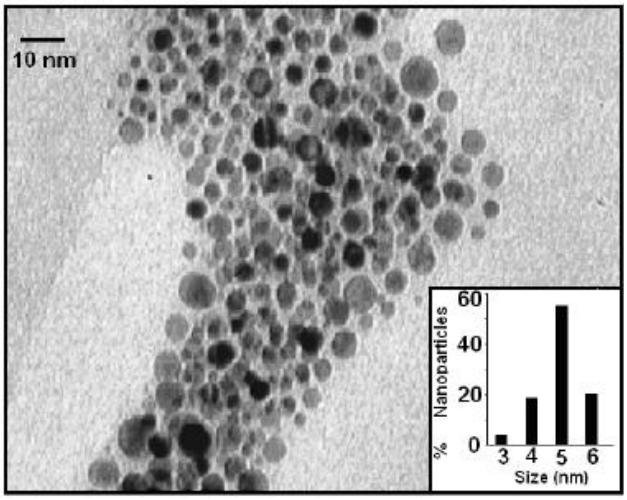

Figure 4: Transmission electron microscopy (TEM) photography of Folate conjugated gold nanoparticles. Inset: histogram of size distribution of the nanoparticles.

2 MATERIALS AND METHODS

2.1 Nanoparticle Cytotoxicity

The cytotoxicity of the Folate targeted nanoconjugate Folate-4Atp-AuNP was tested on two cancer cell lines, HeLa and MCF7. HeLa cells characteristically overexpress the Folate-receptor while MCF7 cells generally express the Folate-receptor at a low level. Both cell lines were subjected to incubation with the nanoconjugate in concentrations ranging from 1 – 100 µg/ml and for 1, 2 or 4 hours. Cell survival was measured using a MTT-Tetrazolium assay.

2.2 Intense Pulsed Light

In order to cause heating of the nanoconjugates we proposed to use an intense pulsed light (IPL) source. We examined the effects of multiple pulses of light on each of the cancer cell lines we tested. The light source was operated under fixed parameters (Energy Fluency 15 J/cm^2, Cut-off filter: 560 nm, Pulse Duration: 3 ms). The cell lines were subjected to numerous pulses of light (10, 15, 20, 30,

and 40) to determine the amount of IPL the cell could withstand before significant loss of cell viability occurred.

2.3 Photothermal Therapy

The maximum number of pulses that did not cause loss of cell survivability was chosen for subsequent photothermal treatment of HeLa and MCF7 cell lines. Again concentrations of the nanoparticle from 1 – 100 µg/ml were incubated with either HeLa or MCF7 cells for 1, 2 or 4 hours. After the incubation the cells were exposed to the IPL for a set number of pulses (determined in previous section) and cell survivability measured with a MTT-Tetrazolium assay.

3 RESULTS

3.1 Nanoparticle Cytotoxicity

After incubating HeLa and MCF7 cells with varying concentrations of the Folate conjugated nanoparticles for 1, 2 or 4 hours cell survivability was measured [15]. Figure 5 shows the reults of the survivability assay. No significant impact on cell survival was observed in either cell line upon incubation with the nanoparticles up to 100 µg/ml for 1, 2 or 4 hours,

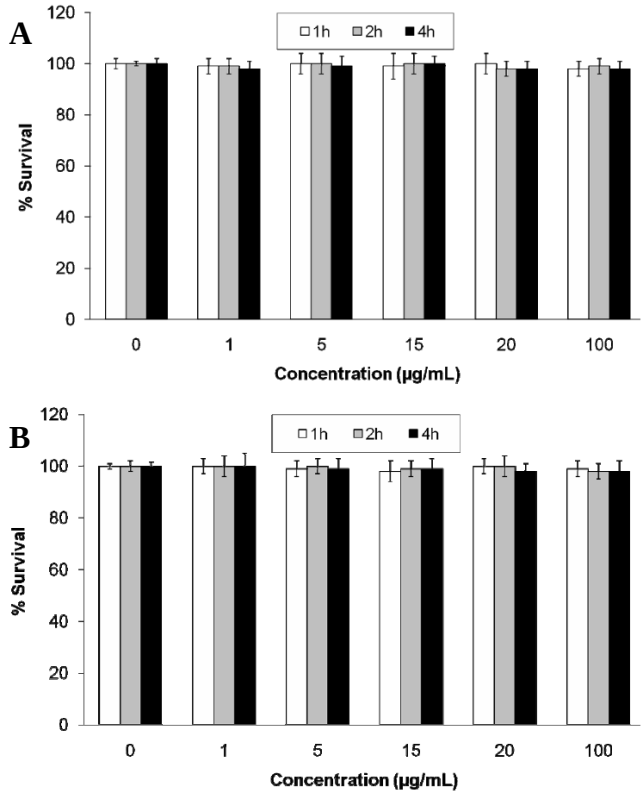

Figure 5: Percentage survival of A) HeLa and B) MCF7 cells incubated with Folate-4Atp-AuNP for 1, 2 or 4 hours at 1 and 100 µg/ml.

3.2 Intense Pulsed Light

Both HeLa and MCF7 cells were exposed to multiple pulses of the IPL and cell survival measured [Figure 6]. No significant impact on cell survivability occurred up to 20 pulses of the light [15]. Beyond 20 pulses cell viability decreased dramatically to only ~45% for HeLa cells and ~54% for MCF7 cells after 40 pulses. Therefore, the IPL was run for only 20 pulses during the photothermal studies.

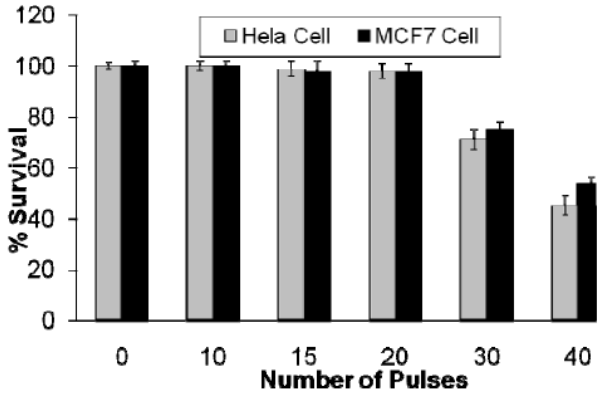

Figure 6: Cell viability of HeLa and MCF7 cells after IPL treatment.

3.3 Photothermal Therapy

The combination of the Folate targeted nanoconjugate and IPL was investigated with varying concentrations and incubation times [Figure 7]. In HeLa cells significant impact on cell survivability was observed in all concentrations of the nanoconjugate tested [15]. For the same incubation times, concentrations beyond 15 µg/ml did not increase the amount of cellular lethality caused by the nanoconjugate. An incubation time of 4 hours allowed for the greatest decrease in cell viability (~98%) with only 5 µg/ml of the nanoconjugate. In MCF7 cells the greatest amount of cell lethality (~26%) was observed in the 100 µg/ml nanoconjugate and 4 hours incubation treatment. At the nanoparticle concentration and incubation time that caused nearly a 98% decrease in HeLa cell viabiltiy only caused at most 10% decrease in MCF7 cell viability.

4 DISCUSSION

Two cancer cell lines were used to examine the ability of Folate targeted nanoparticles to selective cause cell death after exposure to intense pulsed light. HeLa cells were chosen because they characteristically overexpress the Folate-receptor. Likewise, MCF7 cells were chosen because they typically do not express the Folate-receptor. The Folate-4Atp-AuNP nanoparticles did not cause significant cytotoxicity themselves in either HeLa or MCF7 cell lines when tested *in vitro*. The intense pulsed light source was shown not to be harmful to cells in culture when exposed to 20 pulses, which was subsequently used when

testing the ability of Folate-4Atp-AuNP to invoke cell death via photothermal therapy. The combination of IPL with the nanoconjugates caused the greatest decrease in cell viability after incubating the cells for 4 hours with the nanoparticles. The ability of the Folate targeted nanoparticles to selectively target cells overexpressing the Folate-receptor is demonstrated by the increased amount of cell killing in HeLa cells over MCF7 cells.

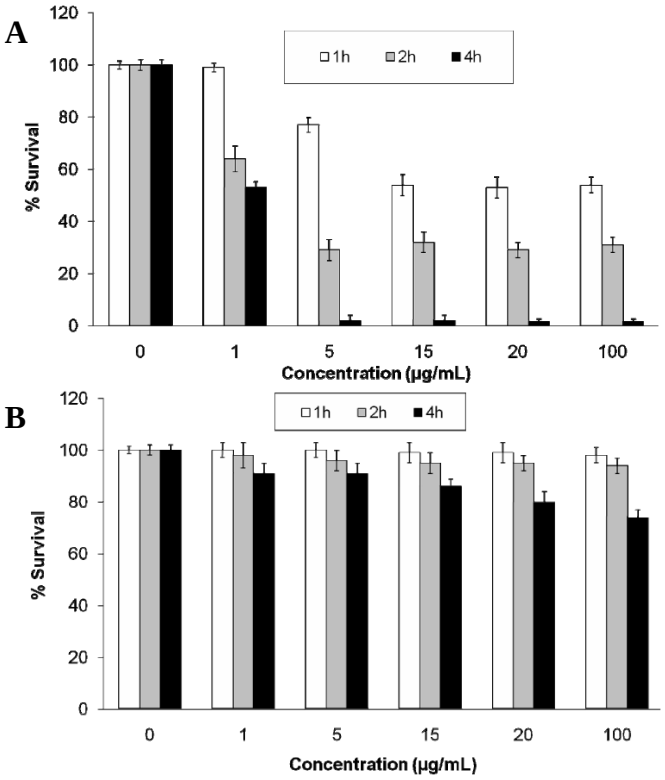

Figure 7: Cell viability of A) HeLa and B) MCF7 cells with Folate-4Atp-AuNP and exposure to 20 pulses of IPL compared to concentration of the nanoconjugate.

5 FUTURE WORK

Additional studies are required to confirm the ability of Folate conjugated nanoparticles ability to selectively target and destroy cancer cells expressing the Folate-receptor. Enhanced imaging techniques will show if the nanoconjugates enter cancer cells or remain bound to the Folate-receptor on the cell membrane. Further enhancements to the conjugate design are required to make the particles more suitable for *in vivo* use, such as: increasing particle size to take advantage of the Enhanced Permeability and Retention (EPR) effect of tumores and limiting diffusion of the particles into healthy tissues; and PEGylation of the nanoconjugate to prevent recognition by the reticuloendotehlial system and increase circulation time [18]. And finally techniques to deliver the IPL to tumors must be estabilished. One possibility is through optical fibers [19] or even tuning the particles to react to near infrared wavelengths for deep non-invasive light penetration [18,20]. Much work must still be performed before *in vivo* testing of the Folate targeted nanoconjugate can begin.

6 CONCLUSIONS

Folate conjugated nanoparticles were able to selectively target and cause hyperthermia in cancer cells which overexpress the Folate-receptor. Neither the nanoconjugates themselves nor the IPL itself caused signigicant cell death, but upon combination caused significant cell death in cells expressing the Folate-receptor.

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