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Folate Targeted Agents for Diagnostic and Therapeutic Uses

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Graduate Literature Project

In fulfillment of M.S. Analytical Chemistry Degree

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Dedicated to everyone who believed in me and supported me throughout my academic career

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Abstract

Folic acid has been frequently exploited for target therapies geared toward overexpressed folate receptors on malignant cells. The folic acid/folate-receptor high affinity interaction can be used to not only target but image cancer cells. Folate-linked nanoparticles represent a potential new drug carrier for tumor cell-selective therapeutics. Folate targeting, nanoparticles in therapeutics, and cancer cell imaging will be outlined in this literature report.

Introduction

Folate, a naturally occurring compound found in leafy green foods is essential to the mammalian folic acid cycle (1) *See Figure 1*. It is involved in critical processes for the transfer of one-carbon units to amino acids, nucleotides, and other biomolecules (2). These functions are all necessary processes which are important in DNA synthesis, replication, cell division, growth and survival (3). Folate also plays a key role in the methylation of many compounds; including DNA, RNA, proteins, and phospholipids (3). Folic acid is being used to treat certain types of cancer, such as cervical and colorectal cancer (4). Folic acid displays multiple desirable characteristics for the use in targeting cytotoxic drugs and imaging agents to cancer tissue (5) and has become an area of particular interest and study in the research and treatment of cancer.

Cancer cells, unlike normal human cells, overexpress folate receptors which have a high affinity for folic acid (6). These receptors can be used for uptake of folic acid in cell (6). There are two major forms of folate receptors: folate receptor alpha (FR-α) and folate receptor beta (FR-β) (7). FR-α primarily exist on cancer cells (8). There are two major forms of folate receptors; Folate receptor alpha (FR-α) and folate receptor beta (FR-β) (7). FR-α primarily exist on cancer cells (8). FR-α, also known as folate receptor 1 (FOLR1), is a protein encoded by the FOLR gene family (1). It binds folic acid on the surface of the cell and allows it to cross the cell membrane via receptor mediated endocytosis (1). It has a high affinity for folate and folic acid analogs at a neutral pH (9). FR-β exists on monocytes and macrophages in a cluster on chromosome 11 and also has a high affinity for folic acid and its derivatives (10). Both FR-α and FR-β are capable of uptake of folate and its derivatives but neither can differentiate malignant

cells from non-malignant cells (8), thus making it challenging to specifically target cancer cells. Therefore, cancer therapies that exploit cell specific ligands to deliver attached cytotoxic drugs selectively to malignant cells are of high interest.

Folate Therapy

Receptor-Mediated Endocytosis

Once folate conjugates are bound to a cell surface folate receptor (FR), they are transported into the cell through a process called receptor-mediated endocytosis (RME) (11). Endocytosis is the process by which cells internalize molecules specific to the molecule being internalized (12). Endocytosis occurs by cell engulfment rather the actual penetration of the cells (12). The cell membrane RME process allows cells to take up very specific macromolecules called ligands (12). Receptor-mediated endocytosis is classified as “active transport” (13). In order for active transport to occur, energy in the form of adenosine triphosphate, ATP, is needed to enable transportation of the molecules (14).

Receptor mediated endocytosis depends solely upon the cell specific receptors on the surface of the cell membrane which only attach to specific components found in the extracellular space (12). The specificity results from a very high affinity between the receptor and ligand (15). During receptor mediated endocytosis the ligand attaches itself to the receptor on the surface of the cell (12). Once bound the complex is then internalized (12). The region of the plasma membrane containing the receptor ligand complex is engulfed by the cell, becoming a transport vesicle (12). After the ligand has been internalized and remain in the cytosol of the cell where it will then be released (12). This process of cell uptake is being used in medical applications due

to its specificity. When targeting cancer cells, the targeted delivery of a therapeutic compound aims to enhance circulation, cellular uptake, improve therapeutic benefit with disease specificity (16) and decrease systemic toxicity. Figure 2 illustrates the receptor mediated endocytosis process.

Folate Targeting

There are many applications of folic acid targeting and folic acid therapeutic agents for treatment of malignant disease (15). For the purpose of this study, we will focus on folate targeted nanoparticles. Folate targeted nanoparticles are designed to carry large quantities of therapeutic cargos in compartments characterized by a diversity of sizes, shapes, rheological properties, and chemistries (15). Once a folate-linked nanoparticle arrives at an FR tumor cell, ligation of the particle to folate can only enhance its therapeutic efficacy, since folate will not only increase retention of the nanoparticle in the tumor mass but also facilitate uptake of the particle by FR-mediated endocytosis (15). In addition, accumulation of nanoparticles in solid tumors can occur due to poor drainage of the particles via the lymphatic system. The ideal approach for the therapeutic exploitation of folate receptors in cancer treatment is the Trojan horse approach, whereas the FOLRs and the cellular receptor-mediated endocytosis are utilized as portals of entry to deliver chemotherapeutic drugs (17). In order for this to be effective the drug must contain at least one of a limited number of chemical moieties SH, COOH, OH, or NH2 which allow for the release of the covalently bonded drug once inside the cell (15). The utilization of cellular endocytosis provides unprecedented specificity of drug delivery that typically exceeds the uptake of free drugs and significantly improves the direct bioavailability

while reducing the toxicity of folate-conjugated drugs or free drug nanoparticles (17). Nanoparticles have taken on a major role in folate targeted drug therapies and imaging.

Nanoparticles in Therapeutics

Therapeutic approaches that exploit nanoparticles (NP) to deliver drugs selectively to cancer cells are considered one of the most promising avenues in the area of cancer therapeutics and imaging (17). The use of nanoparticles in cancer treatment have several advantages over conventional chemotherapy treatments (3). They can protect drugs from being degraded in the body before they reach their target, enhance the absorption of drugs into tumors and cancerous cells, allow for better control over the timing and distribution of drugs to the tissue, and prevent drugs from interacting with normal cells avoiding side effects (3). Nanoparticles, are solid, colloidal particles consisting of macromolecular substances that are being developed to: improve drug bioavailability, abrogate treatment-induced drug resistance, and reduce nonspecific toxicity in the field of medicine (18). These particles are microscopic and are being used in the active targeting of cancer cells (19). Active targeting involves drug delivery to a specific site based on molecular recognition (18). This is typically achieved by coupling a ligand, such as folate to a NP so that the ligand can interact with its receptor at the target cell site (18).

Folate-linked nanoparticles represent a potential new drug carrier for tumor cell-selective targeting (11). Delivery of imaging and therapeutic agents using folate targeted nanoparticles can offer advantages over direct drug conjugation to folic acid (20). One advantage of utilizing NPs is their ability to overcome various biological barriers and to localize into the target tissue (18).

Secondly, large quantities of drug can be internalized at each folate receptor, allowing treatment of cancers with lower potency drugs that may otherwise have desirable properties (20).

Due to NP's small size they can easily penetrate cellular membranes through active and passive mechanisms allowing them to act as colloidal drug delivery systems (17)(11). Colloidal drug delivery systems such as liposomes and nanoparticles provide strategies including controlled release of therapeutic agents, prevention of drug degradation during circulation, and avoidance of toxic effects. Like liposomes, nanoparticles carry a large payload which enables targeted cells to receive a higher concentration of drugs (11).However, while liposomes largely encapsulate their therapeutic cargos, nanoparticles carry their active components on their surfaces (11).

Folate-derivative iron nanoparticles have been targeted to tumor tissues as MRI contrast agents due to their strong paramagnetic properties (11). In order for nanoparticles to serve as a moiety in active targeting a recognizable ligand needs to be tethered to the NP surface (17). For almost two decades metallic nanoparticles have successfully been used for cancer detection, imaging and treatment (21). Due to their high electron density metallic nanoparticles can be easily observed by electron microscopy and used in laser and radiofrequency therapy as energy releasing agents (21).

Folate Imaging

Detection methods using folate conjugates are non-invasive, making its use in locating and determining the severity of folate receptor–positive cancer cells very appealing (11). Major detection methods include optical imaging, magnetic resonance imaging (MRI), and ultrasound

imaging (11). Gold nanoparticles (AuNPs) are also being explored for their use in folate receptor–targeted cancer imaging (11). Other methods also creating interest are single photon emission computed tomography (SPECT) and positron emission tomography (PET) tracers (11). The success of such imaging strategies depends on both the specificity of the targeting ligands and the capacity of the tumor-specific receptors to bind sufficient quantities of imaging agent (22). Imaging agents act in conjunction with ligands. All ligand-targeted imaging agents must: (a) have high affinity for their cell surface receptors, (b) target a receptor that is significantly upregulated on cancer cells, (c) clear rapidly from normal (receptor negative) tissues, and (d) be taken up in sufficient quantities by receptor-expressing tissues to achieve good contrast (22).

Adequate tumor-specific uptake can generally be achieved if the receptor is highly abundant on the malignant cell (22). Recent efforts to develop tumor-specific imaging agents have focused on the use of targeting ligands that deliver attached radio-emitters/contrast agents to receptors that are over-expressed on cancer cells (22). The well characterized chemistry of folic acid allows for: (1) attachment of the vitamin to virtually any common chemical functionality, (2) purification and characterization of the targeted agent by standard organic chemistry methods, (3) storage of the drug for long periods without loss of function, and (4) production of the imaging agent at relatively minimal expense (22).

Conclusion

Folate imaging agents and nanoparticle medicines offer the advantage of allowing unmodified drug delivery to a cell in quantities 10 to 100 times higher than that resulting from the administration of free drug administration (20)(23). The specificity of drug uptake can be

easily monitored with an imaging agent and quantitatively measured by competition with free folates/folic acid (17). These methods are still under development. Coupling imaging with smart delivery is the ideal method for targeted treatment of cancer.

Figures

Folic Acid

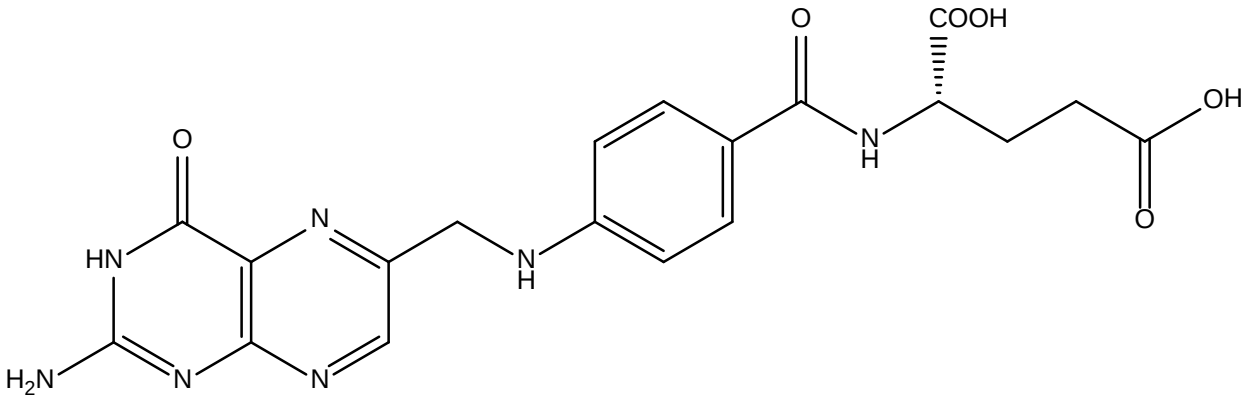

FIGURE 1. Folic acid structure (6)

Endocytosis of Folic Acid with Drug Conjugate

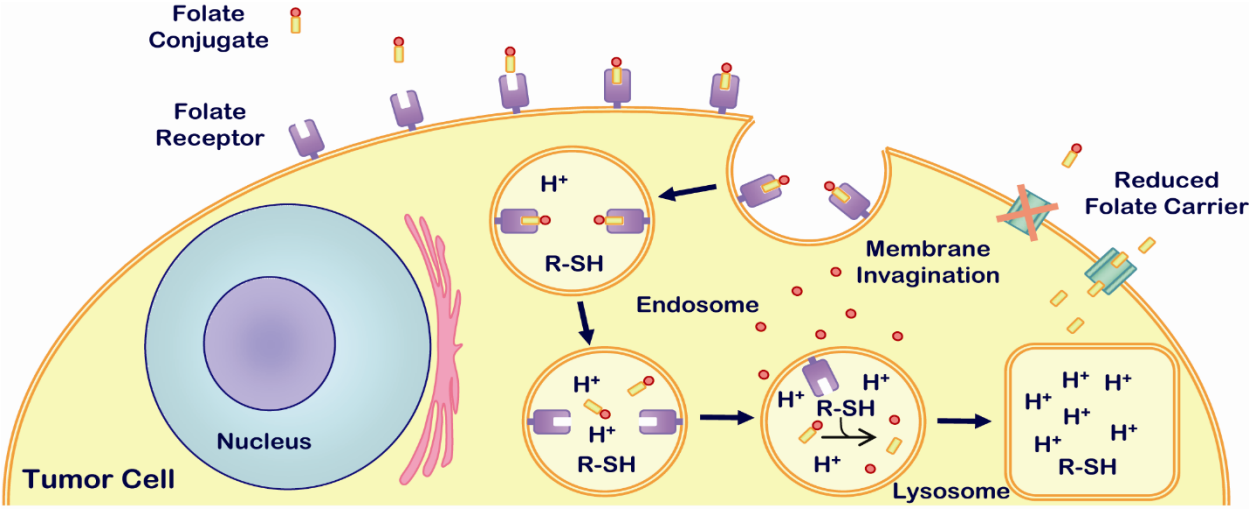

FIGURE 2 Reprinted with Permission: Folate Receptor-mediated endocytosis of folate drug conjugates (24).

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