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# Folate receptor-mediated Therapeutics and Imaging

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**Abstract**

Cancer is the second leading cause of death globally. Before a probable treatment approach is adopted based on early-stage diagnosis, there is a requirement for specific constructive and efficacious strategies. Identifying solid tumors through targeted detection of cancerous cells is one of the most reliable approaches to treatment. Over the past decade, the investigation of folate receptors (FR) has led to the development of several novel cancer treatments. Many tumor types exhibit high overexpression of FR, which was found to be associated with tumor progression and prognosis. Monitoring FR expression levels can prove a useful criterion as it is correlated with the advancement and prognosis of tumors. Due to the strong affinity of the FR towards folate and folate-conjugates, it can be designated as a tumor-associated antigen that is involved in the active transportation of the bound cargo within cells through an endocytic process. A wide range of payloads, from tiny radioactive imaging agents up to massive DNA-containing formulations, may be delivered to FR-positive cells using folate or an analog of it as the ligand. The FR targeting anticancer therapies are being developed currently and the response-predictive aspect of FR expression could serve as a biomarker for these treatments. By boosting delivery to the target tissue as well as an improved target-non-target tissue ratio, targeted drug delivery systems aim to increase the therapeutic windows of molecules. In turn, this results in a decreased minimum effective dose of the drug, lower drug toxicity, and improved treatment efficacy at comparable plasma concentrations. Targeted delivery is especially appealing for drugs with small therapeutic windows and/or those activated at very low concentrations due to the few targeted receptor sites on the specified target tissue. This review discusses recent developments in FR-mediated targeting for therapeutics and its applications in imaging, as well as highlights their potential and anticipated challenges.

*Keywords:* cancer, folate receptor, folic acid, anticancer therapy, tumor marker, endocytosis

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## Introduction

Receptor-targeted drug delivery is affected by the constitution of the targeting ligand and the properties of the accompanying cargo and may offer the following two benefits over standard non-targeted therapies: both receptor-mediated targeting and receptor-targeted delivery have the potential to lessen collateral toxicity to normal tissues by (i) increasing the total absorption of the desired drug by the treated cells, and (ii) reducing drug deposition in non-pathologic cells. Because it can help accomplish these benefits, there is a spike in folate receptor (FR) use for various targeted drug-delivery applications. While it is yet to be confirmed whether FR-targeting significantly enhances the net uptake of low molecular weight chemotherapeutic compounds, except for some antifolates, it has been shown to increase intracellular delivery of attached biomolecular parcels. These parcels include proteins, liposomes, viruses, gene therapy vectors, antisense oligonucleotides, imaging compounds, polymeric drug carriers, and neutron activation agents [1-11].

There might be possibilities that these targeting ligands match the effectiveness of folate in efficiently delivering a few of the drugs discussed above, but FR controlled endocytosis still enjoys an edge that is difficult for rival antibody- and ligand-targeted technologies to match. As a result, cells often internalize antibodies, hormones, and other relevant ligands to remove them from the surface of the cells which means that as soon as a hormone has undergone signaling through its receptor, it must be relieved and managed in a way that it gets destroyed so to prevent uninterrupted signaling for ensuring the stability and life of the cell. Therefore, the majority of targeted ligands are absorbed and directed toward the lysosomes for apoptosis. As a consequence, the major cargo associated with folic acid is not transported to lysosomes, but rather discharged into the cytosol or stored in endocytic regions because it is required for critical cell functions [12, 13]. However, in none of these instances is a sizable portion digested or eliminated. In some cases, the linked cargo associated with the folate seems to be directly transported to the nucleus. This property is crucial for delivering macromolecular or hydrolytically sensitive medications as they are prone to inactivation due to digestion by various hydrolytic enzymes localized in the lysosomes [14].

Folic acid and its reduced byproducts are essential for eukaryotic cells in one-carbon transfer processes that contribute to the production of nucleotide bases. It is not surprising that cellular absorption of the vitamin is crucial for cell survival and growth. The absorption of folates by cells is facilitated by a low-affinity reduced folate carrier (Km 1 mM), which is present in almost all body cells, and a high-affinity

glycosylphosphatidylinositol-linked FR (KD 100 pM), which is more limited in distribution [15]. The FR is involved in the effective transportation of both folic acid and various kinds of cargo associated with folate. However, the reduced folate carrier helps only certain reduced forms of folic acid to travel (i.e., chemotherapeutics, liposomes, imaging agents, proteins, nanoparticles, etc.). Figure 1 shows how conjugated folates adhere to an FR on cell surface and aids in the release of initial quantity of the therapeutic load into the cytoplasm, which is also referred to as the final stage in the receptor-mediated absorption of folate drug ligand-conjugates [16]. As a result, some (but not all) folate conjugates are released from their receptors when the compartments of the endosome are acidified to a pH of 5 after the formation of endocytic vesicle following invagination of the membrane and internalization [17].

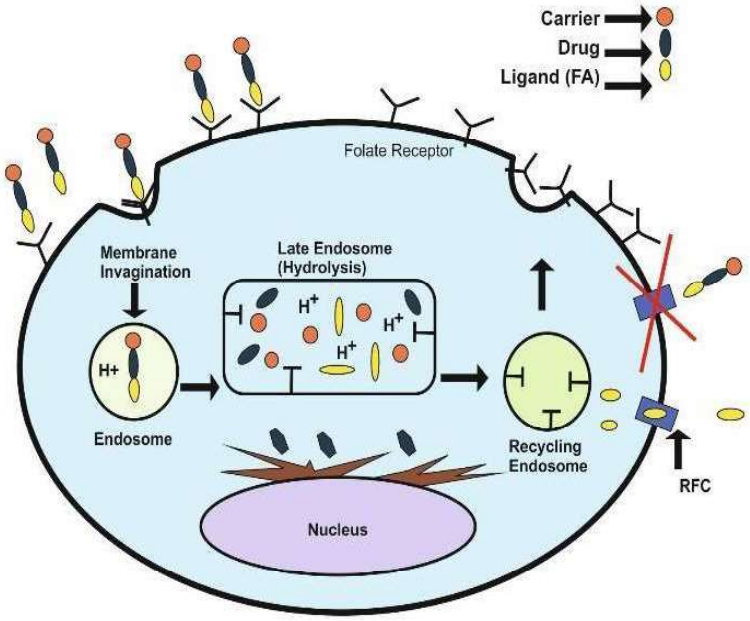


***Figure 1:*** *Mechanism of Folate Endocytosis.*

The separation of the membrane-bound FR from released conjugate/free medicines is subsequently made possible by the transportation of acidic components of the endosomal compartments for recycling. The endosome is observed to be breached by released folate conjugates by an unidentified mechanism, leading to drug deposition in the cytoplasm. The transport of more folate-linked medications into the cell is made possible via membrane-bound FR, which is mostly recycled and comes back to the cell surface. The recycling proportion of cancer cells fluctuates in real-time, from one cycle every 4 hours to one cycle every 12 hours, this recycling rate may be higher in other FR-positive cells. This introduction of hydrolytically sensitive items like genes and ribozymes into cells is made possible because only a few of the folate conjugates enter

lysosomes for degradation [18]. As mentioned previously, FR expression is minimal in healthy cells but is frequently abundant in cancer cells. In epithelial malignancies of the brain, nose, throat, mammary gland, lung, ovary, colon, and prostate, for instance, overexpression of FRs is observed [19, 20]. In instances of acute and chronic myelogenous leukemia i.e. hematological cancers of myeloid origin, FRs are also overexpressed [21, 22]. It has been found that FR expression and a tumor's grade and histological stage are strongly correlated. FR expression is typically significantly higher in highly undifferentiated metastatic tumors compared to localized, low-grade malignancies.

**Function of Folate**

**One-carbon metabolism**: The body's sole apparent use for folate coenzymes is to facilitate the transfer of one-carbon molecules. Folate coenzymes participate in several processes essential to the metabolism of nucleic acids and amino acids as donors and acceptors of one-carbon units.

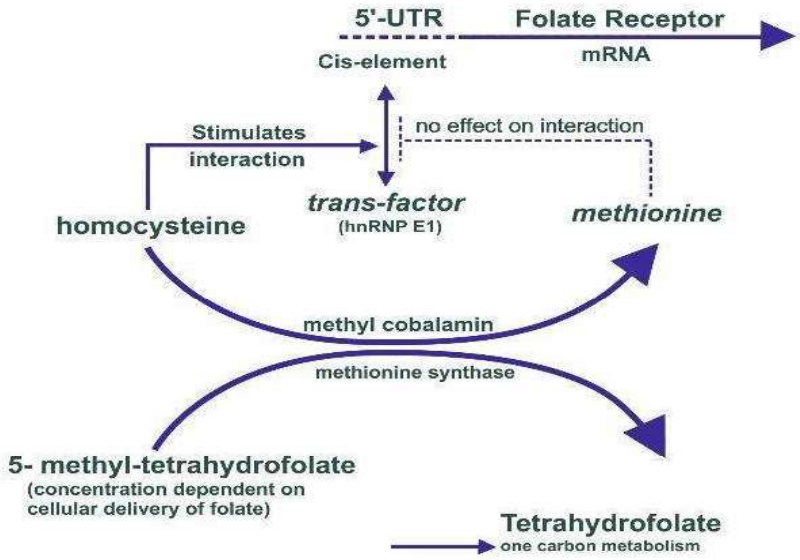


***Figure. 2:*** *The figure explains how disrupted folate metabolism and the concerted translational control of folate receptors influence each other.*

**Nucleic acid metabolism:** Through two distinct routes, folic acid coenzymes are essential for DNA metabolism. Folate coenzymes are required for the synthesis of DNA through its precursors. The biosynthesis of methionine requires a folate cofactor, and the production of S-adenosylmethionine depends on the formation of methionine (SAM). One carbon unit methyl group donor, SAM, is utilized in a broad range of methylation activities at the biological level, which includes the methylation of particular locations in several DNA and RNA. DNA methylation can be a crucial target for preventing cancer as it plays an important role in the same.

**Amino acid metabolism:** The body requires the assimilation of several significant amino acids which is achieved by successful metabolism in the cells, which calls for the use of folate coenzymes. A folate coenzyme and a vitamin B12-dependent enzyme are the two essential requirements for the production of methionine from homocysteine. The thymidylate synthase reaction step is responsible for the detrimental effects of folate deprivation that are evident in the fast-increasing red blood cell metabolism. Henceforth, there is a direct correlation between the disturbed levels of folate metabolism and the control of folate receptor expression (Figure 2). As in cancer chemotherapy, when there is a need to inhibit rapidly growing cells, dihydrofolate reductase (DHFR) is inhibited.

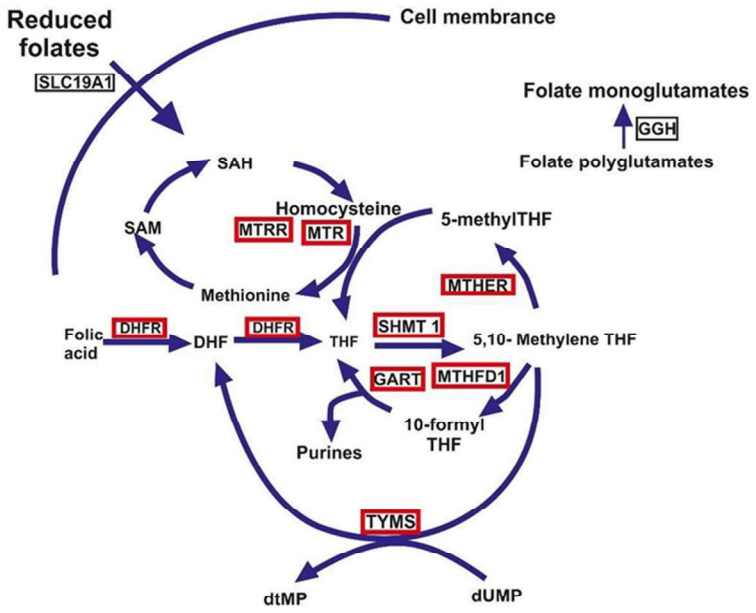


***Figure. 3:*** *Folate Metabolism.*

**Folate Metabolism**

One-carbon molecules need to be transported to various metabolic destinations, and folic acid acts as a carrier. A variety of oxidation states exists for this one-carbon component, and from a clinical perspective, methyl-tetrahydrofolate (methyl-THF) and methylene-THF are the two most significant forms of folic acid. The relevance of these chemical forms and the sequence of the metabolic pathway experienced by these intermediates are depicted below. The various reactions are then interconnected to highlight the main one-carbon transfer processes. The first step in the folate metabolism pathway involves the transfer of the methyl group from serine. The synthesis of 5, 10-methylene-THF from THF is mediated by the transfer of the methyl group from serine to glycine (Figure 3). DHF and THF

obtained from natural sources are then polyglutamated and are required to be enzymatically transformed to mono glutamate to be absorbed from the small intestine. In another approach, 5, 10-methylene-THF can be transformed into 5, 10-methenyl-THF, and finally, 10-formyl-THF.

For the reasons listed, vitamin-based drug delivery systems offer significant benefits over other drug delivery methods, such as monoclonal antibodies. The effective delivery of the conjugate into the tumor is possible only due to the Folate's relatively lower size, (molecular weight: 441) whereas typical monoclonal molecular weights exceeding 150,000 makes it simpler to reach cancer cells. The possibility that the folate drug combination will attach to the receptor is increased since the folate affinity for its receptor on cancer cells is 100x higher than when the affinity for monoclonal antibodies is compared. A significant quantity (20–60 million) of folate drug conjugates can be introduced into the cell in 4 hours because the folate receptor on cancer cells can be easily recycled. The lysosomes inside the cell do not break down folate conjugates. Cells possess compartments called lysosomes that break down molecules to eliminate potentially hazardous compounds. Lysosomes do not attack folate conjugates because folate is transported into the cell to be consumed rather than destroyed. Folate is a natural chemical that does not cause the body to launch an immune reaction, which is crucial in the availability of various types of medications i.e. drug delivery, consisting of several genes and proteins. Consequently, it may be given more than once. However, unless they are humanized (a costly procedure), monoclonals cause a probable response of the immune system that prevents the immature meetup of antibodies with the cancer cell. Last but not least, folate is easily obtainable, simple to make, stable during synthesis and storage, and is known to be a crucial constituent of a large number of medications.

**Folate Receptor as a Tumor Marker**

The folic acid (FA) and folic acid receptor (FR) appear to have a 1:1 stoichiometry and the latter possesses a single-chain glycoprotein structure on the cell surface. This structure mediates a relatively high visible affinity binding and the absorption of the vitamin by receptor-mediated endocytosis. The primarily circulating folate coenzyme, (6S)-5-methyltetrahydrofolate, and several antifolate medications all bind to the folate receptor. The Folate receptor has been identified, described, and cloned in three different isoforms. Several isoforms of FR have been identified from Human (i.e. hFR-α, hFR-β, and hFR-γ) and Murine (i.e. mFR-α and mFR-β) sources.

A variety of Folate receptor structures consists of 16 cysteine residues conserved sequences that share approximately 70% sequence identity and are termed isoforms. These FR

isoforms are all associated with prominent functions. With Kd values of 10-10 mol/L and 10-9 mol/L, respectively, FR-α and FR-β demonstrate remarkably comparable affinities for FA despite the difference in their carboxy-terminal sequences. However, the stereo-specificities of FR-α and FR-β for reduced folate coenzymes are different, with FR-α having a 50-fold greater affinity than FR-β for the physiological (6S) dia-stereo-isomer of N5-methyltetrahydrofolate [15, 21, 23]. Importantly, even the FR's α-isoform binds FA ten times more strongly than any of the vitamin's more reduced forms do.

There is an uneven distribution of FR isoforms throughout the body's numerous tissues and cell types. Instead, FR-α is mostly expressed on healthy epithelial cells and is overexpressed in cancerous tissues made of the same cell types. The Folic acid receptor is considered a preferred target for the delivery of tumor-specific drugs because:

(1) The levels of the Folic Acid receptors are elevated in a variety of human cancers, such as brain, breast, myeloid cell, lung, kidney, and ovarian. (2) The folate receptor is located on the membrane facing the external apical face of the polarized epithelia, which can severely restrict access to it in normal tissues that express it.

(3) The density of the Folate receptor is contemplated to be an efficient biomarker since its level increases along with the cancer progression. Folate-linked therapies can therefore be used to target malignancies that are hardest to cure using traditional means. Folic acid is found to be coupled with both medications of low molecular weight and macromolecular complexes as means of directing the molecular conjugates to cancerous cells to take advantage of these characteristics of folate receptor expression. In vitro studies have revealed that adding folic acid to macromolecules improves their delivery to cancer cells that express the aforementioned folate receptors.

**FR as a diagnostic and therapeutic target**

A plethora of advantages can be enlisted for using the FR as a diagnostic and therapeutic target [24, 25]. In the majority of instances of proliferating non-tumor tissues, FRα is found to be not available or inaccessible to circulatory pathways as it is located on the luminal surface of epithelial cells. In contrast, in the cases of malignant tissues, FRα is accessible through circulation as it is expressed all over the cell. FR carries the potential capability of binding to a molecule of small and comparatively innocuous nature, i.e., folic acid that forms chemical conjugation with other molecules and is known to significantly infiltrate the developed solid tumors. Once FR is bound to a folate conjugate, it gets internalized into the cell, and the FRα is recycled rapidly to the surface of the cell via the FR-mediated endocytic pathway [25, 26].

**Folate-conjugated nanoparticles in the detection and treatment of tumors**

The principal application of FR in recent nanotechnology breakthroughs is the development of imaging techniques for detecting tumor localization and therapeutic drugs. Folate-conjugated IO characteristics for the treatment of intracellularly localized hyperthermia of solid tumors that are abundant in FR are being investigated [28]. Other recent methods that have been tested in vitro and make use of nanoparticle technology include the use of iron oxide nanoparticles [27], fluorescent silica nanoparticles conjugated with folate [29], hydrophobic nanocrystals [30], destruction of cancerous cells by near-infrared agents that are transported via folate-containing carbon nanotubes [31] and lipoprotein-based nanoplatforms that alters the de novo route taken up by the lipoproteins from their normal receptor to the cancer-associated FR i.e, reroutes it [32].

**Customized cancer therapies utilize the role of FR expression**

Certain tumor subtypes express FR, which may be useful as a biomarker for a therapeutic approach that is FR-directed as a component of a tailored strategy toward effective treatment. The creation of reliable, relevant, and usable measuring assays is necessary for the assessment and confirmation of tumor biomarkers [33]. Numerous semiquantitative and quantitative techniques are required for the evaluation of FRα levels in tumor biopsy tissue samples. But many such methods have inherent difficulties, especially in a therapeutic situation [34-41].

The in vivo use of an imaging agent that acts as a companion, which enables the noninvasive, whole-body, real-time evaluation of FRα expression, is an appealing alternative for making it easier to monitor FR status throughout the treatment without the involvement of invasive procedures for obtaining tissue biopsies. The expression of FR in tumors can vary because the molecular features of malignancies might alter over time [42-44]. The link between folic acid and FR binding provides a valuable carrier that enables probes involved in imaging to be targeted to FR-expressing cells. The probe exhibits high-affinity binding towards the FR upon conjugation with a folic acid molecule, which results in the emission of an imaging signal. Magnetic resonance imaging, fluorescence imaging, single-photon emission computed tomography (SPECT), computed tomography, positron emission tomography (PET), and ultrasound imaging is the currently available techniques that may identify probe/folic acid conjugates. Etarfolatide (EC20), one of the several folate conjugates that have been examined from the perspective of tumor imaging, is of special interest [45-52]. A short and linear folate-linked peptide and 99mTechnetium (Tc) make up the imaging agent complex etarfolatide [53]. A typical radiographic tracer is 99mTc. After being introduced into the bloodstream, the folic

acid complex localizes to tissues that express FR and attaches to the FR with a high affinity. 99mTc-etarfolatide is highly absorbed by FR-positive tumors (17% of the injected dose per gram of tissue) and rapidly accumulates within the target tissue [45]. To see the expression of FR, utilize SPECT [54] the kidneys filter out extra probe conjugates. This lessens the general background and makes it possible to acquire the photographs rapidly [45].

**Targeting FRα Imaging and theranostics**

The field of cancer imaging is exploring FRα and its tumor specificity. Many alternate approaches have been developed and this has efficiently reinforced the imaging of FRα-positive tumors, for this FRα-targeted contrast-enhanced MRI has been explored widely. For example, folic acid is attached to a polyamidoamine dendrimer to develop a folate-conjugated dendrimer polychelate. As a result, there is an improvement in contrast enhancement relative to non-targeted contrast agents. This causes targeted tracers to accumulate in FR-expressing tumors [55]. A folic-acid-derived molecule combined with a carboxylate-containing oxide of iron exhibited high retention in FR-positive tumor cells in a study involving breast cancer cell lines and xenograft models [56]. Combining heparin-folic acid micelles and superparamagnetic iron oxide nanoparticles produced identical results [57]. Radiolabeled folate derivatives are also currently being researched. 111In-diethylenetriaminepentaacetic acid-folate was delivered in phase I/II trial to get a full-body single-photon CT emission image of women diagnosed with endometrial cancer or ovarian cancer. When combined with other radiography techniques, the radiotracer concentration present in all probable malignantly developed lesions demonstrated good levels of sensitivity for the identification of malignancy [50]. In xenograft models of mice with ovarian cancer exposed to radiolabeled mirvetuximab soravtansine (an FR that targets ADC), an antibody-based companion diagnostic molecule, 89Zr-DFO-M9346A, is being employed. Up to this point, this drug has shown a distinct level of encouraging tumor-to-background resolution in this animal [58]. The intraoperative imaging of tumors in mouse models has been possible for more than ten years thanks to non-radiolabeled techniques like fluorescent probes connected to folate [59]. These methods must be optimized appropriately to help surgeons do better resections and enable visible improvements in patients with FR-expressing tumors, such as those with lung, ovarian, and breast malignancies [60–62].

**Conclusions**

The FR is involved in the development and progression of cancer to a great degree. As a result, therapies that target FR require dependable methods for detecting tumors that are positive for

FR to assist in identifying individuals who are candidates for treatment. The folate receptor is emerging as a potentially effective diagnostic and therapeutic target in cancer treatment. In many cancerous cells, folate receptors found on the cell surfaces are overexpressed to a high degree. The overexpression can be exploited by therapeutic agents to target malignant tissues through various pathways directly. Even if treatments that make use of FR have shown to be effective, it is still necessary to conduct additional research and testing on such treatments in human beings.

One of the most important things that need to be done is to research the right amount of dosage and the possible long-term consequences of using nanoparticle-based drug delivery as the treatment approach for cancer. For patients who take folic acid supplements, there is a need for additional research into the interactions that can occur between the effects of folate and antifolate. Countries that already fortify their meals with folic acid should also consider this recommendation. The successful utilization of folate conjugates highlights the promising potential of these receptors for cancer management.

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