Folate receptor targeted drug delivery- from the bench to the bedside

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Abstract: Targeted drug delivery is a promising strategy for improving tumor therapy. Folate receptor (FR) is an established ovarian cancer marker that is also frequently found to be overexpressed in other major epithelial tumors. Meanwhile, functional FR-β is expressed in myeloid leukemias and in inflammatory macrophages, including tumor-associated macrophages. FR based tumor targeted drug delivery has been a very active area of research and recent advancement of this technology towards clinical translation is highly significant. FR targeting can be achieved via anti-FR antibody or by folate conjugation, each with distinctive advantages and pitfalls. Several monoclonal antibodies directed against FR-α have been developed. In addition, a large number of imaging agents, drug molecules, and drug carriers have been conjugated to folate for targeting the FR, mostly for targeting FR-α found on the surface of epithelial tumor cells. Only recently, however, have FR-targeted agents moved into Phase III clinical trials, exemplified by the monoclonal antibody farletuzumab and the low molecular weight folate conjugates, folate-¹⁸⁵⁵Tc (EC20) and folate-desacetylvinblastinelatex (EC145), representing each of the two competing strategies for targeting the FR. This review will focus on the activities related to clinical translation of FR-targeted agents. The reader will gain a better understanding of the landscape of clinical translation of this exciting technology and key findings in the clinical trials. The anticipated approval of these agents and the ongoing clinical development of several other folate conjugates should usher in a new era of clinical translation and validation of FR-targeted imaging and therapeutic agents for tumor imaging and therapy.

Keywords: Folate receptor; cancer; leukemia; targeted drug delivery; liposomes.

Introduction

Nowadays, various nanotechnologies, such as nanoparticles, liposomes, and nano-devices, have been widely used in drug delivery, and one hot topic for nanotechnology-based drug delivery is targeted delivery (1-9). Folate is a water soluble vitamin (vitamin B9) which is extensively exploited in targeted drug delivery. Reduced folates are generally transported across the cellular membrane, has K values in the μM range (17-18). Reduced folates are generally transported into cells through the RFC and are subsequently retained in the cytoplasm by polyglutamylammonium by the folyl-polyglutamylation synthase. RFC, however, has limited interaction with folate derivatives due to its relatively low affinities for the oxidized form of folate, such as folic acid, and its inability to transport them across the cellular membrane. In contrast, FR-α binds folate derivatives with high affinity and mediates their internalization by endocytosis via a pathway that is also associated with receptor recycling. FR-α has been shown to be overexpressed in various types of cancers and, therefore, has been studied extensively as a tumor cellular surface marker for targeted drug delivery. FR-targeted agents have received the widespread attention because they can decrease drug toxicity and enhance therapeutic effects. Folate targeting shows considerable promise for the development of tumor-specific therapeutic agents. FR-targeted drug delivery has been discussed in a number of recent review articles (19-21). This article will attempt to summarize important work in the literature on this topic with emphasis on recent development in the clinical translation of FR-targeted agents.

FRs as cellular markers

Expression of either isoform of FRs is highly restricted among human tissues. Among normal tissues, FR-α expression is mostly limited to the apical plasma membrane of the kidney and certain epithelial tissues. This may render them kinetically inaccessible to FR-targeting agents in the blood, unless there is transepithelial transport, which occurs in the kidney for molecules under 40 kDa, and/or epithelial cell depolarization, which frequently occur in the tumor. In addition, FR-α expression is frequently amplified in many epithelial lineage cancers, making it a useful biomarker for tumors (15,22-24). In particular, FR-α has been identified as a marker for non-mucinous ovarian carcinomas, of which over 90% show overexpression (22,25-26). Other cancer types such as endometrial, lung, colorectal, pediatric ependymomas, mesotheliomas, and renal cell carcinomas also show FR-α expression (23,27-30). Interestingly, elevated FR-α expression has also been shown to be a negative prognostic factor for breast and endometrial cancers (22,31). High FR-α expressing tumors have more rapidly proliferation and are
more resistant to therapy. FR-β, which shares ~ 70% sequence homology with FR-α, is most frequently found in a non-folate-binding isomorph on normal granulocytes, possibly due to post-translational modification. Meanwhile, functional FR-β is found in myeloid leukemias and in activated macrophages associated with inflammation and tumor (32-34). Therefore, FR-β is potentially used as a marker for myeloid leukemia, tumor-associated macrophages, and chronic inflammatory diseases, such as rheumatoid arthritis. The tissue selective expression of the FR isoforms suggests their potential utility as cellular markers for targeted delivery of imaging and therapeutic agents to cancer, leukemia, and chronic inflammatory diseases (34-39).

There are two general strategies for targeting the FR. The first is based on anti-FR antibody and the latter based on folic acid as a high affinity receptor ligand and that folate conjugates retain high affinities for the FRs (Figure 1). Significant progress has been made following both types of strategies.

**Immunotherapy based on FR-α specific antibody**

Several anti-FR-α antibodies have been developed for therapeutic targeting of FR-α positive tumors (40). MOv18 and MOv19 are murine monoclonal antibodies recognizing two non-competing epitopes of FR-a, which have been developed by Miotti et al. (41-43). A chimeric version of MOv18 (c-MOv18) has been studied in ovarian cancer for immunotherapy as a therapeutic antibody (44-45). Other variants of MOv18 include a bi-specific antibody targeting FR-α and CD3 (a T-cell marker) for T-cell therapy, and I-131 or At-211 radiolabeled antibody for radioimmunotherapy (46-47). More recently, another monoclonal antibody, farletuzumab, aka MORab-003, has been developed by Morphotek Inc., currently a subsidiary of Eisai Inc. (48-49). It is a fully humanized antibody derived from murine antibody LK26 developed at Memorial Sloan-Kettering Cancer Center. Farletuzumab is currently being studied in a Phase II clinical trial for recurrent platinum-resistant ovarian cancer (50-54), either alone or in combination with platinum/taxane chemotherapy. In preclinical studies, farletuzumab has been shown to elicit robust antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and to inhibit ovarian tumor xenograft in nude mice. In a Phase II clinical study, a majority of the patients treated with farletuzumab showed tumor regression or stable disease, had normalized CA-125 levels, and 20% of the patients showed a second remission that was as long as or longer than the first. These clinical data were very promising and could ultimately lead to the approval of the first FR-targeted antibody therapy for cancer. In addition, In-labeled MORAb-003 has shown promise as an agent for radioimmunoscintigraphy and radioimmunotherapy (55). In 2011, Morphotek announced the initiation of a farletuzumab Phase II study as first-line treatment of non-small cell lung cancer (NSCLC) (48,56).

The advantage of the anti-FR antibody strategy is that tumor targeting is not affected by the level of FR saturation by folate in the circulation and that the antibody is presumably FR subtype specific. The drawback of this strategy is that antibodies are macromolecules characterized by an extended systemic circulation time and slow kinetics of extravasation and clearance. This reduces the achievable target-to-non-target tissue ratio, and may elicit a host immune response in the form of a neutralizing antibody that may ultimately undercut its therapeutic efficacy.

**Folate conjugates as FR-targeted therapeutics**

Folic acid retains high affinity for FRs following derivatization via one of its two carboxyls. Folate conjugation, therefore, presents another strategy for FR-targeted drug delivery. This method has been successfully applied to small molecules, macromolecules, and nanocarriers (57-63). For nanocarriers, which include liposomes, polymeric nanoparticles, and various types of nucleic acid vectors, the drug carrier typically is conjugated to folate, usually through a PEG linker to overcome steric hindrance surrounding the FR on the cell surface (64-68). Among macromolecules, folate has been conjugated to protein toxins (69), antibodies (70-75), polymeric drug carriers (76-80), and prodrug converting enzyme (81). Clinical translation activities, however, have so far been focused on low molecular weight (MW) folate conjugates and have been carried out at Endocyte Inc. These agents, including imaging agents, haptens (for immunotherapy), and chemotherapy agents, are characterized by rapid tissue distribution and fast systemic clearance, thus resulting in very high tumor-to-background tissue targeting ratios. Meanwhile, due to the renal clearance of folate conjugates and the expression of FR-α in the apical membrane of the renal proximal tubules, low MW folate conjugates have shown persistent accumulation in the kidneys. Endocyte has developed a series of products based on folate conjugates at various stages of preclinical development or clinical trial for cancer imaging and therapy (Table 1). For FR specific antibody, studies have shown that 111In-labeled MORAb-003 is an promising agent for radioimmunoscintigraphy and radioimmunotherapy. For folate conjugates, EC20 currently is to enable pre-selection of patients that are highly FR+, so EC20 imaging may be useful for predicting therapeutic response to chemotherapy in general. It is important to note that these folate conjugates target both FR-α and FR-β and FR binding is competitively inhibited by excess free folate.

**FR-targeted imaging agents**

111In-DTPA-folate was the first FR-targeted low molecular agent to enter clinical trial, for non-invasive imaging of recurrent ovarian carcinomas (82-84). Due to the relatively long (2.8 days) half-life and high cost of 111In, a 99mTc
Table 1. FR-targeted drugs that have been studied in clinical trials.

<table>
<thead>
<tr>
<th>Technology Platform</th>
<th>Agent</th>
<th>Trial Stage</th>
<th>Key References</th>
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<tr>
<td>FR–α specific antibody</td>
<td>MOv18</td>
<td>Phase I/II</td>
<td>24</td>
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<tr>
<td></td>
<td>cMOV18</td>
<td>Phase I</td>
<td>27-28</td>
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<tr>
<td></td>
<td>Farletuzumab (Morab-003)</td>
<td>Phase III</td>
<td>31-32</td>
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<tr>
<td>Folate Conjugates</td>
<td>EC20 (Folate-Scan)</td>
<td>Phase III</td>
<td>68-70</td>
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<td></td>
<td>EC145</td>
<td>Phase III, Phase II</td>
<td>59-64</td>
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<tr>
<td></td>
<td>EC0489</td>
<td>Phase I</td>
<td>89</td>
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<td></td>
<td>EC17/EC90</td>
<td>Phase I/II</td>
<td>90</td>
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<tr>
<td></td>
<td>EC0225</td>
<td>Phase I</td>
<td>65</td>
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(half-life 6 hr)-based imaging agent is much preferred, especially given the rapid clearance kinetics of a low MW folate conjugate. Accordingly, ⁹⁹ᵐTc-EC20-folate (Folate scan), which was based on a folate derivative of a small Tc chelating peptide, was synthesized and evaluated clinically as a diagnostic imaging agent for solid tumors (based on targeting FR-α) and for imaging of chronic inflammatory diseases such as rheumatoid arthritis and osteoarthritis (based on targeting FR-β). Rather than diagnosis, the primary purpose of EC20 currently is to enable pre-selection of patients that are highly FR+ and thus constitute the best candidates for FR-targeted therapy (Figure 3). In the treatment, platinum resistant ovarian cancer patients are first given EC20 for FR imaging. This process will identify patients with highly EC20 positive tumors with high FR expression. So these patients are then given EC145 as an FR-targeted therapy in conjugation with Doxil. This will play a better curative effect. EC20 has been a component of > 13 clinical trials in > 500 patients with ovarian, endometrial, renal, pituitary, pulmonary cancers, and has been proven valuable for predicting response to FR-targeted chemotherapy. It should also be noted that FR expression level has also been identified as a prognostic factor for the biological aggressiveness of ovarian and breast cancers. Therefore, EC20 imaging may be useful for predicting therapeutic response to chemotherapy in general.

FR-targeted chemotherapy agents

The first folate conjugated cytotoxic agent to be evaluated in tumor therapy was a maytansinoid conjugate (85). Since then a series of chemotherapy agents have been conjugated to folate for FR targeting, with varying degree of success. These include folate conjugated 5-fluoro-2’deoxyuridine-5’-O-monophosphate 10-mer (86), carboplatin(87), paclitaxel, and several microtubule poisons (88). Key requirements for this approach to work are that the folate conjugate has to be water soluble and that the conjugate must be degraded inside the cell to generate the active drug. The general principle of folate conjugate design is shown in Figure 4A. This means a hydrophilic (typically ionic) and pH and/or enzyme cleavable linker would be needed between folate and the drug moieties. In addition, the drug moiety must have very high cytotoxicity, with an IC₅₀ value in the sub-nanomolar range for the FR targeting strategy to work. This is because there are typically a very limited number of copies of the FR on the surface of each targeted tumor cell. Several conjugates have recently been taken into clinical trial by Endocyte.

EC131 is a folate conjugate of a maytansine with a hydrophilic linker that increased its solubility (85,89). EC140 and EC145 (Figure 4B) are folate conjugates of desacetylvinblastine monohydrizine (DAVLBH), which is a derivative of vinblastine, a microtubule destabilizing

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Figure 2. Structure of EC20.

Figure 3. Stratification of patients based on FR expression level.

Platinum resistant ovarian cancer patients are first given EC20 for FR imaging. This will identify patients with highly EC20 positive tumors, thus having high FR expression. These patients are then given EC145 as an FR-targeted therapy in conjugation with Doxil.

Figure 4. Structure of folate-conjugated chemotherapy agent. A. General design of a therapeutic folate conjugate. The spacer is typically designed to be highly ionized to increase the solubility of the whole molecule. The cleavable segment can be sensitive to low pH, lysosomal enzyme, or the reducing intracellular environment. The drug should be a highly potent agent with sub-nanomolar cytotoxicity. B. Structure of folate-desacetylvinblastinehydrazide (EC145), currently in Phase III clinical trial.
agent (90-96). A hydrophilic linker based on Asp-Arg-Asp-Asp-Cys was incorporated in these conjugates. Of these two, EC145 (Figure 4) showed better activity than EC140. This was possibly due to a higher intracellular cleavage rate of the linker in the former. EC145, therefore, has been selected as the first folate-drug conjugate to enter into clinical trials. This drug has completed a Phase II trial in which it was used in combination with PEGylated lipo-somal doxorubicin (Doxil) on platinum-resistant ovarian cancer patients. The results showed an 85% (2.3 month) increase in median progression-free survival (PFS) in the total population and a 260% (4 month) increase in PFS in the subset of patient that had high FR expression based on imaging study using EC20. This agent is now in a multi-centered Phase III trial.

Following EC145, several additional agents have also entered clinical trial recently. EC0225 is a folate conjugated to two different drugs(97), a vinca alkaloid and mitomycin. BMS-753493 is a folate conjugate of epothilone A, a microtubule stabilizing agent (98-100). Other agents that have reached clinical trial include EC0489, an analog of EC145(101), and EC17(102), a folate conjugate of a hapten, which is an immunotherapy agent used in conjunction with preimmunization of the patients against the hapten.

There are several advantages of an FR-targeting based on a low MW folate conjugate compared to the strategy based on an anti-FR antibody. Firstly, a low MW folate conjugate is rapidly distributed into tumor tissues and is rapidly cleared from systemic circulation, reducing concentration in the non-target tissues. This typically leads to a much better tumor-to-normal tissue ratio. Secondly, folate conjugates are capable of targeting both FR-α and FR-β. This potentially enables the targeting of tumors that are low in FR-α and high in FR-β (e.g., tumors that are highly infiltrated by tumor-associated macrophages that are FR-β+), as well as chronic inflammatory diseases such as rheumatoid arthritis and osteoarthritis. Thirdly, in contrast to antibodies, the low MW conjugates are typically not immunogenic and are not subject to denaturation and the associated loss of biological activity. Finally, it is possible to produce these agents by total synthesis as a single chemical entity. These disadvantages of folate conjugates include potential interference of FR targeting by circulating folate, which may be influenced by a patient’s diet, and the high degree of accumulation in the kidneys due to FR–α expression in the apical membrane of the proximal tubules. Despite the claim by some that low MW folate conjugates actually by-pass the kidney, the high level accumulation of folate-based radiopharmaceuticals, e.g., EC20 (103-105) is a strong indication that kidney uptake of the folate conjugates are very high. Furthermore, the accumulation of these conjugates in the kidneys has been found to be persistent rather than transient. While this fact alone does not prevent clinical translation of folate conjugates that inherently have limited renal toxicity, this may reduce the prospect of clinical application of low MW folate conjugated radiopharmaceuticals for radiotherapy. It is worth noting that, despite obvious concerns over renal toxicity, FR–α in the kidney did not mediate severe renal toxicity in the completed clinical trials on EC series chemotherapies. Finally, the lack of FR subtype specificity of folate conjugates can also be viewed as a negative if such specificity is desired. There are ongoing studies that are aimed at developing subtype-specific conjugates that are based on folate analogs rather than folic acid itself.

FR-targeted immunotherapy – FolateImmune

FolateImmune is a combination of EC17, a folate-fluorescein (as a hapten) conjugate, and EC90, a fluorescein-keyhole limpet hemocyanin (KLH, a carrier protein) conjugate, and an adjuvant GP1-0100. It has been studied in a Phase 1b study in patients with refractory or metastatic cancer. The patients were first vaccinated with EC90’adjuvant, and were treated with EC17, along with low dose interleukin-2 and interferon-α. EC17 is designed to induce anti-hapten antibody mediated antibody-dependent cellular cytotoxicity and/or phagocytosis (85, 90). This approach has certain potential advantages over the farletuzumab strategy since EC17 which “activates” the therapeutic mechanism is a small molecule, therefore is very inexpensive and is rapidly clearing, thus can potentially achieve high tumor-to-normal targeting ratios.

Expert Opinion

FR-targeted drug delivery is one of the most studied strategies in the field of drug delivery. A large number of drugs and drug carriers have been conjugated to folate for FR targeting. But some obstructions must still be overcome before its wide application. In the clinical treatment, FR screening using 99mTc-etarfolatide scintigraphy of patients is a very promising tool to select patients who will benefit from the specific therapeutic agents. Despite promising data obtained in in vitro and in vivo studies, there are only isolated examples of clinical translation. This is because preclinical studies generally involve the use of cell lines and animal tumor models characterized by very high FR expression levels, such as KB cells. The requirement for clinical translation must take into account the moderate level of FR expression found in clinical situations. The recent movement of farletuzumab and EC145 into Phase III trial suggests that FR targeting is finally nearing a clinical reality. With that one can anticipate the approval of the first FR-targeted drug in the not too distant future. Meanwhile, there are remaining issues that warrant continued research, such as the problem of FR isoform selectivity and undesirable accumulation of low MW folate conjugate in the kidneys. Looking forward, I fully anticipate more and more FR-targeted drugs to enter the clinical pipeline as either monotherapy or as a therapeutic combination with existing therapy. FR-targeting will, therefore, become a prime example where the combination of strong scientific rationale meets sound developmental strategy, ultimately leading to success in the clinic.

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