Folic Acid Conjugates for Nuclear Imaging of Folate Receptor–Positive Cancer

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The folate receptor (FR) is overexpressed on a variety of tumor types, whereas its distribution in normal tissues and organs is highly limited. Exploration of the utility of the FR revealed its promising potential for targeting with folate-based radiopharmaceuticals. Herein, we report the principle of the FR-targeting strategy and summarize the development of several folic acid radioconjugates useful for SPECT and PET of cancer diseases. The potential applicability of folate radiopharmaceuticals for FR-targeted radionuclide therapy is also discussed.

Key Words: folic acid; folate receptor; SPECT; PET; cancer

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Because the availability of efficient and reliable tools for non-invasive diagnosis of diseases is crucial for their management and, thus, for the improvement of a patient’s quality of life, identification of targets that are specifically associated with diseased cells is of primary interest. In this respect, the folate receptor (FR) has been intensively studied over almost 2 decades because of its frequent overexpression in cancer cells and its ability to bind and internalize folic acid and conjugates thereof (1).

Folates and folic acid in its oxidized form are water-soluble vitamins of the B-complex group that are exogenously required for optimal health, growth, and development. Folate vitamins act as cofactors for enzymes that are involved in the biosynthesis of DNA and RNA, the amino acid metabolism and epigenetic processes. Thus, folates play a key role for cellular survival and proliferation, whereas impairment of the folate-dependent systems causes several pathophysiologic conditions. Because the hydrophilic nature of folates precludes passive diffusion through the plasma membrane, efficient transport mechanisms are necessary to allow cells the uptake of these essential nutrients. In normal cells, transport is accomplished primarily through the reduced folate carrier (2) and the proton-coupled folate transporter (3). The third uptake system is the high-affinity FR, a glycosyl phosphatidyl inositol–anchored glycoprotein (38–45 kDa) that binds preferentially folic acid (K₄d ≈ 10⁻⁹ M) and 5-methyltetrahydrofolate and is internalized via endocytosis (4).

In healthy tissues, FR expression is restricted to the lungs, the kidneys, the placenta, and the choroid plexus, where it is confined to the apical surface of polarized epithelia (5). Importantly, the FR is often present in large numbers on epithelial cancers, including tumors of the ovary, cervix, endometrium, lung, kidney, breast, colon, and brain (5,6). Investigations of a variety of FR-positive cancer types revealed that of all the types tested, those of ovarian origin displayed elevated FR levels most frequently (5). The FR is also expressed on hematopoietic malignancies of myeloid origin, including chronic and acute myelogenous leukemias (7). Other tumors, such as sarcomas, lymphomas, pancreatic and testicular cancer, and cancer of the bladder, prostate, and liver, do not commonly upregulate the FR (5).

PRINCIPLE OF FR-TARGETED CANCER RADIOIMAGING

The concept of the FR-targeting strategy makes use of the vitamin folic acid as a molecular Trojan horse for selective delivery of attached probes to FR-expressing cancer cells (Fig. 1). Compared with other targeting agents, such as monoclonal antibodies or peptides, folic acid offers several advantages. It is small (441 Da), stable over a broad range of temperatures and pH values, and thus amenable for site-specific chemical modification. It is inexpensive, nonimmunogenic, and binds to the FR with high affinity even after conjugation to a diagnostic or therapeutic cargo.

Because folic acid–targeted imaging agents can serve as non-invasive diagnostic tools to assess the location and severity of FR-positive cancer, a variety of folic acid–conjugated imaging agents have been developed and evaluated in vitro and in vivo. Folic acid conjugates of probes for optical imaging, MRI, and nuclear imaging by SPECT and PET are reported in the literature (Fig. 1) (8,9). Because of the outstanding features of SPECT and PET (e.g., high sensitivity) the overall usage of nuclear medicine procedures is expanding rapidly. Thus, recently, folate-based SPECT and PET tracers have attracted the greatest interest.

FOLIC ACID RADIOCONJUGATES

Radiofolates for SPECT and Potential Therapy

One of the first designs of a folic acid radioconjugate for SPECT used deferoxamine for chelation of the γ-emitting radioisotope ⁶⁷Ga (10). Tumor targeting was successfully achieved with ⁶⁷Ga-deferoxamine–folate in mice bearing FR-positive tumor xenografts (~8.5 percentage injected dose per gram [%ID/g], 4 h after injection). However, significant hepatobiliary excretion of the tracer led to unfavorable abdominal accumulation of radioactivity (11). With the aim of

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designing a more hydrophilic tracer, a folic acid conjugate with a diethylenetriamine pentaacetic acid (DTPA) chelator has been developed for radiolabeling with the SPECT isotope $^{111}$In ($\gamma$-radiation; energy $[E] = 171$ keV, 245 keV, half-life $[t_{1/2}] = 2.8$ d). $^{111}$In-DTPA-folate was found to clear about 97% through the kidneys, with negligible uptake in the peritoneal cavity ($^{111}$In-DTPA-folate was complexed by a short folate-linked peptide (Cys-Asp-Dap-o-Glu-Pte) (Fig. 2) (I5). The in vivo experiments of $^{99m}$Tc-EC20 were performed on a BALB/c mouse model with syngeneic FR-positive M109 tumors. On the basis of excellent preclinical data that showed a high tumor uptake (~17.7 %ID/g, 4 h after injection) and elimination primarily via the kidneys, $^{99m}$Tc-EC20 was introduced into the clinic, where it has been used to image several hundred patients to date. In one clinical trial, imaging with $^{99m}$Tc-EC20 was performed on 155 patients with a variety of solid tumors (I6). The results demonstrated that 68% of the patients showed uptake of $^{99m}$Tc-EC20 in their tumors. Accumulation of $^{99m}$Tc-EC20 was also noted in the kidneys and bladder of almost all patients, consistent with the expression of FRs on the proximal tubules of the kidney and the urinary excretion of the tracer. Mild to marked uptake of radioactivity in the liver was blockable by inhibitors of organic anion transport, suggesting mechanisms other than FR binding (I6). The authors concluded that $^{99m}$Tc-EC20 imaging is a safe, noninvasive procedure that may identify FRs in recurrent or metastatic diseases without the need for biopsy to identify patients who may benefit from treatment with FR-targeted therapy.

A completely different strategy was approached while developing organometallic $^{99m}$Tc-folates (I7). The water- and air-stable organometallic tricarbonyl complex $^{99m}$Tc(\(\text{H}_2\text{O})_3(\text{CO})_3\))$^+$ was previously proved to be a versatile reagent for labeling a variety of bioconjugates with $^{99m}$Tc. Importantly, this strategy potentially allows the synthesis of isostructural compounds for diagnosis and therapy while using the “matched” pair $^{99m}$Tc/$^{188}$Re ($^{188}$Re: $\beta$-decay, average $E_{[\text{av}]} = 763$ keV, $\gamma$-radiation, $E = 155$ keV, $t_{1/2} = 17$ h) (I8). A series of organometallic $^{99m}$Tc-folate derivatives has been developed and evaluated. In vivo, these tracers exhibited specific uptake in FR-positive tumors (~2–4 %ID/g, 4 h after injection) and kidneys. Undesirably, $^{99m}$Tc(CO)$_3$ radiofolates accumulated to a significant extent in the intestinal tract as a consequence of the hydrophobic character of the tricarbonyl-moiety. The most promising organometallic folate tracer, $^{99m}$Tc(CO)$_3$-histidine-folate (Fig. 2), was used for preclinical imaging studies of tumor-bearing mice with a small-animal multipinhole SPECT/CT scanner (I9). This study allowed precise determination of radioactivity uptake and distribution in FR-expressing tissues and organs such as KB tumor xenografts, kidneys, salivary glands, and the choroid plexus in the brain.

More recently, a folate conjugate with a DOTA chelator has been synthesized while using a “Click”-chemistry approach (Fig. 2) (20). This radioconjugate was successfully radiolabeled with $^{111}$In for SPECT and with $^{177}$Lu ($^{177}$Lu: $\beta$-decay, $E_{[\text{av}]} = 134$ keV, $\gamma$-radiation, $E = 113$ keV, 208 keV, $t_{1/2} = 6.7$ d) for potential therapeutic application. In preclinical studies with tumor-bearing mice, the new folate radiotracer displayed an excellent overall tissue distribution with specific accumulation in FR-positive KB tumors ($^{111}$In, ~5.8 %ID/g, and $^{177}$Lu, 7.5 %ID/g, 4 h after injection) but with minimal radioactivity retention in nontargeted organs and tissues (21). Significant uptake was, however, observed in the kidneys, resulting in the same low tumor-to-kidney ratios (<0.15) that were observed with previously evaluated radiofolates.

### Radiofolates for PET

The development of a folate-based PET agent is an attractive concept because PET would be the most accurate method for noninvasive
diagnosis of cancer, particularly of small metastases. Mathias et al. reported the radiosynthesis of the first PET folate tracers, 66Ga- and 68Ga-deferoxamine-folate (22). 68Ga is a generator isotope with a short half-life (89% β+ -decay, Eav $= 830$ keV, t1/2 $= 68$ min), whereas 66Ga has a relatively long half-life but an unfavorably high positron energy (56% β+ -decay, Eav $= 1,740$ keV, t1/2 $= 9.5$ h). In this study, FR-positive tumors and kidneys were clearly visualized on small-animal PET images of a KB tumor–bearing mouse 25 h after injection of 66Ga-deferoxamine-folate. However, the same drawback of a high intestinal accumulation of radioactivity that was reported for 67Ga-deferoxamine-folate also hampered a further development of these PET folates.

The design of a 18F-radiolabeled folate tracer is a promising approach because, compared with other radionuclides, 18F (97% β+ -decay, Eav $= 250$ keV, t1/2 $= 110$ min) displays excellent decay characteristics for PET. The first 18F-folate tracer reported in the literature was a folic acid conjugate with 4-fluorobenzylamine as a prosthetic group, referred to as 18F-fluorobenzylamine-folate (3a). 18F-fluorobenzylamine was coupled with ester-activated folic acid to obtain $\gamma$- and $\alpha$-18F-fluorobenzylamine-folate isomers in a ratio of 4:1. The last reaction step yielded 15%–44% 18F-fluorobenzylamine-folate tracer after purification via high-performance liquid chromatography. PET studies performed with 18F-fluorobenzylamine-folate in tumor-bearing mice were successful in visualizing KB tumor xenografts (~6.5%ID/g, 2 h after injection). Beside uptake in FR-expressing kidneys, massive radioactivity uptake was observed in the gallbladder (>250%ID/g, 2 h after injection) and the intestinal tract. To address the drawback of a low radiochemical yield experienced with 18F-fluorobenzylamine-folate, a more versatile radiosynthetic strategy was approached that used a Click-chemistry reaction (24). The folate precursor, folic acid-$\gamma$-(4-azido)-butylamide, was prepared according to a previously described method (25). The radiosynthesis of the 18F-Click-folate (Fig. 2) comprised 2 main reaction steps. First, the prosthetic group, 6-18F-fluoro-1-hexyne, was produced from the corresponding tosylate precursor with an excellent radiochemical yield (70%–85%) and purity (>95%). The second reaction step comprised the 1,4-triazole formation by Cu(I)-catalyzed cycloaddition of the 6-18F-fluoro-1-hexyne and folic acid $\gamma$-(4-azido)-butylamide. This Click reaction succeeded without the need for protection groups and directly provided the final 18F-Click-folate (20). In vivo studies performed with KB tumor–bearing mice revealed a relatively high and FR-specific tumor uptake (~3%ID/g, 45 min after injection) and a reasonable tumor-to-kidney ratio. However, the strongly lipophilic character of the 18F-Click-folate resulted again in high accumulation of radioactivity in the bile (>600%ID/g, 45 min after injection) and in the intestinal tract. Because both of these 18F-PET tracers provided suboptimal results, further investment in the design of 18F-PET folates will be necessary for optimization of both the radiosynthesis and the in vivo properties of the tracer.

**FIGURE 2.** Chemical structures of folic acid radioconjugates: 99mTc-EC20 (M $= 99m$Tc) (1), 99mTc(CO)$_3$-histidine-folate (M $= 99m$Tc) (2), 18F-fluorobenzylamine-$\alpha$-folate (3a), 18F-fluorobenzylamine-$\gamma$-folate (3b), 18F-Click-folate (4), and DOTA-Click-folate (M $= 111$In, 177Lu) (5).
REFERENCES


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