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Review

The folate receptor as a rational therapeutic target for personalized cancer treatment

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ABSTRACT

Conventional cancer treatment modalities have several limitations including lack of sufficient efficacy, serious untoward toxicity, as well as innate and acquired drug resistance. In contrast, targeted imaging agents can identify patients with receptors overexpressed on the surface of cancer cells, thus allowing appropriate selection of patients for personalized treatment with a desirable targeted therapeutic. The folate receptor (FR) has been identified as a new molecularly targeted entity, which is highly overexpressed on the surface of a spectrum of solid tumor cells, including ovarian, kidney, lung, brain, endometrial, colorectal, pancreatic, gastric, prostate, testicular, bladder, head and neck, breast, and non-small cell lung cancer. Folic acid conjugation is a novel approach for targeting FR-expressing tissues for personalized treatment. With the development of FRα-targeted therapies comes a concomitant prerequisite for reliable methods for the quantification of FRα tissue expression. Therefore, attaching a radioactive probe to folic acid to target diseased tissue has become a novel and powerful imaging technique. Currently available diagnostic tools frequently require invasive surgical biopsy. In contrast, the noninvasive single-photon emission computed tomography-based companion imaging agent, ^{99m}Tc-etarfolatide (^{99m}Tc-EC20), is in development for use as a companion diagnostic with the FRα-targeted folate conjugate, vintafolide (EC145), to identify patients whose tumors express FRα. Vintafolide is a folic acid conjugate of *Vinca* alkaloid (desacetylvinblastine hydrazide) that targets FRα-expressing tumors, thereby disrupting microtubule polymerization. ^{99m}Tc-etarfolatide is taken up by FR-positive tumors and allows for noninvasive, whole-body monitoring of FRα expression status throughout treatment. The combination of vintafolide plus etarfolatide has been evaluated in three Phase 2 studies for the treatment of various solid tumors, including ovarian, endometrial, peritoneal, and platinum-resistant ovarian cancer, as well as lung cancer. Patients with FR-positive tumors, as identified by etarfolatide uptake, have had better clinical outcomes than patients with FR-negative tumors, indicating the potential of etarfolatide as a companion biomarker for predicting vintafolide response. Targeted therapies combined with a reliable companion diagnostic test represent a novel approach toward efficient personalized medicine for malignant and nonmalignant disorders. Furthermore, the recent availability of the crystal structures of FRα and FRβ in complex with folates and antifolates forms a realistic basis for the rational design and implementation of novel FR-targeted drugs for the treatment of cancer and inflammatory disorders.

Contents

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1. Introduction

Conventional chemotherapeutic agents target and eradicate rapidly dividing cells (Vanneman and Dranoff, 2012). However, they are limited by significant untoward toxicities to healthy tissues, frequently emerging inherent and acquired drug resistance in various solid tumors, and a narrow therapeutic index (Gonen and Assaraf, 2012; Livney and Assaraf, 2013; Straussman et al., 2012; Vanneman and Dranoff, 2012). In pioneering studies published in 1986, it was first shown that targeted recombinant antibodies to the Her2/neu oncogene product can specifically block the proliferation of tumor cells overexpressing neu in nude mice (Drebin et al., 1986). Over the years, conventional cancer treatment has been greatly improved by the addition of an increasing number of new molecularly targeted agents, which inhibit molecular pathways crucial for tumor growth, maintenance, and metastasis (Vanneman and Dranoff, 2012). Such targeted and personalized cancer treatment uses the detailed molecular characteristics of a tumor and its microenvironment to allow tailored therapies to improve treatment outcomes, reduce toxicity to healthy tissues, and overcome drug resistance, thereby improving the benefit/risk profile (Gonen and Assaraf, 2012; Livney and Assaraf, 2013). However, lack of sufficient efficacy, reported side effects, and multidrug resistance phenomena often reduce the potential clinical impact of such therapies (Banerjee and Kaye, 2013; Sia et al., 2013; Straussman et al., 2012).

Another challenge associated with targeted therapies involves determining which genetic aberrations are driving disease and whether or not they are potential therapeutic targets (Awada and Aftimos, 2013; Burrell et al., 2013; Gerlinger et al., 2014; Mendelsohn, 2013). Moreover, mutated genes in cancer cells may be able to bypass the canonical molecular pathway or may contain irrelevant genetic alterations, both of which would allow them to evade the activity of targeted therapeutic treatments (Mendelsohn, 2013; Straussman et al., 2012). Hence, biomarkers can be readily used for patient stratification, thereby selecting patients who are likely to respond to a targeted therapy; however, such predictive biomarkers are generally lacking and are urgently needed (Awada and Aftimos, 2013; Banerjee and Kaye, 2013; Baumann et al., 2012; Jonsson and Bergh, 2012; Mendelsohn, 2013). Careful selection of the best imaging modality to identify suitable biomarkers is also necessary (Awada and Aftimos, 2013). The discovery and development of molecularly targeted drugs have led to the emergence of companion diagnostics for patient selection (Gonzalez de Castro et al., 2013). Along with them comes the critical need to ensure their robust clinical validation, in which the detection of specific biomarkers can be linked to patient outcomes (Gonzalez de Castro et al., 2013). As a result of these challenges, the search for rational therapeutic targets for cancer treatment is ongoing.

2. Role of the folate receptor in folate metabolism and cancer

Folates occur in an oxidized form as folic acid, or in physiologically and biosynthetically active reduced tetrahydrofolate forms (Gonen and Assaraf, 2012; Ifergan and Assaraf, 2008). Reduced folates are absolutely essential for the synthesis of DNA and RNA, amino acid metabolism, and methylation reactions; therefore, reduced folates are needed for cell growth, proliferation, and survival (Gonen and Assaraf, 2012). Dietary folates are absorbed in the upper small intestine (Zhao and Goldman, 2013), metabolized in the liver to 5-methyltetrahydrofolate, the major circulating form of folate (Gonen and Assaraf, 2012; Ifergan and Assaraf, 2008), and subsequently circulated through the bloodstream and delivered to the various tissues and organs.

Cellular uptake of folates proceeds through three main routes (Gonen and Assaraf, 2012; Ifergan and Assaraf, 2008). In the first route, uptake occurs via the proton-coupled folate transporter (PCFT/SLC46A1), which is responsible for intestinal folate absorption at the acidic pH of the upper small intestine (Ifergan and Assaraf, 2008; Qiu et al., 2006; Zhao and Goldman, 2013). The key role of PCFT in obligatory intestinal folate absorption has been established in studies with hereditary folate malabsorption (HFM), a congenital disorder in which loss-of-function mutations in PCFT result in low folate levels in the blood and cerebrospinal fluid (Lasry et al., 2008; Qiu et al., 2006; Zhao et al., 2007). HFM manifests within the first few months after birth with anemia, recurrent or chronic diarrhea, hypogammaglobulinemia, severe infection, and failure to thrive (Lasry et al., 2008; Qiu et al., 2006; Zhao et al., 2007). The second route involves the ubiquitously expressed reduced folate carrier (RFC/SLC19A1), which is the primary pathway for reduced folate uptake into various tissues under physiological pH (Gonen and Assaraf, 2012; Ifergan and Assaraf, 2008). Unlike PCFT, which is a proton-driven folate co-transporter, RFC functions as an anion exchanger capable of recognizing reduced folates (but with very low affinity for folic acid) and organic phosphates (Matherly et al., 2014; Zhao and Goldman, 2013). Finally, as folates cannot directly penetrate the cell membrane because of their hydrophilic anionic nature, cellular uptake also occurs via endocytosis through folate receptors (FRs) (Gonen and Assaraf, 2012; Ifergan and Assaraf, 2008; Zhao et al., 2007; Elnakat and Ratnam, 2006).

FRs are glycosylphosphatidylinositol-anchored proteins that bind folic acid and 5-methyltetrahydrofolate with high affinity (K_d: 10^{-9}–10^{-10} M) (Elnakat and Ratnam, 2006; Ifergan and Assaraf, 2008; Parker et al., 2005). Of the four known isoforms (α, β, γ, and δ), FRα and FRβ are anchored to the plasma membrane and bind folic acid with the highest affinity (Elnakat and Ratnam, 2006; Gonen and Assaraf, 2012; Ifergan and Assaraf, 2008; Parker et al., 2005). Cells that express FRα are more efficient in folate uptake because FRα binds folic acid with a binding affinity of 0.34 nM and 5-methyltetrahydrofolate with a binding affinity of 1 nM (K_d ~10^{-9}–10^{-10} M) (Della-Longa and Arcovito, 2013). In normal tissues and organs, FRα expression is restricted to only a few sites, which include kidney, lung, choroid plexus, and placenta, where FRα is confined to the luminal surface of polarized epithelia and, therefore, is not in contact with circulating folates or intravenously administered folic acid conjugates (Elnakat and Ratnam, 2006; Gonen and Assaraf, 2012; Muller, 2012; Parker et al., 2005; Weitman et al., 1992). FRβ expression is restricted mainly to the placenta and white blood cells of myeloid lineage, including activated macrophages (Elnakat and Ratnam, 2006; Gonen and Assaraf, 2012; Jager et al., 2012).

Numerous studies have shown that FRα is markedly overexpressed on the surface of various tumor types, including ovarian,

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