Advanced Drug Delivery Reviews 64 (2012) 749–755

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Combination of pet imaging with viral vectors for identification of cancer metastases ☆

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ARTICLE INFO

Article history:
Received 6 February 2011
Accepted 24 April 2011
Available online 30 April 2011

Keywords:
Positron emission tomography
Oncolytic virus
Melanoma
Lymph node metastases
Molecular imaging
Gene therapy
Reporter genes

ABSTRACT

There are three main ways for dissemination of solid tumors: direct invasion, lymphatic spread and hematogenic spread. The presence of metastases is the most significant factor in predicting prognosis and therefore evidence of metastases will influence decision-making regarding treatment. Conventional imaging techniques are limited in the evaluation and localization of metastases due to their restricted ability to identify subcentimeter neoplastic disease. Hence, there is a need for an effective noninvasive modality that can accurately identify occult metastases in cancer patients. One such method is the combination of positron emission tomography (PET) with vectors designed for delivery of reporter genes into target cells. Vectors expressing the *herpes simplex virus-1 thymidine kinase* (*HSV1-tk*) reporter system have recently been shown to allow localization of micrometastases in animal models of cancer using non invasive imaging. Combination of *HSV1-tk* and PET imaging is based on the virtues of vectors which can carry and selectively express the HSV1-*tk* reporter gene in a variety of cancer cells but not in normal tissue. A radioactive tracer which is applied systemically is phosphorylated by the HSV1-tk enzyme, and as a consequence, the tracer accumulates in proportion to the level of HSV1-tk expression which can be imaged by PET.

In this paper we review the recent developments in molecular imaging of micrometastases using replication-competent viral or nonviral vectors carrying the HSV1-tk gene using PET imaging. These diagnostic paradigms introduce an advantageous new concept in noninvasive molecular imaging with the potential benefits for improving patient care by providing guidance for therapy to patients with risk for metastases.

Contents

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Therapeutic Strategies for Controlling Metastasis and Recurrence of Cancers; Contribution of Drug Delivery Technologies".

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0169-409X/$ – see front matter
doi:10.1016/j.addr.2011.04.007

1. Introduction

Cancer is a prevalent disease worldwide and a primary cause of mortality in the western world [1]. Tumors frequently metastasize to regional lymph nodes and distant organs, and the presence of metastases is considered the most significant factor in predicting prognosis [2]. In melanoma and in carcinomas of the bladder, breast or head and neck, the risk for regional metastases is frequently high enough to necessitate lymph node dissection or sentinel nodal sampling even in the absence of clinical or radiologic evidence of disease [3]. Surgery for removal of regional lymph nodes is indicated not only for ablation of cancer, but also for evaluation of the need for adjuvant therapy [4]. In addition, the presence of distant metastases will influence decision-making regarding treatment in most patients [5]. However, clinical imaging techniques used today are limited in the evaluation and localization of metastases due to their restricted ability to identify subcentimeter neoplastic disease.

The imaging modalities utilized in clinical practice for cancer staging include ultrasound, computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) scanning [6]. Nevertheless, none of these methods can reliably detect micrometastases and differentiate them from inflammatory or infections processes [6–8]. Hence, there is a need to develop an effective noninvasive modality that can accurately identify occult metastases in cancer patients [9].

The purpose of this manuscript is to review the recent developments in molecular imaging of micrometastases using vectors carrying the *herpes simplex virus-1 thymidine kinase (HSV1-tk)* gene using PET imaging.

2. Molecular imaging of cancer

Molecular imaging provides visualization in space and time of normal or abnormal cellular processes at a molecular or cellular level. A leading advancement in molecular imaging of cancer was the introduction of PET. PET is a technique that allows the measurement of tissue function in vivo, utilizing the detection of gamma rays leaving the body after a radiotracer has been administered. The sensitivity of modern PET scanners allows detection of picomolar amounts of radioactive tracers in vivo. The most widely used tracer for studies of tumor metabolism in oncology is [^{18}F]2 fluoro-2-deoxyglucose (FDG). FDG has a low rate of dephosphorylation and so it is transported, phosphorylated and metabolically trapped in tumor cells as fluorodeoxyglucose-6-phosphate. Malignant cells, in general, show increased glucose utilization [10], probably due to an increased number of glucose transporter proteins [11]. As a result, FDG uptake, which reflects glucose metabolic rate, is increased in cancer cells. However, FDG accumulation is not specific to tumors and can be present in various conditions including inflammation, infection and other benign processes, resulting in a low positive predictive value of the test in such settings [12–15]. Most recent studies have indicated that even combination of FDG-PET and CT scanning is not yet sufficiently sensitive to be able to reliably detect occult lymph node metastases [16]. In addition, conventional PET-CT imaging also has a low sensitivity for distance metastases < 10 mm in size [17]. Hence, several studies have concluded that FDG-PET cannot be routinely used for axillary staging of operable breast cancer due to a high rate of false negative result [17]. More accurate methods are therefore required for imaging of micrometastases in cancer.

3. Reporter systems: receptors, transporters and enzymes

A high-quality imaging modality should have adequate spatial resolution in the range of millimeters to micrometers and the sensitivity to detect selective biochemical events such as those taking place in a cancer cell. An ideal method would allow the detection of a single cancer cell among billions of normal cells composing the human body using a non-invasive modality. One such method is the combination of PET or optical imaging with vectors designed for delivery of reporter genes into target cells, also called reporter imaging systems.

There are three main classes of reporter imaging systems: receptors, transporters and enzymes. A membranous receptor which is commonly used for imaging of endocrine cancers is the somatostatin receptor (hSSTrs) [18]. In the transporter group, the human norepinephrine transporter (hNET) transgene carried by a recombinant vaccinia virus (GLV-1h99) resulted in specific uptake of the radiotracer [131I]-meta-iodobenzylguanidine (MIBG) in orthotopic mesothelioma and pancreatic ductal carcinoma tumor models [19]. In the enzyme group, the wild-type *HSV1-tk* gene and its mutation *HSV1-sr39tk* were extensively studied as candidate reporter systems for imaging of cancer [20].

4. HSV1-tk-PET imaging system

HSV1-tk-PET imaging is based on the virtues of HSV which can selectively infect a variety of cancer cells but not normal mammalian cells [21,22]. After entering the cell, the virus readily expresses its early genes, among them the *HSV1-tk* gene product. The HSV1-tk enzyme, like mammalian thymidine kinases, phosphorylates thymidine to thymidine-monophosphate (TdR). But unlike mammalian TK1, viral HSV1-tk can also phosphorylate thymidine analogs, including 2′-deoxy-2′-fluoro-5-iodo-1-[β]-D-arabinofuranosyluracil (FIAU), 2′-fluoro-5-ethyl-1-[β]-D-arabinofuranosyluracil (FEAU) or other acycloguanosine analogs. Following its injection as a non-phosphorylated, uncharged compound to the circulation, [^{18}F]FEAU enters the cellular membranes of malignant and normal cells alike. However, upon its entry to cancer cells, [^{18}F]FEAU is phosphorylated by the HSV1-tk enzyme, and as a consequence, it is trapped inside the infected cancer cell, but not in normal cells resistant to the virus. [^{18}F]FEAU is then accumulated in proportion to the level of HSV1-tk expression which can be imaged using PET as depicted in Fig. 1.

4.1. HSV, an efficient reporter gene delivery system

Noninvasive reporter gene imaging has been successfully applied using expression systems mediated by viral or nonviral vectors.

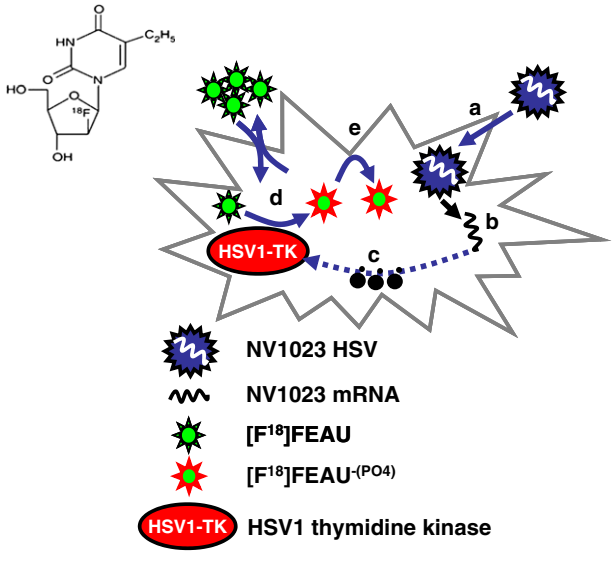

Fig. 1. Schematic demonstration of genetic imaging using HSV1-tk expressing system. After entering the cell (*a*), virus readily expresses early genes (*b*), among them the herpes simplex virus type 1-*thymidine kinase* (HSV1-*tk*) gene product (*c*). As opposed to the mammalian *TK*, the HSV1-*tk* gene product has the ability to catalyze the phosphorylation of [^{18}F]FEAU, a radioactive tracer administered systemically (*d*). Phosphorylated [^{18}F]FEAU, is trapped inside HSV1-*tk* expressing cancer cells (*e*); it accumulates in proportion to the level of HSV1-*tk* expression and can be imaged using PET. Adopted from reference no. [64].