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Progress and problems with the use of suicide genes for targeted cancer therapy☆

Zahra Karjoo [a], Xuguang Chen [a], Arash Hatefi [a,b,*]

[a] Department of Pharmaceutics, Rutgers, The State University of New Jersey, Piscataway, NJ 08855, United States
[b] Rutgers Cancer Institute of New Jersey, New Brunswick, NJ 08903, United States

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ABSTRACT

Among various gene therapy methods for cancer, suicide gene therapy attracts a special attention because it allows selective conversion of non-toxic compounds into cytotoxic drugs inside cancer cells. As a result, therapeutic index can be increased significantly by introducing high concentrations of cytotoxic molecules to the tumor environment while minimizing impact on normal tissues. Despite significant success at the preclinical level, no cancer suicide gene therapy protocol has delivered the desirable clinical significance yet. This review gives a critical look at the six main enzyme/prodrug systems that are used in suicide gene therapy of cancer and familiarizes readers with the state-of-the-art research and practices in this field. For each enzyme/prodrug system, the mechanisms of action, protein engineering strategies to enhance enzyme stability/affinity and chemical modification techniques to increase prodrug kinetics and potency are discussed. In each category, major clinical trials that have been performed in the past decade with each enzyme/prodrug system are discussed to highlight the progress to date. Finally, shortcomings are underlined and areas that need improvement in order to produce clinical significance are delineated.

Contents

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* Corresponding author at: Department of Pharmaceutics, Room 222, Rutgers, The State University of New Jersey, 160 Frelinghuysen Road, Piscataway, NJ 08854-8020, United States. Tel.: +1 848 445 6366; fax: +1 732 445 3134.
E-mail address: ahatefi@pharmacy.rutgers.edu (A. Hatefi).

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1. Introduction

Dysregulation of proliferation in cells can lead to overgrowth and production of tumor masses with aberrant leaky blood vessels, hypoxic environment and elevated interstitial fluid pressure due to poor lymphatic drainage [1,2]. This complex tumor pathophysiology therefore demands sophisticated therapeutic modalities for effective treatment. Early attempts to cure cancer were based on using agents that can inhibit cell growth. Unfortunately, severe side effects on normal high proliferating cells such as those in hematopoietic and immune system significantly limited the use of anti-metabolite agents. Despite these challenging adverse effects, the proliferative features of cancer remained an attractive focus for rational design of "targeted therapeutics" in second half of the 20th century. Concurrently, advances in cancer biology and genetics opened new horizons and rapidly translated into targeted drug design in which the molecular aspects of cancer became as important as its proliferative features. Approved in 1996, Imatinib mesylate (Gleevec®) is the first targeted chemotherapy agent designed based on advances in genetics [3]. One year later, FDA approved bevacizumab (Avastin®) which is the first monoclonal antibody against vascular endothelial growth factor receptor. These two drugs among others are the milestones of molecular targeted cancer therapy where the basic biological differences between cancer and normal cells are exploited to effectively target cancer. Unfortunately, these distinctive features are not always available to be exploited. The rationally designed drug may enter other organs which share the same biological features with the tumors [4]. For instance, Imatinib's off-target effects such as hypophosphatemia and hypocalcemia are caused by its inhibitory effect on c-fms tyrosine kinase in osteoclasts and osteoblasts. In general, mere use of *active targeting* strategy has not been sufficient to effectively eradicate cancer.

In parallel to actively targeted antibody-based therapeutics, numerous nanomedicines have been developed in an attempt to not only enhance drug localization at the tumor site and increase drug efficacy, but also decrease chances of multidrug resistance and toxicity [5]. Nanomedicines are designed to take advantage of tumor leaky vessels in order to *passively target* and accumulate in tumor tissues. Doxil® is among the first FDA approved nanomedicines that is mainly used for the treatment of Kaposi sarcoma where tumor vessels are very leaky. This PEGylated liposomal formulation of doxurubicine passively targets and accumulates in the tumors through enhanced permeability and retention effect (EPR) and then releases the drug [6]. Due to its small size, the released doxorubicin can then diffuse throughout the tumor tissue via concentration gradient and significantly impact tumor growth. Although such passively targeted formulations enhance the concentration of drug in tumor interstitial fluid but still a significant number of the liposomal particles are picked up by the reticuloendothelial system. Furthermore, the efficacy of such nanomedicines that rely solely on passive targeting is also limited by the degree of leakiness of tumor blood vessels which varies by cancer type and tumor size. As a result, there is a significant probability to observe toxicity in non-target tissues before the drug concentration in tumors reach the therapeutic level. Hence, *passive targeting* by itself may not be sufficient to render an effective and safe therapeutic outcome. Published data in the past decades suggest that more refined approaches may be necessary in order to overcome the obstacles mentioned above.

2. Cancer gene therapy

In recent years, more sophisticated approaches have emerged that combine passive and active targeting strategies in order to maximize efficacy at the target tumor site while minimizing the potential for off target toxicity. Targeted-shielded nanomedicines (viral and non-viral) carrying gene therapy agents (RNAi or DNA) are newer generation of targeted therapeutics that first accumulate in tumors passively via EPR effect and then due to the presence of ligands can bind to specific antigens on the surface of cancer cells and internalize [7–9]. This approach is especially useful for several gene therapy-based nanomedicines where the target site is inside the cancer cells. Cancer gene therapy is the treatment that is based on the transfer of therapeutic genes into cancer cells in order to slow down or cease the progress of malignancy. Cancer gene therapy can be classified into three categories: corrective gene therapy, toxin/apoptosis-inducing gene therapy and suicide gene therapy. Cancer corrective gene therapy is the approach that applies therapeutic genes into cancer cells to adjust the deranged gene profile and consequently moderate or stop cell proliferation. Tumor suppressor genes such as p53 or genetic interference agents that interfere with cancer cell proliferation (e.g., siRNA or miRNA) are two prominent examples of this approach [10–13]. Toxin/apoptosis-inducing cancer gene therapy is a more straightforward method where the delivered transgene results in production of a toxic protein (e.g., diphtheria toxin or TNF-α) that in turn induces cell death. The main weakness of corrective gene therapy and toxin/apoptosis-inducing gene therapy is that only the cancer cells that have received the therapeutic gene get affected and those that have not received the therapeutic gene continue to proliferate. This becomes especially problematic for nanomedicines that rely solely on these two gene therapy strategies because they cannot penetrate deep into the tumor tissues due to tumor's dense physiological environment and elevated interstitial fluid pressure [14]. As a result, not all cancer cells in tumors can be eliminated and this significantly increases the probability of cancer recurrence. Overall, it appears that off-target toxicity and lack of access to all cancer cells in the tumor environment are among the major obstacles to successful treatment of cancer. Because of these reasons, no passively and actively targeted nucleic acid-based nanomedicine has reached the clinic yet.

2.1. Suicide gene therapy

One idea that has gained significant attraction for cancer therapy with potential to overcome the discussed obstacles is targeted suicide gene therapy. In literature, this approach is also known as gene directed enzyme prodrug therapy (GDEPT). It allows us to combine passive, active, and transcriptional targeting strategies to maximize anticancer activity at the tumor site while minimizing impact on normal tissues. By definition, GDEPT is a two step process where the cancer cells are first transduced by a gene coding for a non-toxic enzyme (suicide gene) followed by administration of a non toxic prodrug [15]. Cell death occurs as a result of prodrug conversion to its toxic metabolite by transduced cells which actively express the suicide gene. In the context of GDEPT, therapeutic index increases by reducing side effects and restricting the toxicity of a chemotherapy agent only to target cancer cells. This approach provides two distinct advantages over the conventional cancer therapeutic strategies such as chemotherapy and radiation therapy.

The first advantage is the ability for transcriptional targeting where the suicide gene is put under the control of a tumor-specific promoter so that the gene expression occurs only in tumor cells but not in normal cells [16]. Consequently, the prodrug gets activated only in tumor environment reducing the probability of observing off-target toxicity. The suicide genes can then be loaded onto targeted vectors (viral or non-viral) and delivered to the tumor cells first passively and then actively [17]. Until early 2000s, variety of cancer/tissue specific promoters such as human telomerase reverse transcriptase (hTERT) promoter, carcinoembryonic antigen (CEA) promoter, osteocalcin (OC) promoter, and hypoxia and radiation responsive elements had been developed [18–21]. The application, advantages and disadvantages of using these promoters are eloquently reviewed by several groups [16,22,23], and summarized in a book chapter by our group [24]. Over the past decade, several new promising promoters have been developed which attracted significant attention (Table 1). Although all these promoters have shown promise, hTERT is the only one that has successfully entered clinical trials [25]. The major obstacle preventing transcriptional targeting