DNA REPAIR 6 (2007) 1197–1209

available at www.sciencedirect.com

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

O6-methylguanine-DNA-methyltransferase (MGMT) gene therapy targeting haematopoietic stem cells: Studies addressing safety issues

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ARTICLE INFO

Article history:
Published on line 11 May 2007

Keywords:
O6-methylguanine-DNA-methyltransferase
Haematopoietic stem cell
Chemoprotection
Gene therapy
In vivo selection
Chromosomal damage

ABSTRACT

As haematopoietic stem cell gene therapy utilizing O6-methylguanine-DNA-methyltransferase has reached the clinical stage, safety-related questions become increasingly important. These issues concern insertional mutagenesis of viral vectors, the acute toxicity of pre-transplant conditioning protocols and *in vivo* selection regimens as well as potential genotoxic side effects of the alkylating drugs administered in this context. To address these questions, we have investigated toxicity-reduced conditioning regimens combining low-dose alkylator application with sublethal irradiation and have analysed their influence on engraftment and subsequent selectability of transduced haematopoietic stem cells. In addition, a strategy to monitor the acute and long-term genotoxic effects of drugs with high guanine-O6 alkylating potential, such as chloroethylnitrosoureas or temozolomide is introduced. For this purpose, assays were implemented which allow an assessment of the generation and fate of primary drug-induced adducts as well as their long-term effect on chromosomal integrity at the single cell level.

1. Introduction

1.1. *Haematopoietic stem cell gene therapy using chemotherapy resistance genes*

Gene therapy promises new treatment options for a number of disabling and life threatening diseases, and the treatment of cancer is one of the prime areas in which major progress is expected. While most gene therapy approaches for cancer target the malignant cell directly, other strategies aim to reduce therapy-induced side effects. Here, myelosuppression is one of the major and frequently dose-limiting sequelae of cytotoxic chemotherapy (CTX), and myelotoxicity is particularly severe after dose-intensified regimens such as used in the treatment of malignant lymphomas, multiple myelomas, germ cell tumours, and other chemotherapy sensitive malignancies. In this context, autologous stem cell support and/or haematopoietic growth factor administration are routinely used to reduce haematologic toxicity. Nevertheless, a 10–20 day period of severe myelosuppression which substantially contributes to the morbidity and mortality of these regimens usually cannot be avoided [1].

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doi:10.1016/j.dnarep.2007.03.021

Transfer of so-called chemotherapy resistance (CTX-R) genes into autologous haematopoietic cells prior to retransfusion is presently investigated as a strategy to reduce the haematological side effects of dose-intensified CTX and/or to permit an increase of dose intensity [2]. A broad spectrum of cellular functions have been shown to protect cells from the toxic side effects of anticancer drugs and quite a number of the related genes are presently evaluated for use in CTX-R gene therapy. Among them are genes coding for the detoxifying membrane-bound efflux pump multidrug resistance protein-1 (MDR-1), for proteins involved in the metabolism of cytotoxic drugs or interfering with their mode of action, such as glutathione S-transferase, cytidine deaminase, aldehyde dehydrogenase, or mutant forms of dihydrofolate reductase, as well as for DNA-repair proteins [2–4].

1.2. O^6-methylguanine-DNA-methyltransferase in haematoprotective gene therapy

Currently, the DNA-repair protein O^6-methylguanine-DNA-methyltransferase (MGMT) appears to be one of the most promising candidates for myeloprotection. While other DNA-repair pathways involve the concerted action of multiple proteins, repair by MGMT is a one-step process in which the cytotoxic alkyl-group at the O^6-position of guanine is transferred to a cysteine residue within the acceptor pocket of the MGMT protein [5], causing the irreversible inactivation of the protein. In comparison with other tissues, bone marrow cells show low MGMT activity [6] and this probably explains, at least partly, the pronounced haematotoxic potential of O^6-alkylating agents. While several investigators have shown the feasibility of haematoprotection by overexpression of wild type MGMT [7–9] the increase in chemotherapy resistance at the cellular level has been only moderate and selection of transduced cells in *in vivo* transplant models has been problematic [10,11]. More recently, mutant forms of MGMT such as $MGMT^{P140K}$ and $MGMT^{G156A}$ which are resistant to the pharmacological MGMT inhibitor O^6-benzylguanine (BG) have been utilised to increase the level of protection for haematopoietic cells [12]: BG is a guanine analogue which binds to the catalytic centre of MGMT and causes irreversible inactivation of the protein [13]. BG has been used in phase II studies to reduce the repair capacity of tumour cells with high MGMT expression and thereby increases their susceptibility to agents which execute their cell killing potential predominantly *via* guanine-O^6 alkylation in DNA. Among those drugs are the chloroethylnitrosoureas (CENUs) 1-(4-amino-2-methyl-5-pyridinyl-methyl)-1-(2-chlorethyl)1-nitrosourea (ACNU), 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), or 1-2-chlorethyl)-3-cyclohexyl-1-nitrosourea (CCNU), or the triazene-derivatives temozolomide (TMZ) or dacarbazine (DTIC). In these studies, severe myelosuppression was shown to be the dose-limiting toxicity of combined BG/BCNU treatment [14,15]. A number of BG-resistant variants of MGMT have been evaluated for their usefulness in haematoprotective strategies in the context of combined therapy with BG and O^6-alkylating agents. The haematoprotective effect of $MGMT^{P140K}$ has been demonstrated in clonogenic progenitor and long-term culture-initiating cells *in vitro* [16,17] as well as in murine *in vivo* models [18–21]. In addition, it has been demonstrated that $MGMT^{P140K}$ confers long-term haematoprotection in large animal transplant models [22,23]. In these studies, also highly efficient and stable *in vivo* enrichment of haematopoietic stem cells (HSC) protected by transgene $MGMT^{P140K}$ expression was demonstrated in the context of BG/alkylator chemotherapy. Additionally, in murine models of monogenetic diseases such as β-thalassemia [24] or protoporphyria [25] efficient and stable selection of genetically repaired cells has been shown when such cells were protected by $MGMT^{P140K}$ gene transfer and selected with BG/TMZ or BG/BCNU.

Clinical use of BG-resistant MGMT mutants, in particular $MGMT^{P140K}$, has been suggested in the setting of dose-intensified treatment strategies for brain tumours, as in this tumour entity BCNU as well as TMZ are among the few active agents. In addition, overexpression of MGMT has been described as a mechanism of drug resistance in this disease entity, arguing for the application of BG to overcome the non-responsiveness [26]. Another potential application of $MGMT^{P140K}$ gene therapy could be malignancies such as malignant melanoma or certain lymphomas in which not only CTX with BCNU or TMZ, but also immunotherapeutic approaches have proven effective, while the therapeutic efficiency of the combination of the two is hampered by CTX-induced immunosuppression. The protection of lympho-haematopoietic cells by $MGMT^{P140K}$ in the context of BG/TMZ or BG/BCNU CTX may allow the circumvention of immunosuppression and thus the delivery of effective immunotherapy in a situation of maximal tumour regression.

In addition, the efficient enrichment of transduced HSC has paved the way for utilizing $MGMT^{P140K}$ as a selection marker in HSC gene therapy of monogeneic diseases with haematologic manifestation. Here, additional "selection genes" are required for those diseases in which the therapeutic gene itself does not confer a selective advantage to the corrected cells on the level of HSC, and it may therefore be difficult to achieve sustained therapeutic levels of transgenic cells. This applies to hemoglobinopathies such as sickle cell disease or the thalassemias, chronic granulomatous disease (CGD), congenital storage diseases such as mucoviscidosis and Morbus Gaucher, or pulmonary alveolar proteinosis due to genetic deficiency of the common β-chain of the GM-CSF/IL3/IL5 receptors. As clinical studies have shown that high initial transduction rates unfortunately may also increase the risk of insertional mutagenesis and may thereby cause secondary leukemias, such as in the recent human study of gene therapy in X-linked SCID patients [27], the use of low initial transduction rates and $MGMT^{P140K}$ as an efficient selection marker may circumvent these problems.

1.3. Toxicities related to $MGMT^{P140K}$ gene therapy

While the primary aim of MGMT gene transfer in the context of malignant disease is to allow the application of dose-intensified CTX, the use of $MGMT^{P140K}$ as selection marker will certainly require pre-transplant conditioning and post-transplant selection regimens which induce as few toxicities as possible. In particular, myeloablative radiotherapy cannot be justified in most patients with monogeneic diseases. Besides these acute toxicities, also long-term genotoxic effects connected with $MGMT^{P140K}$ gene transfer have to be taken