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Gene manipulation to enhance MIBG-targeted radionuclide therapy

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Abstract

The goal of targeted radionuclide therapy is the deposition in malignant cells of sterilizing doses of radiation without damaging normal tissue. The radiopharmaceutical [^{131}I]*meta*-iodobenzylguanidine ([^{131}I]MIBG) is an effective single agent for the treatment of neuroblastoma. However, uptake of the drug in malignant sites is insufficient to cure disease. A growing body of experimental evidence indicates exciting possibilities for the integration of gene transfer with [^{131}I]MIBG-targeted radiotherapy.

Keywords: Gene therapy; Targeted radiotherapy; MIBG; Noradrenaline transporter

1. Introduction

Targeted radiotherapy of tumors is achieved by the delivery, specifically to malignant deposits, of cytotoxic radionuclides bound to tumor-seeking agents. The advent of monoclonal antibody technology was accompanied by the prospect that all tumors could be targeted by exploiting the differential display of cell surface antigens between malignant and normal tissues. With the notable exception of B-cell lymphoma [1,2], clinical applications of these radiolabeled macromolecules have generally been unsatisfactory due to low tumor specificity of targeted epitopes and limited penetration into tumors. These considerations favor the use of nonimmunogenic small molecules with good penetrative properties and higher uptake in tumors. Such criteria are fulfilled by *meta*-iodobenzylguanidine (MIBG) and sodium iodide (NaI), which are readily available in radioiodinated form. [^{131}I]MIBG and [^{131}I]NaI have been used extensively for the treatment of neural crest-derived tumors (neuroblastoma and phaeochromocytoma) and thyroid carcinoma, respectively.

The recent characterization of the genes encoding the cellular transporters responsible for the active uptake of [^{131}I]MIBG [3] and [^{131}I]NaI [4,5] has raised the possibility of gene transfer to enable the targeting of a wide variety of tumor types with these low molecular weight radiopharmaceuticals. Transfection of the sodium iodide symporter (NIS) gene into a various nonthyroidal cancer cell lines has induced iodide or pseudohalide uptake activity and cytotoxicity in vitro and in vivo [6–12]. Furthermore, tumor-selective up-regulation of NIS expression may be achieved by hormonal manipulation [13] or application of differentiation-inducing agents [14,15]. However, the absence of an iodide organification pathway in nonthyroidal cells has the undesirable consequence of rapid iodide egress, which, despite laudable attempts to enhance retention [16,17], still represents the major limitation of this therapeutic strategy.

An alternative promising radiopharmaceutical with longer intracellular retention time is radioiodinated MIBG. Promotion of its active accumulation by tumor cells by transfer of the noradrenaline transporter (NAT) gene indicates exciting prospects for cancer therapy.

2. Noradrenaline transporter gene transfection to enable targeting of tumor cells using radiolabeled MIBG

One of the most promising, nonimmunologenic, targeting agents is [^{131}I]MIBG. This analog of adrenergic neuron blockers (Fig. 1) [18] is selectively concentrated in neuroblastoma cells via the NAT [19], resulting in specific irradiation of the target tumor cells with relative sparing of

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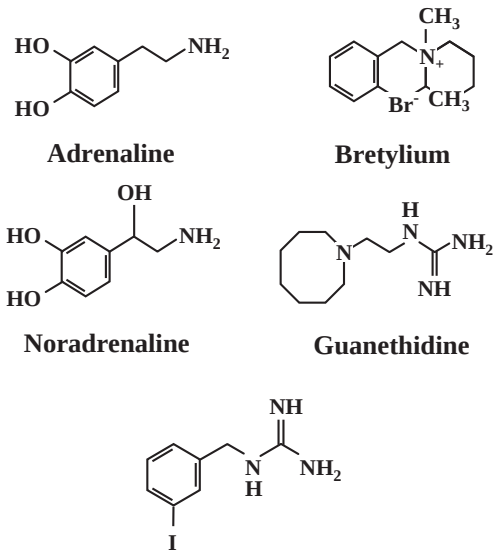

Fig. 1. MIBG is a structural analog of noradrenaline and adrenergic neuron blockers — bretylium and guanethidine.

normal tissues. While cures have generally not been achieved, encouraging results (long-term remissions and palliation) have been obtained with [^{131}I]MIBG used as a single agent in patients with resistant disease [20–22]. However, the most effective way to use this tumor-targeting drug has yet to be defined, and increasingly, [^{131}I]MIBG is administered in combination with more conventional treatments [23].

Cellular accumulation of MIBG occurs by two distinct mechanisms: (i) passive diffusion that is nonspecific, low level, energy independent, unsaturable and takes place in all cells, and (ii) active uptake that is specific, high affinity, saturable, ATPase-dependent and only occurs in cells that synthesize the NAT [24]. Under ideal conditions in vitro, the specific uptake process is about 50 times more efficient than passive uptake [25]. Noradrenaline transporter expression is predictive for MIBG uptake capacity [26], and quantification of NAT mRNA has potential for the selection of patients for MIBG therapy [27].

Therapeutic application of [^{131}I]MIBG is confined to a few NAT-expressing tumors, of neural crest origin, which selectively accumulate the radiopharmaceutical. In order to determine whether [^{131}I]MIBG could be used to treat other tumor types, we cloned NAT cDNA [28], under the control of the strong ubiquitous RSV promoter, into an episomal expression plasmid and used it to transfect a human, glioblastoma, cell line (UVW) that did not express the NAT gene. Transfectants exhibited at least 25-fold enhancement of [^{131}I]MIBG uptake and NAT-transfected clonogens succumbed to [^{131}I]MIBG administration in a dose-dependent manner [29]. The superior toxicity of [^{131}I]MIBG to cells grown as spheroids rather than as monolayers confirmed the efficacy of the β-radiation cross-fire effect [30,31].

Experimental tumors in athymic mice were grown from NAT gene-transfected UVW cells. Treatment with [^{131}I]MIBG produced impressive tumor cure rate and suppression of tumor growth at activities that induced no myelotoxicity [32]. As the dose-limiting tissue in patients treated with [^{131}I]MIBG is bone marrow, these results demonstrate the potential of gene therapy-assisted, MIBG-targeted radiotherapy for the treatment of nonneuroectodermal tumors.

3. Tumor-specific promoters for NAT transgene expression

Achieving tumor-selective expression of radiopharmaceutical transporter genes is necessary for the success of strategies, which combine targeted radionuclides and gene transfer. Establishing differences in gene expression between malignant and normal tissue indicates gene promoter elements that may allow activation of the expression of therapeutic transgenes preferentially in tumors.

Telomerase is an enzyme that is responsible for the maintenance of chromosome termini. It is the most common general marker of cancer cells, and its activity has been detected in every major category of human malignancy, whereas normal somatic tissues have negligible activity [33–36]. Therefore, an attractive method of specifying gene expression to malignant cells is provided by the telomerase promoters. Telomerase comprises an RNA component (hTR) and a protein component (hTERT). Although both constituents are necessary for telomerase activity, the expression of hTERT is confined almost exclusively to tumor cells whereas hTR gene transcription is less stringently limited to malignant cells [37].

We have demonstrated that the hTR and hTERT promoters can drive expression of the NAT transgene in a range of nonneuroectodermally derived tumor cells [38]. Moreover, telomerase promoter-driven NAT gene transfection of SK-N-BE(2c) neuroblastoma cells, which already express the NAT, induced these hosts to express higher levels of NAT mRNA, to actively accumulate more [^{131}I]MIBG (Table 1) and to succumb more readily

Table 1
NAT gene expression and active MIBG uptake by untransfected SK-N-BE(2c) cells and SK-N-BE(2c) cells transfected with the NAT gene controlled by telomerase promoters (hTERT or hTR)

Cell line	MIBG uptake (cpm × 10^3 per 10^6 cells)	NAT copies per GAPDH copies
SK-N-BE (2c)	980±84	4.2±0.3
SK-N-BE (2c)-hTERT/NAT	1344±153	12.6±1.0
SK-N-BE (2c)-hTR/NAT	1185±127	11.8±0.9

From RNA isolates, NAT and GAPDH expression were assessed by reverse transcription and real-time PCR amplification. Active uptake of [^{131}I]MIBG was determined by subtraction of nonspecific uptake (assessed in the presence of desmethylimipramine) from total [^{131}I]MIBG uptake. Data are means±S.D. of three separate determinations in triplicate.