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BIOLOGY CONTRIBUTION

ENGINEERED MODULAR RECOMBINANT TRANSPORTERS: APPLICATION OF NEW PLATFORM FOR TARGETED RADIOTHERAPEUTIC AGENTS TO α-PARTICLE EMITTING ^{211}At

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Purpose: To generate and evaluate a modular recombinant transporter (MRT) for targeting ^{211}At to cancer cells overexpressing the epidermal growth factor receptor (EGFR).

Methods and Materials: The MRT was produced with four functional modules: *(1)* human epidermal growth factor as the internalizable ligand, *(2)* the optimized nuclear localization sequence of simian vacuolating virus 40 (SV40) large T-antigen, *(3)* a translocation domain of diphtheria toxin as an endosomolytic module, and *(4)* the *Escherichia coli* hemoglobin-like protein (HMP) as a carrier module. MRT was labeled using *N*-succinimidyl 3-[^{211}At]astato-5-guanidinomethylbenzoate (SAGMB), its ^{125}I analogue SGMIB, or with ^{131}I using Iodogen. Binding, internalization, and clonogenic assays were performed with EGFR-expressing A431, D247 MG, and U87MG.wtEGFR human cancer cell lines.

Results: The affinity of SGMIB-MRT binding to A431 cells, determined by Scatchard analysis, was 22 nM, comparable to that measured before labeling. The binding of SGMIB-MRT and its internalization by A431 cancer cells was 96% and 99% EGFR specific, respectively. Paired label assays demonstrated that compared with Iodogen-labeled MRT, SGMIB-MRT and SAGMB-MRT exhibited more than threefold greater peak levels and durations of intracellular retention of activity. SAGMB-MRT was 10–20 times more cytotoxic than [^{211}At]astatide for all three cell lines.

Conclusion: The results of this study have demonstrated the initial proof of principle for the MRT approach for designing targeted α-particle emitting radiotherapeutic agents. The high cytotoxicity of SAGMB-MRT for cancer cells overexpressing EGFR suggests that this ^{211}At-labeled conjugate has promise for the treatment of malignancies, such as glioma, which overexpress this receptor.

Modular recombinant transporters, Epidermal growth factor receptor, ^{211}At, Radionuclide therapy, Nuclear targeting.

INTRODUCTION

Although the mechanisms through which radiation can interfere with cellular proliferation are complex, strong empirical evidence has shown that with both conventional and high linear energy transfer (LET) radiation, increasing the energy deposition in the cell nucleus results in a decreased cell survival fraction (1, 2). Strategies that shift the site of radionuclide decay from the cell surface to the nucleus are advantageous for two reasons. First, they increase the geometric probability (solid angle) that the nucleus will be traversed by the radiation. Even with the multicellular range β-emitter ^{131}I, dosimetry calculations and *in vitro* experiments have shown that shifting the site of decay from the cell membrane to the cytoplasmic vesicles near the nucleus increases the cell nucleus radiation dose and cytotoxicity by a factor of two to three (3). Second, radiation in the subcellular range cannot be effective unless the site of decay is within the range of the cell nucleus. For example, if an α-particle emitter can be localized in the cell nucleus, one can also exploit the cytotoxic potential of the α-particle recoil nucleus created during

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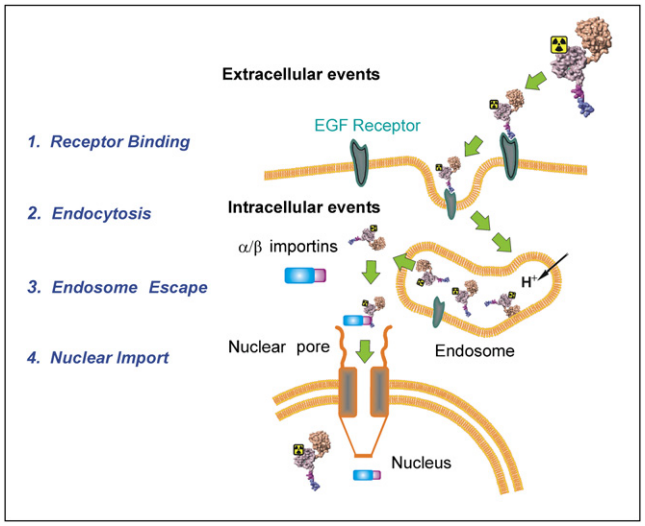

Fig. 1. Steps in targeting of modular recombinant transporter (MRT) from surface to nucleus of cancer cell. EGF = epidermal growth factor.

α-decay, which has a subcellular tissue range of <100 nm and an LET of about 10 times greater than that of the α-particle itself (4, 5). When located outside the cell, α-particle recoil nuclei are not cytotoxic. The radiation dose deposited in the cell nucleus from radionuclides decaying in various cellular sites has been calculated for different cell geometries, and the results have indicated a significant increase in the dose to the cell nucleus for sources located within the cell nucleus (6).

We have confirmed the predicted exquisite cytotoxicity of ^{211}At when localized in the cell nucleus in studies with 5-[^{211}At]astato-2′-deoxyuridine (AUdR) (7, 8). Effective killing of tumor cells, including a human glioma line, could be achieved *in vitro* after only about one to three α-particle hits per cell. This labeled compound allowed us to demonstrate the concept of developing targeted α-particle therapeutics that undergo decay in the cell nucleus; however, AUdR is not suitable for patient treatment because of its lack of tumor specificity and poor *in vivo* stability.

A major challenge in the development of specific and effective cancer treatments is that exploiting a molecular target that is accessible (*i.e.,* cell membrane or extracellular matrix) is critical for achieving tumor selectivity while delivery of the therapeutic agent to the cell nucleus is generally required for maximizing the therapeutic effect. An intriguing approach to this conundrum is to use a hybrid molecule to achieve both goals by linking together peptides with different functionalities. Originally, this was attempted through the use of bifunctional cross-linking reagents (9, 10). We have now used a recombinant technology to develop targeted therapeutic agents that include modules for addressed delivery both to tumor cells and into compartments within these cells that are the most sensitive to the drug (11, 12). These modular recombinant transporters (MRTs) are polypeptides possessing *(1)* an internalizable ligand module providing target cell recognition and subsequent receptor-mediated endocytosis by the cell; *(2)* an endosomolytic module ensuring escape of the MRT from the endosomes; *(3)* a module containing a nuclear localization sequence (NLS), thereby enabling interaction of the transporter with importins, the intracellular proteins ensuring active translocation into the cell nucleus; and *(4)* a carrier molecule for attachment of the drug (*i.e.,* photosensitizer, radionuclide; Fig. 1). A significant advantage of MRTs is the interchangeable nature of the modules, offering the exciting prospect of generating an MRT cocktail using a mixture of ligands tailored to the molecular profile of an individual patient's tumor.

In the present study, we evaluated the potential utility of using an MRT for targeting ^{211}At to cancer cells overexpressing epidermal growth factor (EGF) receptor (EGFR). The results of this study have demonstrated that an MRT with four functional modules retained high affinity and specific binding to EGFR after radiolabeling. An MRT labeled using *N*-succinimidyl 3-[^{211}At]astato-5-guanidinomethylbenzoate (13) was significantly more cytotoxic than [^{211}At]astatide against three different EGFR-expressing human cancer cell lines.

METHODS AND MATERIALS

Cell lines

The human epidermoid carcinoma cell line A431 (14) (ATCC, Manassas, VA) and the human glioblastoma cell lines D247 MG (15) and U87MG.wtEGFR (16) (both provided by Dr. Darell Bigner, Duke University Medical Center) have been reported to overexpress EGFR. The cells were cultured in Zinc Option medium supplemented with 10% (vol/vol) fetal bovine serum and penicillin/streptomycin (100 U/mL) at 37°C in a 5% carbon dioxide atmosphere. All tissue culture reagents were obtained from Gibco/Invitrogen (Carlsbad, CA).

Modular recombinant transporter

The MRT used in these experiments was DTox-HMP-NLS-EGF of 76.3 kDa (heretofore designated as MRT), where DTox is the translocation domain of diphtheria toxin, serving as an endosomolytic module; HMP is an *E. coli* hemoglobin-like protein, serving as a carrier module; NLS is the optimized simian vacuolating virus 40 (SV40) large T-antigen NLS; and EGF is epidermal growth factor and served as the ligand module (12). The MRT was purified to >98% purity on Ni-NTA-agarose (QIAGEN, Hilden, Germany) according to the standard procedure furnished by the supplier. The MRT modules retained their functions. They demonstrated high-affinity interaction with EGFR and α/β-importin dimers, ensuring nuclear transport of NLS-containing proteins, and they formed holes in lipid bilayers at endosomal pH and accumulated in the nuclei of A431 human epidermoid carcinoma cells (12).

Radionuclides

Sodium [^{125}I]iodide and sodium [^{131}I]iodide with a specific activity of 2,200 Ci/mmol and 1,200 Ci/mmol, respectively, were obtained from Perkin-Elmer Life and Analytical Sciences (Boston, MA). ^{211}At was produced at the Duke University Medical Center by bombarding a natural bismuth internal target with 28-MeV α-particles by way of the ^{209}Bi(α, 2n)^{211}At reaction and isolated from the cyclotron target using a dry distillation method (17).