Communications to the Editor

Exploiting Differences in Sialoside Expression for Selective Targeting of MRI Contrast Reagents

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Paramagnetic substances that are targeted to antigens on tumor cell surfaces should aid in the early detection and diagnosis of tumors by magnetic resonance imaging (MRI).[1,2] Some progress has been achieved utilizing these "contrast reagents" conjugated to monoclonal antibodies which bind to cell-surface antigens.[1,3] However, antibody targeting has been hampered by several factors, such as structural heterogeneity of the epitopes, low density of antigenic determinants ($<10^5$/cell), and antibody cross-reactivity in vivo.[1,2] These limitations have directed significant attention to alternative strategies for targeting diagnostic agents.

Tumor cell surfaces exhibit abnormal glycosylation in the form of overexpressed naturally occurring oligosaccharides as well as glycoforms that are normally expressed only during fetal development.[4] Many tumor-associated carbohydrate antigens possess the monosaccharide sialic acid, and indeed, the overexpression of sialic acid has been correlated with the malignant and metastatic phenotypes in epithelial-derived cancers from gastric, colon, pancreatic, liver, lung, prostate, and breast tissue, and in several types of leukemia.[5] The collective display of multiple sialylated antigens on a tumor cell can result in the presentation of up to 10^9 sialic acid residues per cell,[6] and can account for the broad distribution of the high sialic acid phenotype across many different types of cancers. Diagnostic strategies that target cells on the basis of sialic acid expression may therefore find broad utility in tumor diagnosis.

Here we report a strategy for the selective delivery of magnetic resonance contrast reagents to tumor cells that exploits instrinsic differences in sialic acid expression. The approach capitalizes on the unnatural substrate tolerance of the enzymes in the sialoside biosynthetic pathway, which allows the metabolic conversion of peracetylated *N*-levulinoylmannosamine (**1**) to the corresponding *N*-levulinoyl sialic acid (**2**) in human cells (Figure 1).[7,8] Not normally resident on cell surfaces, the ketone group within the levulinoyl side chain is chemically orthogonal to native cell surface components and provides a unique chemical target for covalent reaction with aminooxy- or hydrazide-functionalized probes.[7] Furthermore, ketones have been shown to undergo highly selective reactions with similar nucleophiles in vivo forming stable covalent adducts.[9] We hypothesized that ketone expression levels on cells treated with compound **1** would mirror intrinsic sialic acid expression levels, enabling preferential targeting of highly sialylated cells with an aminooxy-functionalized contrast reagent (Figure 1).

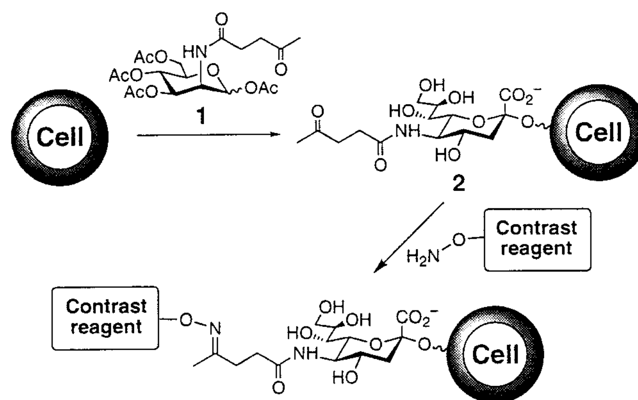

Figure 1. A strategy for the selective targeting of magnetic resonance contrast reagents to highly sialylated cancer cells.

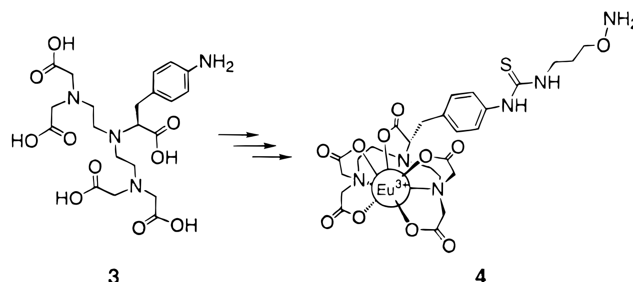

Figure 2. Compound **4** was synthesized from DTPA analogue **3**. The details of the synthesis are provided in the Supporting Information.

We synthesized an aminooxy-terminated analogue (**4**) of the clinically utilized contrast reagent Magnevist (GdDTPA) starting from DTPA derivative **3** reported previously by Rapoport and co-workers (Figure 2).[10,11] A Eu^{3+} ion was substituted for Gd^{3+} in this study because it has favorable fluorescence properties[12] which allowed facile detection and quantification. Cultured Jurkat cells, a heavily sialylated human T-cell lymphoma cell line, were grown in the presence or absence of compound **1** (50 μM) for 3 days and then treated with compound **4** at concentrations ranging

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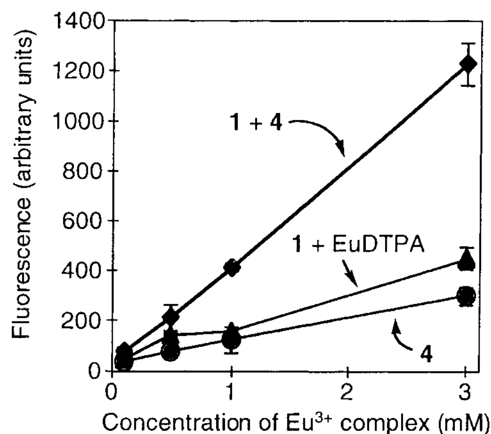

Figure 3. Dose-dependent targeting of compound **4** to cells requires the presence of cell-surface ketones and the aminooxy group of the Eu^{3+} complex. Error bars represent high and low values of duplicates, and similar results were obtained in three separate experiments.

from 0.1 to 3 mM.[13] Cells were then washed and suspended in a fluorescence enhancement solution reported by Lövgren and co-workers[14] and Eu^{3+} fluorescence was quantified. In a control experiment, Jurkat cells were treated with the Eu^{3+} analogue of Magnevist (EuDTPA).[15] As shown in Figure 3, localization of compound **4** on the cell surface was dependent on the presence of both the aminooxy group and cell-surface ketones. EuDTPA stained ketone-coated cells only at background levels, as did compound **4** with normal Jurkat cells that are devoid of ketones.

To investigate the selective targeting of compound **4** to cells on the basis of differential sialic acid levels, we developed an in vitro model of the high- and low-sialic acid phenotypes by selecting a mutant subline of Jurkat cells that exhibited decreased expression of the epitope Siaα2→3Gal found in several tumor antigens.[5,16] The relative amounts of this epitope on the mutant and normal Jurkat cells were determined by flow cytometry using a fluorescein isothiocyanate (FITC)-conjugated lectin, *Maakia amurensis agglutinin* (MAA), specific for Siaα2→3Gal.[17] Mutant cells exhibited over 20-fold lower reactivity with FITC-MAA, while the total number of cell surface sialic acid residues was reduced approximately 2-fold for the mutant cells as determined by the periodate-resorcinol assay (data not shown).[6]

When preincubated with compound **1** (50 μM, 3 days), the heavily sialylated normal Jurkat cells accumulated over twice the number of Eu^{3+} ions after treatment with compound **4** than the mutant Jurkat cells with lower sialic acid levels (Figure 4). Control cells treated with peracetylated *N*-pentanoylmannosamine (**5**), nearly identical to compound **1** but lacking the ketone group, displayed only a background level of contrast reagent staining thereby highlighting the requirement for the ketone as a cell surface target. We determined that approximately 4×10^6 Eu^{3+} complexes are targeted to a normal Jurkat cell. This value is within the range required for detection of relaxation enhancement which has been estimated at 1×10^6 chelates/cell, a value that is difficult to obtain with antibody conjugates.[1,2,18] If a similar level of cell surface localization is manifested in vivo, we calculate that a concentration of Gd^{3+} chelates between 10 and 20 μM can be achieved, which falls within the useful range for MRI of 10−100 μM.[1,2,19]

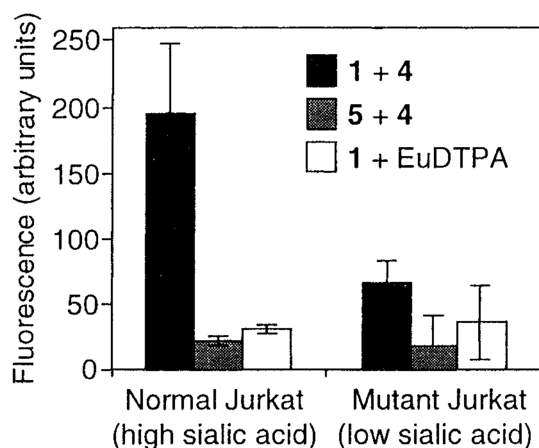

Figure 4. Compound **4** selectively targets cells that are higher in Siaα2→3Gal epitopes. Error bars represent high and low values of duplicates, and similar results were obtained in three separate experiments.

In conclusion, by exploiting the intrinsic promiscuity of the sialic acid biosynthetic pathway and the highly selective reaction of ketones with aminooxy groups, we were able to distinguish cells solely on the basis of subtle alterations in sialoside metabolism. The relaxivity and T_1 values for compound **4** in solution and immobilized on cells are presently under investigation and will allow further evaluation of this approach to tumor detection and diagnosis.

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Supporting Information Available: Synthetic procedures for compound **4** and experimental details for biological assays (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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