Cancer detection in mice using dendrimeric Gd-Peptide Nucleic Acid-Peptide molecular probes

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Introduction

Early diagnosis and staging of cell malignancy with MRI-based molecular probes is currently the topic of intensive research. It has been shown[1] that ^{64}Cu labeled chelator-PNA-peptide chimeras can be specifically accumulated in pancreas tumor xenograft in mice by penetrating through the cellular membranes by IGF1 receptor mediated endocytosis mechanism followed by binding *KRAS* mRNA target and by accumulation of these radioactive probes in the specific cancer cells. Conjugation of Gd-1-(amido)-1,4,7,10-tetraazacyclodecane-4,7,10-triacetate (Gd-DO3A) complex to mRNA-specific antisense peptide nucleic acids (PNA) with short IGFR-specific peptide (FIG. 1) might enable detection of pathogenic mRNAs in cells via MRI. PNAs have great potential for these applications because PNA is resistant to biological degradation and binds complementary mRNA with affinity, specificity, and stability exceeding those of corresponding DNA/RNA duplexes. Internalization of PNA into tumorigenic cells that overexpress insulin-like growth factor 1 (IGF1) receptors can be accomplished with a short IGF1 peptide analog conjugated to PNA. Non-hybridized probes will leave cells with little or no expression of the target mRNA in order to permit a specific image in the targeted cells. To increase the Gd-based contrast in the MRI signal, a novel PolyDiAmino-Propanoate (PDAP) dendrimer with up to 8 DO3A-chelator residues conjugated to PNA-peptide has been designed and synthesized[2]. We then investigated whether a $(Gd\text{-}DO3A)_2$-PDAP[1]-*KRAS* PNA-peptide or $(Gd\text{-}DO3A)_8$-PDAP[3]-*KRAS* PNA-peptide with 2 or 8 Gd ions per probe was taken up by pancreas cancer xenografts, enabling use of such chimeras for MRI experiments.

Methods

Relaxivity of the synthesized $(Gd)_2$-PNA-peptide, $(Gd)_8$-PNA-peptide, and control Gd-DTPA was measured on the 3 T Philips Achieva system by measuring T_1 at Gd(III) concentrations of 0.001, 0.01, 0.025, 0.05, 0.1, 0.25 and 0.5 mM at room temperature using an IR-TSE sequence using a set of inversion times ranging from 22 ms to 900 ms. We used a 4cm-diameter solenoid coil for detection. Relaxivity r_1 was determined based on the equation $(1/T_1)_{obs} = (1/T_1)_d + r_1[Gd]$.

Female NCr nude mice were implanted with malignant cells subcutaneously in the flanks. Tumor size reached 0.5–1.5 cm in diameter 7-14 days after cell implantation. The contrast agents were injected intravenously in the tail vein at a dose of 0.06 or 0.03 mmol Gd/kg for $(Gd)_2$-PNA-peptide and $(Gd)_8$-PNA-peptide, respectively. Images were acquired at 5, 10, 15, 20, 30, 45, 60 min, 2, 3, 24 and 48 h postinjection using a 3D T_1W high resolution isotropic volume exam (THRIVE) sequence. The imaging parameters were: TE 5.6-ms, TR 67-ms 15° flip angle, 65-mm FOV, resolution 0.19 x 0.19 x 1 mm.

Results

The relaxivity of $(Gd)_2$-PNA-peptide (7.00 ± 0.21 $mM^{-1}\,s^{-1}$) and $(Gd)_8$-PNA-peptide (6.83 ± 0.21 $mM^{-1}\,s^{-1}$) was higher than for Gd-DTPA (4.42 ± 0.13 $mM^{-1}\,s^{-1}$) . MRI results with $(Gd)_8$-PNA-peptide showed that 8 Gd ions in Gd-PNA-peptide probes was enough to get good contrast enhancement of the tumor, while $(Gd)_2$-PNA-peptide probes did not show any visible-contrast enhancement of the tumor. At 24 h after injection $(Gd)_8$-PNA-peptide produced significant contrast enhancement of the tumor tissues, which slightly continued to increase at 48 h postinjection (FIG. 2). The normal muscle intensity did not show significant contrast enhancement at 3, 24 or 48 h after administration. Figure 3A shows the percentage contrast enhancement in the different tissues (tumor, muscle, kidney) over time after injection of the contrast agent $(Gd)_8$-PNA-peptide. At 1 h after injection contrast enhancement began to increase and reached a value +32, +75 and +98 % at 3, 24 and 48 h after injection, respectively, that is essentially higher than enhancement in the muscle (+18.1 and +28.8 % at 23 and 48 h) at the same time regions. A tumor/muscle intensity ratio (Figure 3B) shows specific accumulation of the contrast agent in the tumor tissue in comparison with the inert muscle tissue. A plot of the tumor/muscle intensity ratio for $(Gd\text{-}DO3A)_8$-PNA-peptide contrast agent showed an initial spike, followed by slower accumulation continuing through 48 h, where the tumor/muscle intensity ratio reached a value 1.28, 1.71 and 1.77 at 3, 24 and 48 h. In conclusion, our results show that $(Gd)_8$-PDAP[m] dendrimers conjugated to mRNA specific antisense PNA-peptides may be used as MRI molecular probes for cancer detection.

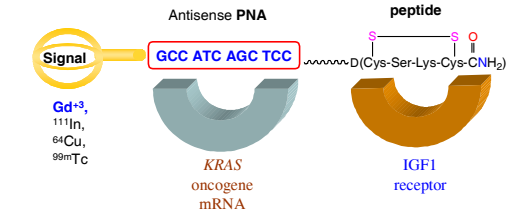


Figure 1. Design of the PNA-peptide chimeras for non-invasive molecular imaging of oncogenenic expression. For delivery of the labeled antisense PNA to the cancer cells, a small version of the IGF1 peptide analog, D(Cys-Ser-Lys-Cys) [D(CSKC)], was conjugated to PNA. We hypothesize that our PNA-peptide probes will bind to the IGF1 receptors on the surface of the cells, penetrate through membrane into the cells and then bind to the specific oncogene mRNA through the antisense PNA. The labeled PNA-peptide probes will then accumulate in the cancer cells but not in normal cells that lack the specific oncogenic mRNA.

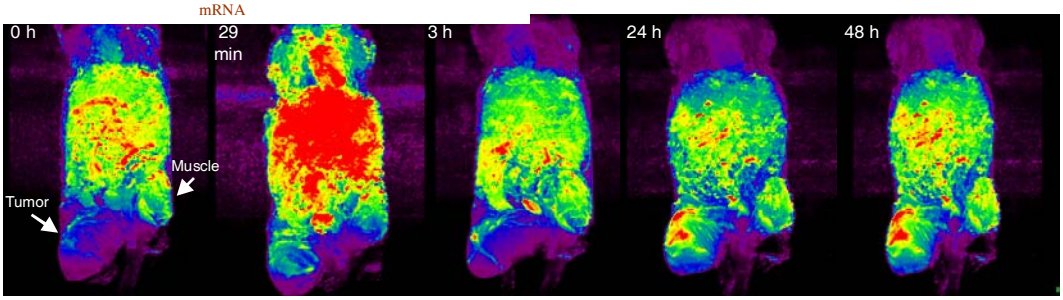
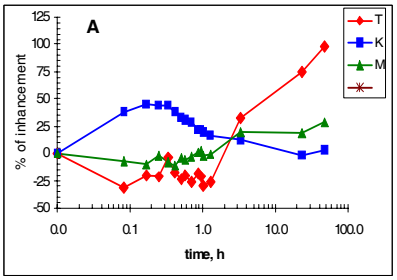


Figure 2. Contrast enhanced images of mouse bearing tumor using dendrimer $(Gd)_8$-*KRAS* PNA-peptide. The images were taken before contrast (0 h) and 29 min, 3 h, 24 h, 48 h postinjection. Preferential enhancement of the tumor with respect to the muscle is seen.



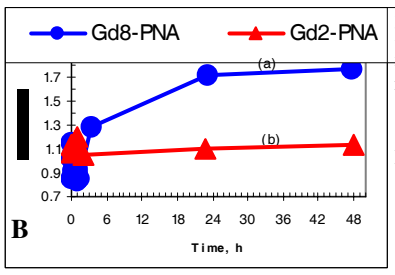


Figure 3. A. MRI contrast enhancement for different tissues (T–tumor, K–kidney, M–muscle) for $(Gd\text{-}DOTA)_8$-*KRAS* PNA-peptide over time after injection in mice bearing tumor xenograft. **B.**Tumor/muscle intensity ratios for $(Gd\text{-}DOTA)_8$-*KRAS* PNA-peptide and $(Gd\text{-}DOTA)_2$-*KRAS* PNA-peptide.

References: **1**. Chakrabarti, A., Aruva, M. R., Sajankila, S. P., Thakur, M. L., and Wickstrom, E. (2005) *Nucleosides, Nucleotides, and Nucleic Acids* 24(5):409-414. **2**. Amirkhanov, N. V. and Wickstrom, E. (2005) *Nucleosides, Nucleotides, and Nucleic Acids*, V. 24, No5-7, p. 423-426.