A novel solubility-switchable MRI agent allows the non-invasive detection of matrix metalloproteinase-2 activity in vivo in a mouse model

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Introduction

The most commonly used cancer prognostic factors are tumoral stage, grade and histology. Still, therapy outcome varies greatly despite similar tumoral characteristics (1). Matrix metalloproteinase-2 was proposed as an additional marker of tumoral progression which could help therapists select an optimal anti-cancer treatment (2). While MMP-2 activity was correlated *in vitro* and *in vivo* with increased invasiveness of cells and malignant progression (3), MMP inhibitors (MMPi) trials ended into failures (4). A number of reasons for this were proposed, including the absence of a tool to monitor MMP activity and MMPi performance. Recently, a contrast agent (CA) incorporating a solubility switch was developed and used to detect MMP-7 expression in a human tumor xenograft mouse model (5). Cleavage of the agent caused a decrease in its solubility which resulted in its accumulation at the cleavage site. We improved on the concept and designed a novel molecule allowing for the non-invasive detection of MMP-2 activity *in vivo*. Our results show that our agent possesses clearly distinct pharmacokinetics in tumors with elevated (wild-type, WT) or low (knockdown, KD) MMP-2 levels.

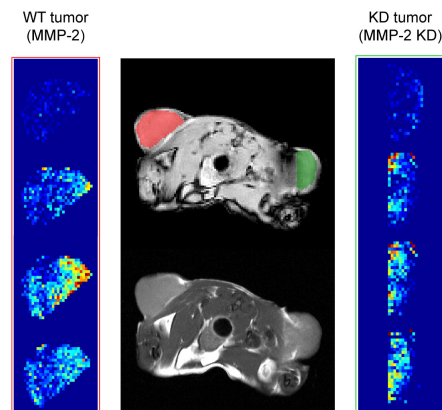

***Figure 1**. T_1-weighted (middle top) after injection of PCA2-switch and the corresponding pre-injection T_2-weighted (middle bottom) axial image for one slice of one animal. The left and right columns are composed of relative ΔR_1 maps for the WT and the KD tumor, respectively at four different time points (0, 16, 42 and 76 min).*

Materials and methods

The novel contrast agent (PCA2-switch, for Protease-modulated Contrast Agent) and a scrambled (i.e., not specifically cleaved) version of this agent (PCA2-scrambled) were both designed and synthesized in our laboratory. Briefly, the agent formulation was Gd-DOTA – $(CH_2)_{12}$ - SPAYYTAA – PEG_8. MC7-L1 cells were modified with shRNA to produce a MMP-2 KD cell line (KD tumors). Two tumors, made of WT or KD MC7-L1 cells, were inoculated subcutaneously in the hind limbs of Balb/c mice (syngenic model) and left to grow for one month. Acquisition of data before, during and after i.v. injection of the PCAs allowed us to evaluate their pharmacokinetics. A small animal 7T scanner (Varian Inc. Palo Alto, CA, USA) with a 40 mm volume RF coil was used. Dynamic contrast-enhanced (DCE) MRI studies included the acquisition of a pre-contrast T_1 ($T_{1,0} = 1/R_{1,0}$) map using a multiple flip angle approach and a gradient echo sequence with TR: 100 ms, TE: 2.49 ms, matrix: 128 × 128, FOV: 30 × 30 mm^2, NA: 4, 10 slices 1.5 mm thick. Sets of T_1-weighted images using the same sequence were acquired continuously before, during and after injection of CA. The R_1 values were calibrated to account for perfusion differences.

Results

As can be seen in figure 1, a significant increase in relative ΔR_1 between 10 minutes and 40 minutes after injection is observed for the WT tumor (left column, 2nd and 3rd images), compared with the KD tumor (right column, 2nd and 3rd images). Figure 2 shows the relative ΔR_1 progression as a function of time in multiple animals (n=8) injected with PCA2-switch. Statistical difference between the pharmacokinetics in WT and KD tumors was confirmed with an unequal variance T-test ($p < 0.05$). In order to test the specificity of the agent, a negative control was performed with PCA2-scrambled. Figure 3 shows the results with both PCA2-switch and PCA2-scrambled in a single animal. As can be seen, similar pharmacokinetics are observed in both tumors with the scrambled agent. On the other hand, PCA2-switch allows the detection of low MMP-2 activity levels in the KD tumor, and high MMP-2 levels in the WT tumor. Variation in the pharmacokinetics between tumors and agents were confirmed by slope comparison in four animals (ANOVA, $P < 0.05$).

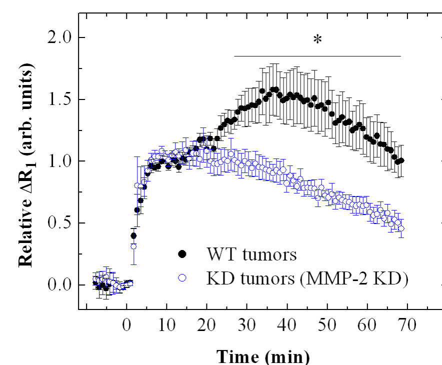

***Figure 2**. Comparison between WT and KD tumors (n = 8) with PCA2-switch. The relative change in relaxation rate (ΔR_1) normalized to the initial increase enables a direct comparison between animals and tumors. Both tumors are perfused by PCA2-switch, but while ΔR_1 decreases after 10 min in the KD tumor, a significant ΔR_1 increase is observed in the WT tumor. The kinetics differ significantly (T-Test, $p < 0.05$) under the line marked with an asterisk.*

Discussion

MMP-2 activity is usually assessed *in vitro* by zymography (6), and MMP-2 expression by immunochemistry (7). While these assays allowed correlating MMP-2 activity and presence with tumor malignancy, they require invasive procedures, limiting MMP-2 activity monitoring in long term studies. Our results indicate that PCA2-switch allows differentiating between low (KD tumors) and high (WT tumors) MMP-2 activity, compared with the pharmacokinetic observed with the scrambled version of the agent.

Conclusion

We designed a novel CA allowing the detection of MMP-2 activity in a tumor mouse model. The agent becomes more hydrophobic after its cleavage by this enzyme, causing its accumulation at the cleavage site and enhancing the MRI signal This activation occurred much less in tumors grown out of MMP-2 KD cells, or with the scrambled version of the agent.

Bibliography

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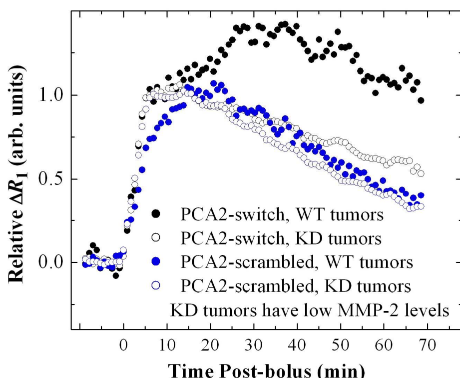

***Figure 3**. Pharmacokinetic comparison of PCA2-switch and PCA2-scrambled in WT and KD tumors in a single animal. The relative change in relaxation rate (ΔR_1) is normalised to the initial increase (not shown in this figure). The post-bolus kinetic is similar in both tumors with PCA2-scrambled (negative control). A slower ΔR_1 decrease is observed with PCA2-switch in the KD tumor, while a ΔR_1 increase is measured in WT tumors.*