Novel solubility switch contrast agents: *in vivo* detection of MMP-7 activity

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Introduction: We propose a novel concept to detect the activity of enzymes and proteases *in vivo* using MRI. The concept is based on the solubility switching of a contrast agent upon interaction with the selected enzyme or protein. This solubility switch, from hydrophilic to hydrophobic, modifies significantly the pharmacokinetic properties of the agent, which can be detected from the associated slow kinetics from the activity site. Using this concept, we designed an agent (PCA7-switch) that was able to detect *in vivo*, in mice, the activity of the secreted matrix-degrading proteinase, matrix-metalloproteinase-7 (MMP-7), an enzyme playing an important role in the tumor progression and metastasis. Control experiments were performed using an agent that did not interact with MMP-7 (PCA7-scrambled), or agents that could be cleaved by MMP-7 but lacking the solubility switch, or a standard contrast agent (Gd-DTPA, Berlex Inc.). Finally, PCA7-switch was injected to mice previously treated with a MMP inhibitor.

Experimental Protocol: Athymic nude mice were injected subcutaneously on the left hind limb with 1.5 x 10^6 parental control SW480 colon cancer cells (MMP-7-negative), and 1.0 x 10^6 SW480Mat cells (MMP-7-positive) on the right hind limb. These cells grow to approximately 1.5 cm^3 tumors within 4 weeks. Three days prior to imaging, the mice were surgically implanted with a jugular vein catheter, via which the contrast agents were injected. The contrast agents were composed of a peptide sequence that could be cleaved by MMP-7, or a peptide sequence that was uncleavable by MMP-7. A Gd-DOTA moiety was linked to one end of the peptide via a hydrophobic alkyl chain. A polyethylene glycol (PEG) chain was coupled to the other end of the peptide, resulting in a global initial hydrophilicity of the compounds. HPLC *in vitro* assays confirmed a change in retention time of the compound after cleavage by MMP-7. The enhancement pattern in the tumors were analysed for both the core and the rim of the tumors.

Results and Discussions: The MMP-7-positive and negative subcutaneous tumors are indicated in Fig. 1. These images show that a signal enhancement is visible in both tumors. The principle of the agent is to exhibit a change in pharmacodynamic properties after cleavage. Thus, the enhancement pattern of both tumors as a function of time must be examined. Fig. 2 shows the comparison of the enhancement patterns for the same animal injected with PCA7-switch followed 2 days later by PCA7-scrambled. Similar patterns are observed in the rim of both tumors, where dense micro-capillaries account for an efficient supply and washout of contrast agent. The situation is quite different in the core of the tumors when PCA7-switch is injected (panel A). An accumulation of contrast agent continuing after 140 minutes is perceived for the MMP-7-positive, whereas a decrease in intensity is observed after 80 minutes for the MMP-7-negative tumor. In contrast, a similar enhancement pattern is observed for both tumors when PCA7-scrambled is injected. Further controls using a cleavable agent without a solubility switch and using Gd-DTPA on a total of 12 mice support these observations. We note that tumor size could not explain the behavior, which was seen in mice having either a larger or smaller MMP-7-positive tumor compared to the MMP-7-negative tumor.

The selectivity for MMP-7 was verified by injected PCA7-switch after a mouse was treated with MMP inhibitors, effectively turning off the activity of MMP-7. In that case, no difference in the enhancement patterns could be seen between tumors.

Our interpretation of the data is that the agents leak out of the neovessels in tumor into the interstitial space, where they can interact with MMP-7. Cleavage products generated in the rim of the tumor, where perfusion is high, can be washed out, even with a reduced hydrophilicity. Cleavage products in the core of the tumor, on the contrary, accumulate and are present on a longer time scale. Uncleavable compounds such as PCA7-scrambled do not show the same accumulation in the core of the positive tumors.

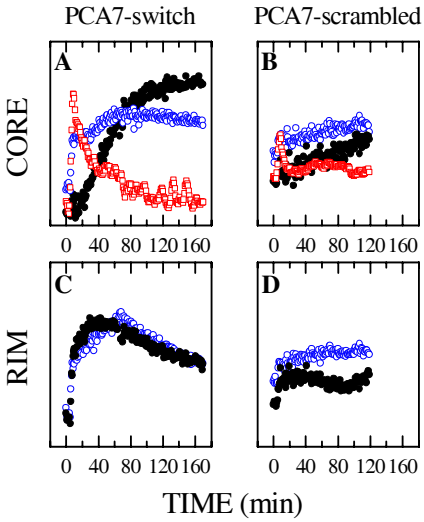

Fig. 2. Comparison of PCA7-switch and PCA7-scrambled in MMP-7-positive and MMP-7-negative tumors. The enhancement patterns in the core (top row) and rim (bottom row) of MMP-7-positive (black circles, 111 mm^3), MMP-7-negative (blue circles, 224 mm^3) tumors and dorsal muscle (red squares).

Conclusion: We detected the activity of MMP-7 *in vivo* using our novel "solubility switch" concept. The concept is flexible since the cleavable peptide sequence can be chosen to achieve a tunable level of specificity for a variety of enzymes.

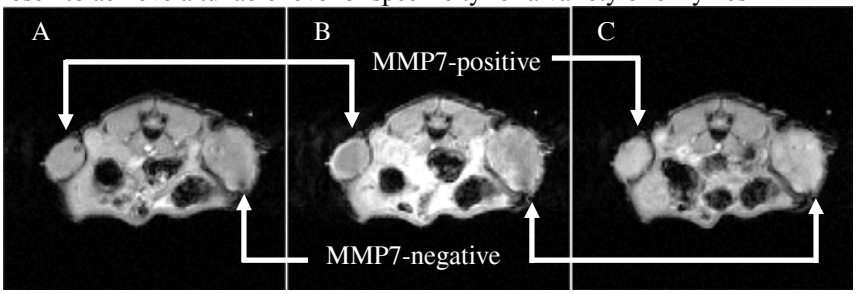

Fig. 1. Representative axial images from a mouse A) before and B) 16 and C) 170 minutes after injection of PCA-7-switch.