A zinc-sensitive MRI contrast agent differentiates healthy from cancerous prostate in a transgenic prostate cancer model

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Introduction: The prostate has the highest levels of Zn(II) in the organism and there are marked differences in content between the healthy, malignant, and benign hyperplastic prostate. Given that accurate differential diagnosis between these conditions is difficult non-invasively, we introduce prostate Zn(II) as a MRI imaging biomarker. In this work we use a Gd-based zinc sensor that can sensitively detect glucose-stimulated intracellular release of Zn(II) in the healthy, and malignant mouse prostate using a transgenic adenocarcinoma of the mouse model.

Methods: **Synthesis:** Gd-CP027 was synthesized and characterized using published protocols1. **In vitro fluorescence:** Normal human prostate epithelial cells (ATCC, VA, USA) were cultured using vendor protocols. Cells were incubated with 75µM ZnSO4 added directly into the medium for 72 hours at 37°C prior to study. The fluorescent zinc probe, ZIMIR, kindly provided by Dr. Li at UT Southwestern was added to the cells to form a final concentration of 1 µM. Cells were then incubated at room temperature for 20 minutes and washed with SAB before glucose challenge. Zn(II) secretion in response to glucose was observed after adding 18mM D-glucose along with 10µM EDTA and 2µM DPAS. Epifluorescence images post-stimulation were obtained with an inverted Nikon wide-field fluorescence microscope. **In vivo MRI:** All animal experiments were performed in accordance to approved guidelines by the UT Southwestern IACUC committee. Ten TRAMP mice of the strain C57BL/6-Tg(TRAMP)8247Ng/J (Jackson Laboratories) were fasted for at least 12 hours; the animals were anaesthetized with isofluorane and catheterized via the tail vein. Two pre-injection 3D T1-weighted gradient echo scans were obtained (TE/TR 1.69/3.34ms, NEX4, Matrix 128x128x128) using a 9.4T Varian MRI scanner. After 50µl of 20% w/v D-glucose IP, 0.07mmol/Kg Gd-CP027 was injected IV using a syringe pump at a rate of 70µl/min. Immediately after injection, a series of 3D T1-weighted scans were obtained to observe the dynamic glucose response and Zn(II) release in the prostate. The imaging protocol was repeated every two weeks until tumors were found.

Results: The zinc sensor displays an increase in T1 relaxivity at 0.5 T of almost 8-fold upon binding to Zn(II) and human serum albumin (HSA) as seen in the schematic in Figure 1A. Although this is dramatically affected at higher magnetic fields the “on” 9.4 T relaxivity of 9.4mM⁻¹s⁻¹ is still sufficiently sensitive to obtain an indirect measurement of prostatic zinc in vivo. Prostate epithelial intracellular zinc is mainly stored in zinc-rich granules and bound to a series to metalloproteins and citrate2, in this work we discovered that the use of D-glucose as an external secretagogue stimulates Zn(II) secretion in the prostate and thus make Zn(II) readily available to our Gd-based sensor. In Figure 1B we see this phenomenon in RWPE-1 cells where the cells were cultured in low glucose conditions and provided with a zinc supplement available for intracellular storage. The zinc fluorescent probe, ZIMIR, anchors its lipophilic side-chains to cellular membranes and therefore is ideal to observe the movement of Zn(II) ions from inside the cell to its surroundings as a response to D-glucose. Figure 1B shows that approximately 5 minutes after addition of D-glucose to the medium we observe clear fluorescence emitted by the probe and thus validating the concept that a sudden increase in D-glucose stimulates the Zn(II) secreting mechanisms in prostate epithelial cells.

Discussion: The use of Zn(II) to differentiate between healthy prostate cells and aberrant cells was tested here by monitoring the spontaneous tumor formation in a TRAMP model. The mice were scanned upon arrival at 7-11 weeks old and subsequently every 1-2 weeks. Figure 1C shows three sets of representative mice, each showing distinctive stages of tumor formation. The bottom panel displays ~50% prostate enhancement as a result of D-glucose IP and Gd-CP027 IV injection, this enhancement may indicate that the levels of zinc are not yet depleted and thus characteristic of healthy prostate. Middle panel, mouse at 11 weeks of age, show a hypotense region in the dorsolateral lobe of the prostate, indicative of cells deficient in Zn(II), top panel, mouse at 19 weeks of age, shows a large tumor stemming from the dorsolateral lobe of the prostate, it is evident that our zinc sensitive contrast agent still enhances the minimal healthy prostate left but fails to enhance the tumor theoretically due to the lack of zinc present in the tissue.

Conclusion: We conclude that our Gd-based zinc sensor successfully differentiates healthy prostate tissue from prostate cancer tissue in the TRAMP model in vivo. Capitalizing on the known differences in Zn(II) content1 and that there is no current technique to differentiate between normal, malignant, and benign hyperplastic prostate tissue non-invasively, this lays the foundation for accurate differential diagnosis using glucose-stimulated release of Zn(II) as an imaging biomarker of disease.


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**Figure 1.** A) Zinc detection mechanism with Gd-based contrast agent. B) **In vitro** epifluorescence of normal human prostate epithelial cells (RWPE-1) cultured with 75µM ZnSO4 for 72 hrs, Zn(II) secretion stimulated by 18mM D-Glucose. Zn(II) secretion observed with fluorescence probe (ZIMIR). C) 3D T1-weighted MRI of the TRAMP prostate cancer model at different stages of disease.