In vivo characterisation of orthotopic prostate tumor and healthy rat prostate metabolism using ^{1}H MRS at 4.7 T

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Introduction

Prostate cancer (PCa) is the second cause of death by cancer, and there is a need for better diagnostic and therapeutic efficacy biomarkers. Magnetic resonance techniques are increasingly gaining recognition as important tools for detection, localisation and staging of primary and recurrent PCa. Indeed, the increasing use of techniques such as perfusion-weighted and diffusion-weighted MRI, as well as magnetic resonance spectroscopy(^{1}H MRS), have been shown to offer some improvement in sensitivity and specificity for prostate cancer localisation. In particular, ^{1}H MRS enables the non-invasive study of prostate metabolites, including citrate (Cit), polyamines (PA), choline-containing compounds (tCho) and creatine/phosphocreatine (tCr), and the (tCho+tCr)/Citrate ratio would appear to be a sensitive biomarker of the presence of cancer in men in the clinic. In addition, to assist the development of new anti-cancer drugs, it is important to identify biomarkers of treatment efficacy early in the preclinical and early clinical phases of drug development. In addition, more realistic animal models are needed i.e. tumors xenografted directly on the prostate gland of rodents, with dedicated measurement protocols, which may be technically difficult due to the specific localisation of the prostate. The aim of this study was to establish such an experimental setting, compatible with drug development protocols (short and repeatable imaging sessions), which allows *in vivo* monitoring of the metabolism of orthotopic prostate cancer model as well as the host gland using conventional T_1 and T_2 weighted MRI and ^{1}H MRS.

Materials and Methods

The evolution of healthy prostate metabolism was assessed on 3 *Nude* rats by MRI/MRS at 7, 9 and 12 weeks of age. Spectroscopy was performed in the dorsal (DP) and ventral (VP) prostate lobes. Tumor metabolism was assessed on *Nude* rats bearing orthotopic PC3-MM2 human prostate tumors. Tumor volume and metabolism were assessed by MRI/MRS 6, 9, 15 and 21 days after injection of PC3-MM2 cells in the ventral lobe of the prostate of 3 *Nude* rats. The metabolism of the DP was also explored in the tumor-bearing rats. *In vivo* imaging and spectroscopy were performed on a 4.7T Pharmascan (Bruker). Animals were maintained under anaesthesia via a constant flow of isoflurane at 2-3% delivered by a nose cone. Sagittal T_1-weighted and axial T_2-weighted images were acquired to assess tumor volume and to allow positioning of the spectroscopy volume of interest. Spectroscopy was achieved using a single voxel PRESS sequence (TE=11ms/TR=2500ms) in voxels of 8 to 30 mm^3 and spectra were acquired with (NA=256) and without water suppression (NA=8). Spectra were analyzed using LCModel (1). Concentrations provided by LCModel were normalised with respect to tissue water. The following metabolites were quantified on all spectra: tCr, inositol (Ins), tCho and three lipid resonances at 2.0 ppm (L20), 1.3 ppm (L13), and 0.9 ppm (L09).

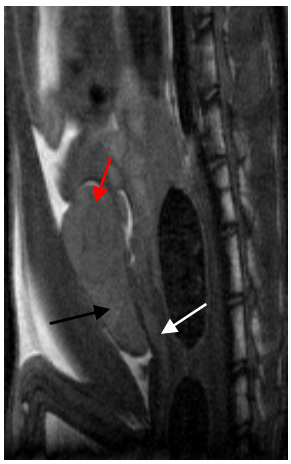

Figure 1: Sagittal T1-weighted image of a rat bearing a PC3-MM2 tumor, 15 days after tumor implantation. Dorsal Prostate lobe (white arrow), Ventral Prostate lobe (black), tumor (red).

Results

The metabolic content of the two lobes of the healthy rat prostate differs considerably, with a significantly lower Ins and tCho content in the VP (Ins: 5.8±3.4; tCho: 5.6±0.9) compared to DP (Ins: 19.1±1.3; tCho: 15.9±0.8; p<0.003). Despite a 3-fold increase in volume of both lobes (DP: 42±8 to154±11; VP: 136±12 to 273±28 mm^3), no significant changes in the metabolic content occurred between 7 and 12 weeks of age.

No significant difference was observed between the DP of healthy rats and of rats bearing PC3-MM2 tumors. PC3-MM2 shows a statistically lower tCr content (3.1±0.9) compared with healthy DP (12.8±1.8) and VP (14.8±2.9), whereas tCho (3.5±0.9) and Ins (4.3±2.8) were close to that found in VP of healthy rats. The metabolic content of the tumor appeared to be stable during the two-week follow up while its volume observed a nine-fold increase (152±48 to 1390±226 mm^3).

Discussion

To our knowledge, there have been very few *in vivo* studies on rodent prostate with or without orthotopic cancer using proton spectroscopy. One striking feature of the rat prostate metabolism as observed by ^{1}H MRS is that it does not resemble in any way that of humans. Whereas the citrate resonance largely dominates the ^{1}H spectrum in healthy human prostate, we have been unable to detect it in healthy rat prostate, confirming previous results from other teams that have measured a lower prostate Cit content in rat than in humans (2). In addition, rat prostate presents two lobes with very different metabolic content. The quantification of tCr does show differences between VP of healthy rats and PC3-MM2 tumor implanted on the VP of the rats. Moreover, the metabolic profiles of the VP and of the PC3-MM2 tumor appear very different (Fig 2). The complexity of these differences highlights the need for an improved characterisation of the metabolic profile, based on the global spectra.

Conclusion

In summary, we have shown that the *in vivo* study of an orthotopic prostate cancer model and healthy prostate is feasible in rats. We suggest a complete follow-up protocol using ^{1}H MRS of the rat prostate. Such baseline data could be important when following the modifications in metabolism during the course of a therapeutic treatment.

References

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2. Costello, Franklin, Urology 50, 3 (1997)

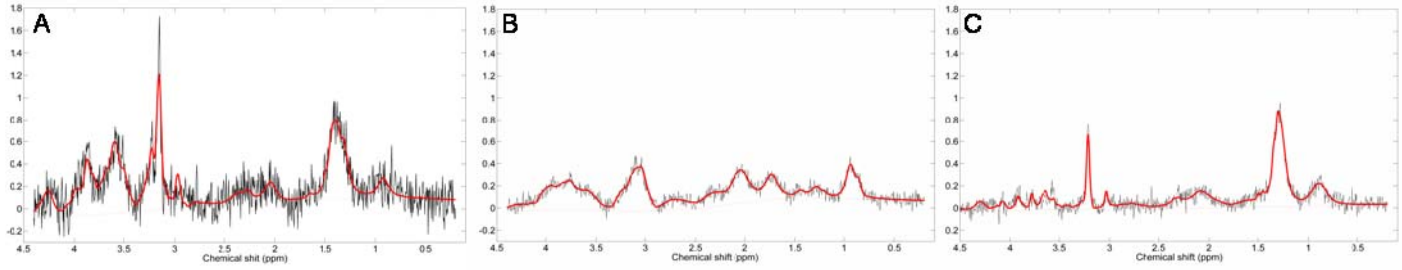

Figure 2: Representative spectra of (A) the dorsal lobe, (B) ventral lobe and (C) PC3-MM2 tumor. Spectra are normalized with respect to water. Original spectra in black and LC-Model fit in red.