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Prostate cancer metabolism assessment by proton magnetic resonance spectroscopy (^{1}H-MRS) and high resolution magic angle spinning spectroscopy (^{1}H-HRMAS) in experimental rat models

P. Provent[1], P. Walker[2], X. Tizon[1], N. Hoffmann[1], S. Parfait[2], V. Lemaitre[1], V. Bari[1], J. Miteran[2], A. Tabard[3], G. Créhange[4], O. Duchamp[1], P. Genne[1]

[1] Oncodesign Biotechnology, Dijon, France; [2] LE2I, Université de Bourgogne, UMR CNRS 5158, Dijon, France; [3] ICMUB , Université de Bourgogne, UMR CNRS 5260, Dijon, France; [4] Centre G-F Leclerc, Dijon, France

INTRODUCTION

Prostate cancer (PCa) is the second cause of death by cancer in men, and there is an essential need for diagnostic and therapeutic efficacy biomarkers. Magnetic resonance imaging (MRI) is frequently used for the detection and staging of PCa in patients. Recent developments in MRI and MR spectroscopy (MRS) have improved our understanding of tumor biology and tumor metabolism and have enabled the characterization of PCa. In this study, we investigate the effect of radiotherapy on the metabolism of rat prostate and on the metabolism of human prostate tumor subcutaneously (SC) and orthotopically (OT) xenografted in nude rats.

OBJECTIVES

- Demonstrate the value of ^{1}H-MRS to measure metabolic profiles and to study the effect of radiotherapy on these profiles in rat prostate cancer models.
- Demonstrate the value of ex-vivo ^{1}H-HRMAS to confirm *in vivo* ^{1}H-MRS results
- Study choline content changes with age and differences in metabolism between dorsal and ventral lobes in healthy rat prostate.

METHODOLOGY

Procedures were performed according to ethical guidelines concerning animal care and handling.

- ***Healthy prostate metabolism assessed by ^{1}H-MRS and ^{1}H-HRMAS***

Nude male rats 5-7 weeks and 10-12 weeks of age underwent *in vivo* spectroscopy. Data were acquired from one voxel in both the ventral and dorsal lobes of the prostate. The ventral and dorsal prostate lobes were then removed surgically and small representative samples were placed in D_2O in an HRMAS insert and frozen until HRMAS analysis.

- ***Radiotherapy effect on prostate tumor model***

Radiotherapy anti-tumor efficacy was evaluated by measuring tumor volume in two human prostate models: PC3-MM2, a hormone-independent model and PAC-120 a primary hormone-dependent model.

- ***Subcutaneous models: PC3-MM2 and PAC-120***

PC3-MM2 cells and PAC-120 tumor fragments were injected/implanted subcutaneously in groups of 12 or 18 *Nude* rats. Tumor-bearing rats were ranked according to tumor volume and randomized 5 days (PC3-MM2) or 39 days (PAC-120) after inoculation in 2 groups. Animals received 5 daily fractions of 2Gy by HDR-brachytherapy (RT group), or no treatment (CTRL group).

- ***Orthotopic model: PC3-MM2***

PC3-MM2 cells were injected in the ventral lobe of the prostate of 10 *Nude* rats. Tumor-bearing rats were ranked according to body weight and randomized 6 days after inoculation. Five rats (RT group) received 5 daily fractions of 2Gy by X6 external beam irradiation, and 4 rats received no treatment (CTRL group). Tumor volume and metabolism were followed by MRI and MRS at treatment start and 4, 9 and 15 days after treatment start in 3 rats in each group.

- ***^{1}H-MRS***

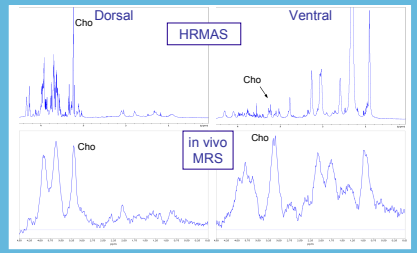
In vivo imaging and spectroscopy were performed on a 4.7T Pharmascan (Bruker). During the imaging protocol, the animals were maintained under anaesthesia via a constant flow of isoflurane at 2-3% delivered by a nose cone. Sagittal T1-weighted and axial T2-weighted images were acquired to assess tumor volume and to allow positioning of the spectroscopy volume of interest within the tumor. Spectroscopy was achieved using a single voxel PRESS sequence (TE=11ms/TR=2500ms/NA=512). Voxel size was between 15 mm^3 and 35 mm^3, depending on tumor size. Spectral data were analyzed using LCModel version 6.2 [1], and peak areas of total Choline, Lipids (0.9 ppm, 1.3 ppm and 2.0 ppm) and resonances at 3.6 and 3.8 ppm were measured. Spectra were corrected for eddy current using a water reference acquisition and concentrations were normalized using water content. Measurements of lipid contents provided by LC Model analysis were discarded because of variable lipid contamination from outside the volume of interest.

- ***^{1}H-HRMAS***

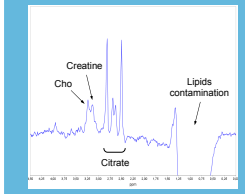
^{1}H-HRMAS was performed on a 14T Bruker Avance magnet equipped with a MAS probe. Samples were maintained at 4°C and spun at 4000 Hz during the experiment. Spectra were acquired after water presaturation (NA=128, SW=12500 Hz).

1. Provencher, Magn Res Med 30, 672 (1993).

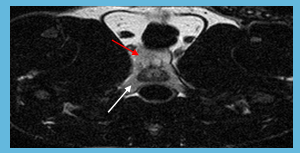
Fig. 1 - Healthy rat prostate: in vivo MRS and HR-MAS

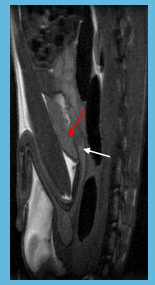


A - Healthy rat prostate: ^{1}H-HRMAS (top) and in vivo ^{1}H MRS (bottom) spectra from dorsal (left) and ventral (right) part of the prostate.

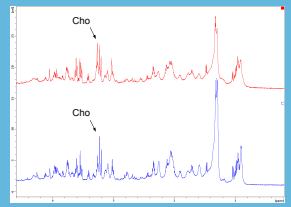


B - Human healthy prostate MRS spectrum.





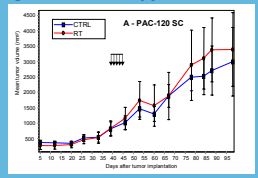
C - Rat prostate morphology: T1 sagittal (top) and T2 axial (bottom) images from a healthy rat. **Dorsal lobe. Ventral lobe.**

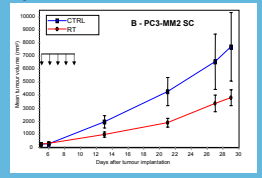


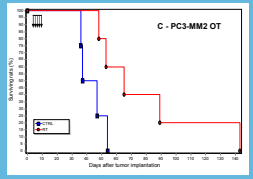
D - Healthy ventral prostate ^{1}H-HRMAS spectra from a 6 weeks (top) and a 11 weeks (bottom) old rat.

- **The rat prostate (fig. 1A) presents a metabolite profile that differs from human prostate (fig. 1B). Citrate, one of the main metabolites detected in normal human prostate is absent from normal rat prostate. High choline level is detected in normal rat prostate whereas elevated choline only appears in pathologic tissue in human prostate.**
- **Rat prostate is divided in ventral and dorsal lobes (fig. 1C). These two tissues exhibit different profiles as measured by MRS (fig. 1A): higher choline concentrations in dorsal lobe and presence of many resonances in the 3.4-3.9 ppm zone.**
- **There are no significant differences in rat prostate metabolism between 6 and 11 weeks old rats (fig. 1D).**

Fig. 2 - Radiotherapy antitumor activity

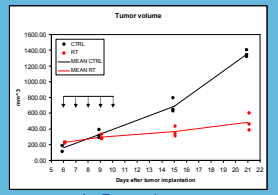




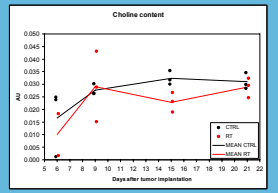


- **Radiotherapy did not show antitumor activity in PAC-120 SC-xenografted tumors (fig. 2A; T/C%=90%).**
- **Radiotherapy showed a significant antitumor activity in PC3-MM2 in both subcutaneous (fig. 2B; T/C%=38%) and orthotopic (fig. 2C; ILS%=58%) tumor xenografts.**

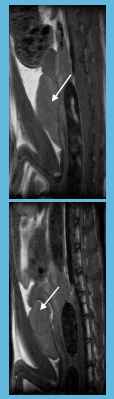
Fig. 3 – MR spectroscopy to follow radiotherapy antitumor activity on OT PC3-MM2 model

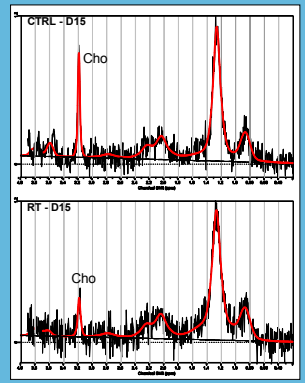


A – Tumor volumes



B - Choline content in arbitrary units as measured by LC Model.





C - Representative sagittal images (left) and in vivo spectra (right) of PC3-MM2 OT tumors, 9 days after treatment start from one rat from the control group and one rat treated by radiotherapy.

White arrows: tumor - Raw spectra, LC Model fit

Radiotherapy induced a decrease in total choline resonance detected at 3.2 ppm 4 days after the end of radiotherapy treatment (D15) in the PC3-MM2 OT model (fig. 3B).

CONCLUSIONS

- **^{1}H-MRS is a robust technique to assess *in vivo* normal and tumoral metabolism, and it might be efficient to detect choline-related metabolic changes after radiotherapy.**
- **Ex vivo and in vivo spectroscopy in rats showed significant differences with human prostate metabolic profile, and these results need to be taken into account for future studies using spectroscopy as a biomarker of therapeutic efficacy in animal models of PCa.**