IN VIVO CHARACTERISATION OF ORTHOTOPIC PROSTATE TUMOR AND HEALTHY RAT PROSTATE METABOLISM USING ^{1}H-MRS AT 4.7 T

X. Tizon[1], P. Provent[1], S. Parfait[2,3], G. Créhange[4], J. Mitéran[2,3], P. Genne[1], F. Brunotte[2,3,4], O. Duchamp[1], P. Walker[2,3]

(1) Onco*design*® , Dijon, France
(2) Université de Bourgogne, LE2I, Dijon, France
(3) CNRS UMR 5158, Dijon, France
(4) Anticancer Center Georges-François Leclerc, Dijon, France

Laboratoire Electronique Informatique et Image — Le2i
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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. Indeed, the increasing use ^{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 in the preclinical and early clinical phases of drug development. In order to improve the predictivity of preclinical experiments, more realistic animal models are needed, for example tumors xenografted directly on the prostate gland of rodents. These animal models require 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 anti-cancer drug development protocols (short and repeatable imaging sessions),
- Allowing *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 singel-voxel ^{1}H-MRS.

MATERIALS & 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 4.7 T Pharmascan (Bruker)

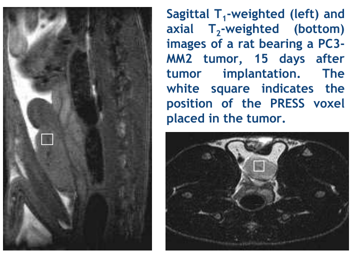
Animals were maintained under anaesthesia via a constant flow of isoflurane at 2-3% delivered by a nose cone.

Imaging protocol

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 mm³ and spectra were acquired with (NA=256) and without water suppression (NA=8).

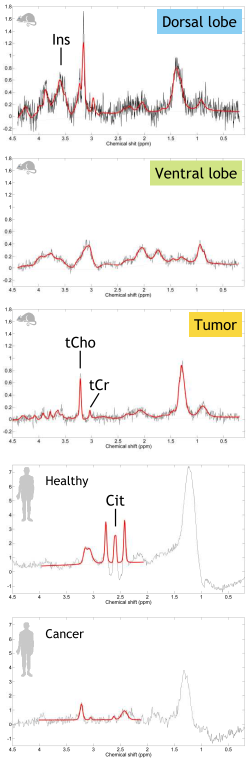
Data analysis

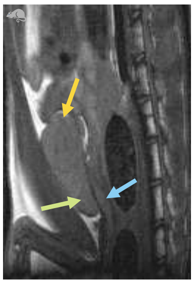
Spectra were analyzed using LCModel [1]. Concentrations provided by LCModel were normalised with respect to tissue water. The following metabolites were quantified on all spectra: tCr, inositol (Ins), tCho and three lipid resonances at 2.0 ppm (L20), 1.3 ppm (L13), and 0.9 ppm (L09).



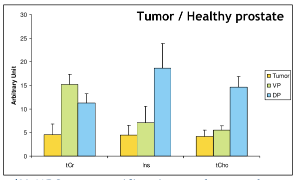
Sagittal T_1-weighted (left) and axial T_2-weighted (bottom) images of a rat bearing a PC3-MM2 tumor, 15 days after tumor implantation. The white square indicates the position of the PRESS voxel placed in the tumor.

RESULT 1: RAT PROSTATE CANCER MODEL IS SIMILAR TO HUMAN PROSTATE CANCER

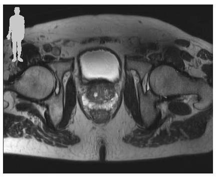




Sagittal T_1-weighted image of a rat bearing a PC3-MM2 tumor, 15 days after tumor implantation. Dorsal Prostate lobe (blue arrow), Ventral Prostate lobe (black), tumor (red). Corresponding ^{1}H-MRS spectra are shown on the left (black: raw data, red: fitted with LCModel).



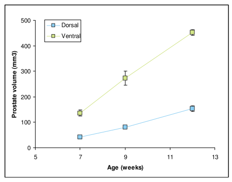
^{1}H-MRS quantification does show differences between the prostate of healthy rats and PC3-MM2 tumor implanted on the VP of the rats.



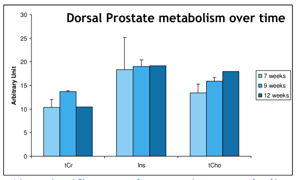
Axial T_1-weighted image of a human prostate. Upper left: healthy ^{1}H-MRS spectrum. Lower left: 1H-MRS spectrum measured in a cancerous prostate (black: raw data, red: fitted with LCModel).

- Citrate in rat prostate is not detectable, confirming previous results [2]
- Tumor metabolism measured by ^{1}H-MRS is different from the metabolism of its host gland, making this biological feature a good candidate to follow treatment effects
- ^{1}H-MRS spectra of human cancer and orthotopic *Nude* rat cancer model are similar

RESULT 2: PERFORMING LONGITUDINAL FOLLOW-UP IS FEASIBLE

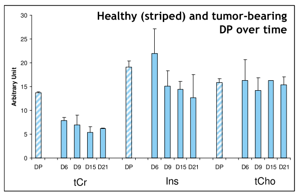


Dorsal and ventral lobe volumes of healthy rats increased 3-fold between 7 and 12 weeks of age.



No significant change in metabolic content was observed in the dorsal lobe of healthy rats between 7 and 12 weeks of age.

- Prostate growth does not influence the metabolic content of the prostate lobes over a period suitable for anticancer efficacy studies

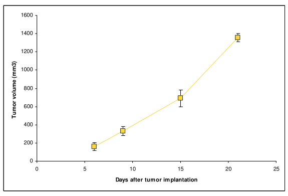


There is no difference in DP metabolism between healthy rats and tumor-bearing rats, measured during tumor growth.

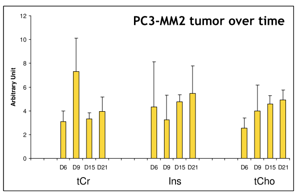
DP metabolism in tumor-bearing rats is also stable during tumor growth.

- Tumor metabolism is stable during its growth
- DP metabolism is also stable, allowing its use to assess treatment toxicity

RESULT 3: TOWARDS MEASURING THE EFFICACY OF A TREATMENT



Rapid growth of PC3-MM2 tumors implanted orthotopically in the ventral lobe of the prostate.



PC3-MM2 tumor metabolism shows no variation during its growth phase, from D6 to D21 after implantation.

CONCLUSION

Healthy rat and human prostates appear different as measured by ^{1}H-MRS. However, the described orthotopic model in rats shows ^{1}H-MRS spectra similar to these of human prostate cancer cases.
The complexity of these quantification tasks highlights the need for an improved characterisation of the ^{1}H-MRS spectra.

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.

This study is part of the Pharmimage® project

1. Provencher, Magn Res Med 30, 672 (1993) 2. Costello, Franklin, Urology 50, 3 (1997)