

# **<sup>13</sup>C HR MAS MRS reveals differences in the glucose metabolism between two breast cancer xenograft models with different gene expression pattern**

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## **Introduction**

Two breast cancer xenograft models have been established from human breast cancer patients and orthotopically xenografted in female SCID mice (1). The models represent a basal-like (poor prognosis) and a luminal-like (better prognosis) genetic profile. Using HR MAS <sup>1</sup>H MR spectroscopy, it has been shown that these two models have distinct metabolic profiles (2). It is well known that tumor cells metabolize glucose at a higher rate than normal cells (3). This is mainly used to form lactate and alanine and is observed even when high concentrations of oxygen are present. Labelled [<sup>13</sup>C]-glucose can be used to measure glucose consumption and transformation with MR spectroscopy. The purpose of this work was to investigate the rate of glucose metabolism in the two breast cancer xenograft models.

## **Methods**

Mice carrying xenograft tumors received a bolus injection of [<sup>13</sup>C]-glucose (29 mg per animal) via the lateral tail vein under isoflurane anaesthesia. 10 or 15 minutes after the injection, the mice were sacrificed and the tumor tissue harvested and immediately frozen in liquid nitrogen. A total of 33 samples were collected (N=19 controls, N=14 [<sup>13</sup>C]-glucose labelled). One dimensional HR MAS <sup>1</sup>H with water presaturation and <sup>13</sup>C power gated proton decoupled MR spectra were recorded using a BRUKER Avance DRX600 with a <sup>1</sup>H/<sup>13</sup>C HR MAS probe. Two dimensional HSQC <sup>1</sup>H/<sup>13</sup>C coupled experiments were obtained on two samples for spectral assignment. All spectra were preprocessed, mean normalized, peak aligned and baseline corrected. Integration was performed to calculate metabolites ratios. A two sided t-test was performed to investigate differences in glucose, alanine and lactate between the groups.

## **Results**

A significant difference in the glucose/alanine ratio ( $p < 0.001$ ) between the luminal-like ( $0.43 \pm 0.33$ , N=9) and the basal-like ( $1.55 \pm 0.57$ , N=10) model was observed in the natural abundance spectra. It was a trend of higher glucose/lactate ratio in the basal-like ( $0.24 \pm 0.10$ ) compared to the luminal-like ( $0.13 \pm 0.18$ ) model, but no significant difference was observed. The difference in alanine/lactate was small between the two models. The [<sup>13</sup>C]-glucose labelled spectra had significantly higher signals from glucose, alanine and lactate compared to the natural abundance spectra indicating an efficient glucose uptake and conversion. In the labelled spectra from samples collected after 15 minutes, significant differences between the two models (luminal-like N=6, basal-like model N=5) were observed for all ratios (glucose/alanine  $p < 0.01$ , glucose/lactate  $p = 0.01$  and alanine/lactate  $p = 0.04$ ). There was a trend of a higher glucose/alanine and glucose/lactate ratios (not significant) between samples collected at 10 (N=3) and 15 (N=5) minutes in the basal-like model.

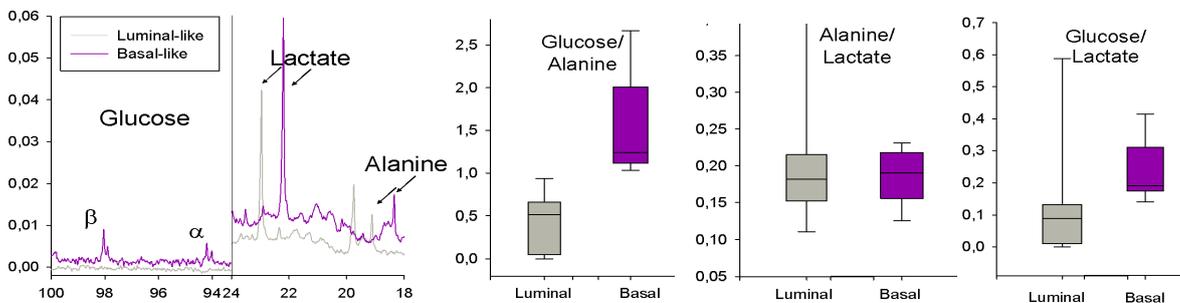


Figure 1: Average <sup>13</sup>C natural abundance spectra (expanded) and box plots of metabolite ratios from the luminal- and basal-like models indicate differences in glucose/alanine, glucose/lactate and alanine/lactate ratios between the two models.

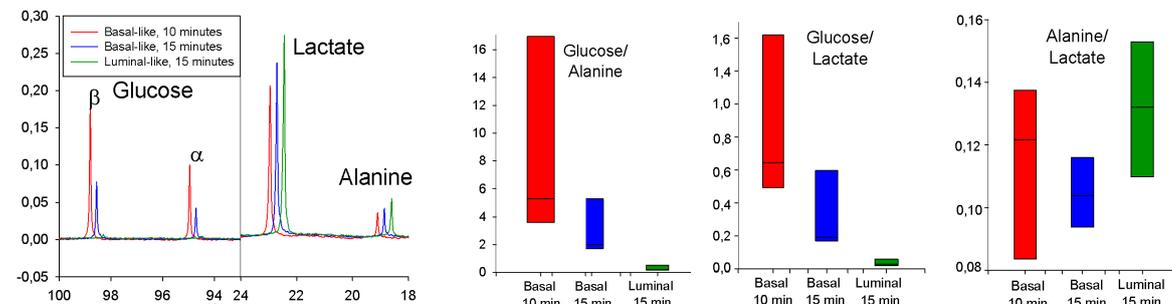


Figure 2: Average [<sup>13</sup>C]-glucose enriched spectra (expanded) and box plots of metabolite ratios from the two animal models showing the glucose/alanine, glucose/lactate and alanine/lactate ratios in samples from the two models harvested 10 or 15 minutes after injection of <sup>13</sup>C labelled glucose.

## **Discussion**

The natural abundance spectra indicate a higher glucose/alanine and glucose/lactate level in the basal-like compared to the luminal-like model. It is likely that a difference in the metabolite ratios between the two models is due to a lower concentration of glucose in the luminal-like model, glucose could in fact not be detected in several spectra. It was only a small difference in the alanine/lactate ratio between the two models.

There is a clear signal enhancement in glucose, lactate and alanine in the <sup>13</sup>C labelled spectra compared to the natural abundance spectra, indicating that glucose has been taken up and metabolised to lactate and alanine. No other <sup>13</sup>C labelled signals could be observed (data not shown). The significant difference in glucose/alanine and glucose/lactate ratio between the luminal-like and the basal-like model could be explained by a lower uptake of glucose and/or a higher rate of glucose metabolism towards lactate and alanine in the luminal-like compared to the basal-like model. The luminal-like model tends to convert almost all of the glucose to lactate and alanine within 15 minutes. In the basal-like model conversion from glucose to lactate and alanine is observed also between 10 and 15 minutes.

In conclusion, ex vivo <sup>13</sup>C MRS measurements demonstrate differences in the glucose metabolism for two different phenotypic breast cancer xenograft models.

## **References**

(1) Bergamashi et al. Mol Oncol 2009; 3:469-82 (2) Moestue et al. Proc Int Soc Magn Reson Med, Honolulu 2009, #2323 (3) Warburg Science 1956; 123: 309-14