Metabolic response to everolimus in patient-derived xenografts of triple negative breast cancer

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BACKGROUND

- **Triple negative breast cancer (TNBC)**
  - -Lack of detectable expression of hormone receptors (ER and PgR) and HER2 amplification. No current targeted therapy.
  - -Large overlap with the basal-like gene expression subtype, which is associated with the highest activation of PI3K/Akt/mTOR signaling.
- **Everolimus**: mTOR inhibitor
  Potential candidate for TNBC targeted therapy.

AIMS: To investigate:
1) metabolic changes as an effect of Everolimus treatment,
2) metabolic differences between responders and non-responders,
3) the potential of metabolic profiles to discriminate tumors expressing or not expressing proteins involved in PI3K/AKT/mTOR signaling.

METHODS

Study Design

13 TNBC patient-derived xenograft (PDX) models (n=103 animals)

- Everolimus Treated (n=4 animals per model, $n_{Treated}$=50)
- Controls (n=4 animals per model, $n_{Controls}$=53)

1-Metabolic profiles were obtained by **high resolution (HR) magic angle spinning (MAS) magnetic resonance (MR) spectroscopy**.

2-Relative levels of **17 metabolites** were calculated by integration, and lactate/glucose, taurine/creatine, and glycerophosphocholine/phosphocholine ratios were subsequently determined.

3-Naturally ocurring differences were explored using principal component analysis (**PCA**).

4-Classification models were built using partial least squares-discriminant analysis (**PLS-DA**), with double cross validation including all samples from the same PDX model in either the training or test set, validated with permutation testing.

5-Univariate linear mixed-effects models (**LMM**) were built with metabolite level as the response variable, the fixed effects of *treatment group* (treated or controls) and *response group* (responder or non-responder), and PDX model as a random effect. Significance was considered for multiple testing corrected p-value (q-value) ≤0.05.

RESULTS AND DISCUSSION

PLS-DA of 20 metabolic integrals or ratios revealed differences between treated and control xenografts (**Figure 1A**) with an accuracy of 67% (p=0.003), with treated xenografts showing higher glucose, glutamine, and alanine and lower phosphocholine, glycerophosphocholine, and lactate/glucose (**Figure 1B**).

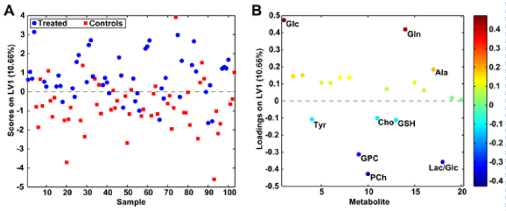

Figure 1. PLS-DA scores (**A**) and loadings plots (**B**) of treated vs controls (n=103). Loadings are colored according to latent variable (LV) 1. Glc: glucose; Tyr: tyrosine; GPC: glycerophosphocholine; PCh: phosphocholine; Cho: choline; GSH: glutathione; Gln: glutamine; Ala: alanine; Lac: lactate.

In accordance with PLS-DA, **LMM** revealed significant differences in the same 6/20 metabolites for the fixed effect *treatment group* after multiple testing correction, exhibiting similar changes as observed in PLS-DA loadings. No metabolites were significant for the *response* fixed effect (**Table 1**).

Table 1. LMM results for the fixed effects of *treatment group* and *response*.

Metabolite	Response			Treatment group		
	Estimate	Std. Error	q-value	Estimate	Std. Error	q-value
Glc	-0.049	0.116	9.25E-01	0.130	0.038	4.45E-03*
Asc	0.186	0.063	2.51E-01	0.016	0.019	6.63E-01
Lac	-0.057	0.055	8.39E-01	0.015	0.018	6.63E-01
Tyr	0.045	0.065	9.09E-01	-0.012	0.021	8.82E-01
Gly	0.008	0.077	9.76E-01	0.015	0.017	6.63E-01
mI	-0.054	0.126	9.25E-01	0.007	0.018	9.24E-01
Tau	0.002	0.055	9.76E-01	-0.004	0.021	9.24E-01
sI	0.028	0.118	9.59E-01	0.038	0.040	6.63E-01
GPC	0.093	0.165	9.25E-01	-0.035	0.025	4.80E-01
PCh	0.032	0.093	9.25E-01	-0.129	0.028	1.26E-04**
Cho	0.054	0.051	8.39E-01	-0.006	0.022	9.24E-01
Cr	0.103	0.112	8.39E-01	-0.003	0.019	9.28E-01
GSH	-0.054	0.057	8.39E-01	0.007	0.027	9.24E-01
Gln	0.115	0.098	8.39E-01	0.144	0.021	2.59E-08**
Succ	0.040	0.041	8.39E-01	0.019	0.018	6.63E-01
Glu	-0.081	0.061	8.39E-01	0.007	0.019	9.24E-01
Ala	-0.071	0.075	8.39E-01	0.060	0.015	7.02E-04**
Lac/Glc	-0.008	0.132	9.76E-01	-0.115	0.041	2.05E-02*
Tau/Cr	-0.103	0.128	8.83E-01	0.000	0.015	9.87E-01
GPC/PCh	0.059	0.172	9.25E-01	0.095	0.025	1.63E-03*

Metabolite increase (positive estimate) or decrease (negative estimate) is shown for responders and treated xenografts in relation to controls and non-responders, respectively. * and ** indicate significance (q≤0.05 and q≤0.001, respectively).
Std.: standard; Glc: glucose; Asc: ascorbate; Lac: lactate; Tyr: tyrosine; Gly: glycine; mI: myo-inositol; Tau: taurine; sI: scyllo-inositol; GPC: glycerophosphocholine; PCh: phosphocholine; Cho: choline; Cr: creatine; GSH: glutathione; Gln: glutamine; Succ: succinate; Glu: glutamate; Ala: alanine

Responders and non-responders could not be discriminated by multivariate **PLS-DA** for either control or treated xenografts (**Table 2**). Similarly, **PCA** of integrals or ratios of control xenografts did not reveal any separation between responders and non-responders (**Figure 2A**).

Table 2. Classification results from PLS-DA.

Samples included in the model	Variables used	Discriminated Classes	n	No. of LVs	Class. Accuracy (%)	Sensitivity/ Specificity (%)	Permutation p-value
All	Metabolite integrals	Treated vs Controls	103	1	67	66/68	0.003*
Controls	Metabolite integrals	Responders vs Non-resp.	53	1	47	63/32	0.613
Treated	Metabolite integrals	Responders vs Non-resp.	50	1	57	68/46	0.237
Controls	Spectral metabolic profile	INPP4B+ vs INPP4B-	53	1	69	74/65	0.005*
Controls	Spectral metabolic profile	PTEN+ vs PTEN-	53	1	64	79/50	0.055
Controls	Spectral metabolic profile	pAKT+ vs pAKT-	49[#]	1	51	17/85	0.468

Sensitivity/Specificity are reported for Treated/Responders/INPP4B+/ PTEN+/ PAKT+.
[#]4/53 samples belonged to a PDX model with undetermined PAKT expression, and were therefore excluded.
* indicates significant p-values (≤0.05).
n: number of samples; LVs: latent variables; Non-resp.: non-responders; Class.: classification

Separate **PCA** of integrals or ratios of responders (**Figure 2B**) and non-responders (**Figure 2C**) showed clear groupings in the scores plot by PDX model, reflecting the metabolic heterogeneity among the studied PDX models. Initial metabolic differences within the two groups may thus hinder prediction of response to everolimus treatment.

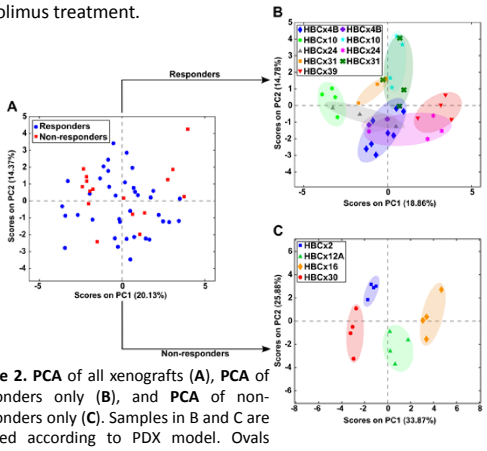

Figure 2. PCA of all xenografts (**A**), **PCA** of responders only (**B**), and **PCA** of non-responders only (**C**). Samples in B and C are colored according to PDX model. Ovals were drawn manually to illustrate clusters.

Individual metabolite differences between treated and control xenografts were determined in responders (n=73) and non-responders (n=30) separately, using **LMM** to correct for repeated PDX model measurements. In responders, the same metabolites were significantly different as those for *treatment group* (**Table 1**) when performing LMM on the whole cohort, while only 2/6 of these were significant for non-responders (**Figure 3**).

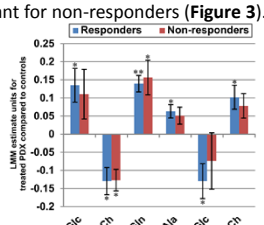

Figure 3. LMM estimates for treated xenografts compared to controls for responders and non-responders. * and ** indicate significance (q≤0.05 and q≤0.001, respectively). Glc: glucose; PCh: phosphocholine; Gln: glutamine; Ala: alanine; GPC: glycerophosphocholine.

PLS-DA results on spectral profiles of controls (n=53) to classify expression or no expression of INPP4B and PTEN, which negatively regulate mTOR, as well as the PI3K downstream member pAKT (determined by immunohistochemistry), are summarized in **Table 2**. Expression of INPP4B was successfully discriminated with an accuracy of 69% (p=0.005) (**Figure 4A**), associated with increased phosphocholine, glycine, creatine, alanine, and lactate, and decreased glycerophosphocholine and taurine (**Figure 4B**). While metabolic profiles were unable to predict pAKT expression, PTEN expression discrimination approached significance.

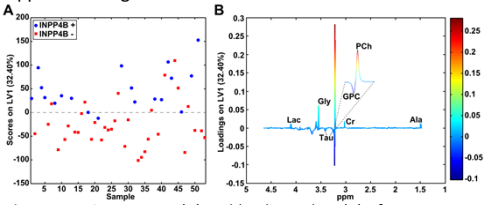

Figure 4. PLS-DA scores (**A**) and loadings plots (**B**) of INPP4B+ vs INPP4B- controls. Loadings are colored according to latent variable (LV) 1. Lac: lactate; Gly: glycine; Tau: taurine; GPC: glycerophosphocholine; PCh: phosphocholine; Cr: creatine; Ala: alanine.

CONCLUSION

Clear metabolic differences between everolimus-treated and control xenografts were detected, indicating reduced glycolytic lactate production and glutaminolysis after treatment, consistent with PI3K/AKT/mTOR signaling pathway inhibition[1]. Although inherent metabolic heterogeneity between different PDX models seemed to hinder prediction of response to everolimus, following treatment significant metabolic changes in glucose, alanine, lactate/glucose, and glycerophosphocholine/phosphocholine were detected in responders, but not in non-responders. Metabolic profiles reflected INPP4B, but not PTEN or pAKT, status, which may provide complimentary insight into PI3K pathway regulation.

References: [1] Foster, R *et al.*, Multiple Metabolic Alterations Exist in Mutant PI3K Cancers, but Only Glucose Is Essential as a Nutrient Source. PLoS ONE 2012; 7(9): e45061. doi:10.1371/journal.pone.0045061

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