

Title: A Tumour-Nerve-Adipose Metabolic Dependency Axis involving N-Acetyl Aspartate (NAA) and Glutamate Carboxy Peptidases (PSMA)

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Background: The metabolite N-Acetyl Aspartate (NAA) is found in extremely high quantities in nerve tissue and is the second most abundant metabolite in the central nervous system after water, closely followed by glutamate. NAA has been shown to alter adipose tissue function, support tumour cells and alter immune response in the tumour micro environment. There is an emerging role for NAA in multiple cancer types including ovarian, breast, HCC, glioma and prostate cancer. N-Acetyl Aspartate Glutamate is the highly abundant glutamatergic neurotransmitter closely related to NAA via Glutamate Carboxy Peptidases (GCPII or PSMA) which cleave off glutamate found on the cell surface leaving NAA as its product. NAAG is the third most abundant neurotransmitter in the mammalian nervous system and intraosseous nerves are in high abundance in the bone marrow space of lumbo/sacral vertebrae where NAAG has a role in metastasis bone pain.

Methods: Multi-omics pathway analysis (metabolites, protein and RNA) of pre clinical orthograft models of CRPC (CWR/22RV1, LNCAP/LNCAPAI, VCAP/VCAPCR) highlighted NAA GCPII/PSMA and NAT8L, the NAA synthetic enzyme (Figure 1). Combinations of inhibitors (enzalutamide - AR, mifepristone -GR and 2MPA -PSMA) and metabolite supplementation of NAA/G were used to induce mitochondrial stress and study the response to NAA/G. Explant culture of intact primary tumour from prostatectomy using the "Lisa Butler" technique was used with automated IHC analysis using Halo. Matched High resolution 7T MRI Spectroscopy (MRS) imaging of NAA/NAAG was performed in ex vivo prostatectomy specimens. Bone metastatic tumours from pathological fracture repair were piloted using the same explant technique assessing viability between fresh and cryopreserved cultures.

Results: The NAA/G axis appears to be a conserved response to Mitochondrial stress. In the absence of a stressor NAA/G supplementation has no clear phenotype. However NAA/G supplementation in the presence of metabolic stressors results in rescue of mitochondrial dysfunction (stressors - AR/GR blockade, H2O2, ROS and radiation). This effect is conserved for NAA only and not replicated by acetate or aspartate supplementation. NAA supplementation of clinical tumour and non tumour specimen revealed tumour specific changes in Ki67 and AR staining. Our bone metastatic model showed an intact tumour micro environment with PSMA positive tumour in proximity to bone marrow adipocytes and CD3 positive T cells. Cryopreservation appears to maintain cellularity and PSMA staining.

Conclusions: The NAA/G axis is biologically active in prostate cancer cells and clinical explants. Tumoural NAA, primarily from nerve tissue, represents a conserved metabolic dependency on the tumour-nerve-adipose environment both locally and may also be present in bone metastatic disease.

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Conflicts of Interest: No conflicts of interest to declare

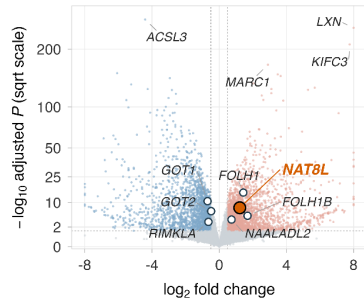
Figure 1

Each CRPC model has its own transcriptional response — *NAT8L* is up in all three

DESeq2, gene level. Significance: adjusted $P < 0.05$ and $|\log_2\text{FC}| \geq 0.5$. Open circles = NAA/NAAG/PSMA machinery reaching significance.

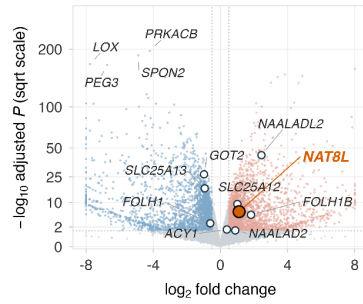
22RV1 vs CWR

▲ 2515 up · ▼ 2386 down · 18071 tested



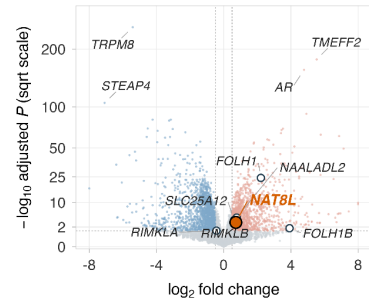
LNCaP-AI vs LNCaP

▲ 2761 up · ▼ 2544 down · 18454 tested



VCaP-CR vs VCaP

▲ 1406 up · ▼ 1534 down · 16552 tested



Axes are shared across all three panels; y is square-root scaled. Points beyond $|\log_2\text{FC}| = 8$ are drawn at the boundary. Labels: *NAT8L*, significant NAA/NAAG genes, and the strongest hits per panel.