Quantitative assessment of citrate secretion by ^{13}C-labeling in human prostate cancer cell line LNCaP

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Introduction Healthy epithelial cells in the prostate have the unique capability to produce and secrete large amounts of citrate. Key in this production is the inhibition of aconitase (citr->isocit) by zinc allowing the accumulation and secretion into the lumina of citrate.[1,2]. Citrate is the product of a condensation reaction of oxaloacetate with acetyl-CoA in the Krebs cycle. Both glucose and pyruvate can fuel this reaction via both acetyl-CoA and oxaloacetate (Fig.1). Metastasis PCa cell line LNCaP produces citrate in contrast to most other PCa derived cell lines.[3] Aim of this study is to determine an index for citrate secretion versus Krebs cycle consumption from simple ratios in NMR spectra of ^{13}C-labeling experiments in LNCaP.

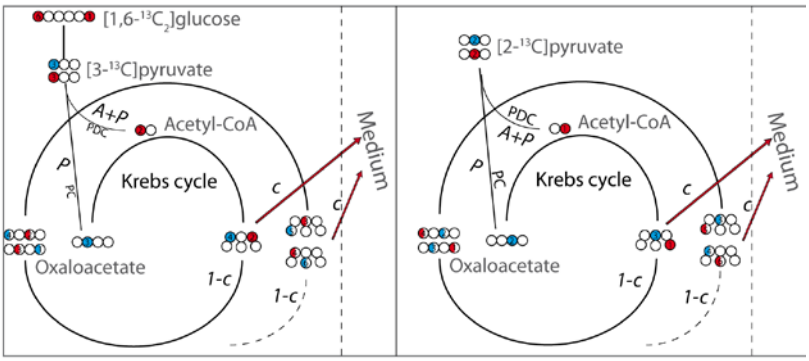

Fig.1 Fate of ^{13}C-labels originating from [1,6-$^{13}C_2$]glucose or [2-^{13}C]pyruvate in Krebs cycle with respect to citrate production (pyruvate carboxylase (PC), pyruvate dehydrogenase complex PDC, c excretion probability).

Methods LNCaP (~10x10^6 cells) were grown in RPMI-1640+Gln+S/P supplemented with [1,6-$^{13}C_2$]glucose (11 mM) or [2-^{13}C]pyruvate (7 mM). After 48h the media were collected and analyzed by ^{1}H- and ^{13}C-NMR spectroscopy. Using *j*MRUI AMARES, the resonances of citrate ^{13}C2/4 and ^{13}C3 ([1,6-$^{13}C_2$]glucose experiments) and citrate ^{13}C1/5 and ^{13}C3 ([2-^{13}C]pyruvate experiments) were fitted.

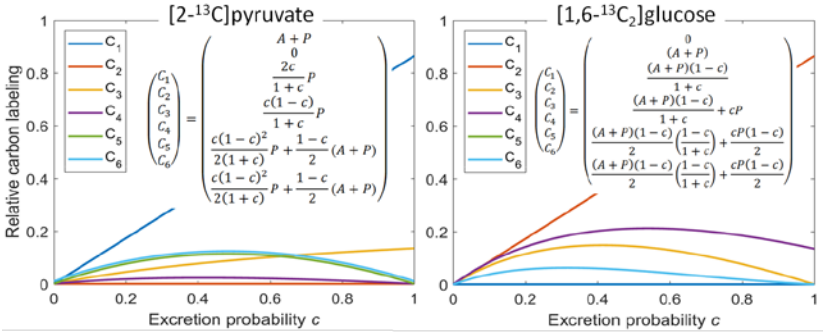

Fig.2 Carbon labeling of citrate versus excretion probability *c*.

Results and discussion. Citrate was detected by ^{1}H- and ^{13}C-NMR and ^{13}C-labeled citrate was observed after 48h in the medium supplemented with [1,6-^{13}C]glucose and [2-^{13}C]pyruvate. The ratio R_1= {$C_{2/4}$-C_3}/{$C_{2/4}$} was found to be 0.75 after glucose labeling. Using [2-^{13}C]pyruvate resulted in ^{13}C-labeled citrate at C1 and C3. The ratio R_2={$C_{1/5}$}/{C_3} is found to be ~6.
Using these experimental ratios R_1 and R_2 as input we calculated the average number of cycles ^{13}C-labels complete before secretion and the contribution of anaplerosis via PC in LNCaP. At the first labeling, *P* labels will be present in oxaloacetate and *A*+*P* labels in acetyl-CoA. With *P* the fraction of pyruvate being converted into oxaloacetate. If we define *c* as the probability of excretion, the contribution of the first labeling to the total citrate pool in the medium is (*A*+*P*)*c* labels at C2 and *Pc* labels at C4. (1-*c*) of the citrate continues down the Krebs cycle and can be labeled again resulting in different labeling of the citrate carbons. Summing the contributions of all cycles results in the labeling for the different carbons shown in fig 2. Using these expressions and the experimental ratios allows us to calculate the average number <*n*> of Krebs cycles completed to be 1.25, the probability *c* that citrate is secreted from the cycle to be 0.44 and the contribution *P* of pyruvate carboxylase to the citrate production to be equal to 0.135.

Conclusion We detected several pathways for carbon supply to produce citrate in metastatic PCa cell line LNCaP and determined a labeling index describing citrate production that may be valuable as biomarker for transition from healthy prostate cells to malignant cells.

References [1]Costello, L.C., e.a., Mol. Cancer 2006;5(17) [2]Bertilsson, H., e.a., Clin Cancer Res 2012;18(12) [3]Cornel, E.B., e.a., Prostate 1995;5(26)