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The Impact of MCT1-driven lactate import on the stem cell phenotype of pancreatic ductal adenocarcinoma (PDAC) cells

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Purpose: A particular condition in tumor metabolism and hallmark of tumor aggressiveness - as seen in pancreatic cancer (PDAC) - is the *reverse Warburg* phenotype which is characterized by the uptake of lactate, ketone bodies and pyruvate by certain tumor cells. Being part of a metabolic flux between neighboring tumor cells and stromal cells, these metabolites are taken up through the lactate-carrier MCT1/SLC16A1 and serve not only as energy substrates (mainly through oxPhos) but also as epigenetic modifiers. Consequently, stemness features are also affected by the MCT1 driven uptake of these metabolites, yet the exact modalities are poorly understood.

Methods: In the differentially differentiated PDAC cell lines Panc1, BxPC-3 and T3M4, the expression of MCT1 and the effect of lactate treatment as well as MCT1 inhibition by 7ACC2 on the expression of stemness transcription factors and iPSC markers were explored. In addition, histone H3 modifications and their dependence on MCT1 driven lactate import was elucidated. Using the A818-6 cell line and its corresponding 3D tumor hollow spheres, the impact of lactate import on shifting the stem cell fate as well as on histone H3 acetylation and the cells' sphere forming capability were explored.

Results: We could show a marked difference in the stemness marker profile in Panc1, T3M4 and BxPC-3 cells, where the expression of KLF4 as well as Nestin, Sox2 and Nanog is significantly higher in BxPc-3 and T3M4 (both MCT1 positive) than in Panc1 cells (MCT1 negative). MCT1-driven lactate uptake increased the expression of the stemness markers Nestin, Sox2 and Nanog in both T3M4 and BxPC-3 and further elevated the highly expressed KLF4 in T3M4 cells. Moreover, protection from gemcitabine induced apoptosis by lactate treatment was seen in BxPC-3 and T3M4 but not in Panc1 cells. Furthermore, exposure to lactate increased the level of acetylated H3 in BxPc-3 and T3M4 cells, whereas treatment with 7ACC2 or MCT1 siRNA reduced H3 acetylation in these cells. Whilst in A818-6 monolayer cells MCT1 is expressed at high levels accounting for significant lactate uptake, the corresponding A818-6 hollow spheres lack MCT1 expression and lactate uptake. Accordingly, H3 acetylation was greater in monolayer cells than in hollow spheres. Moreover, qPCR analysis detected higher expression level of the pancreatic stem cell markers CD133 and Oct4 in A818-6 spheres, as well as the iPSC marker Sox2 and the transcription factor Nanog, but not KLF4. Lactate pre-treatment of monolayer cells led to an increase in size and number of the formed hollow spheres as well as an increase in Sox2 and CD133 and notably also KLF4.

Conclusion: The flux of lactate into reverse Warburg PDAC cells contributes to H3 modifications and thereby to epigenetic control. This relates to shifting the cells into a more stem cell like phenotype and increasing their resistance, invasiveness and 3D tumorsphere formation capacity.