SIRT1 and mTOR interplay in bladder cancer: a potential therapeutic target

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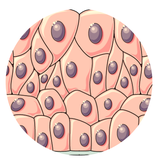
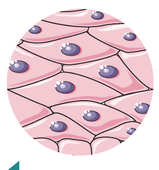
INTRODUCTION

Bladder cancer (BC), a tumour with high heterogeneity, has a high incidence and recurrence rate, with frequent alterations in several signalling pathways. The mammalian target of rapamycin (mTOR) pathway is altered in 72% of BC cases. Interestingly, the role of SIRT1 in tumorigenesis has been a matter of controversy since it can act both as a tumour promoter or tumour suppressor, suggesting that it has different roles according with the characteristics of the tumour. In recent studies, mitochondrial functions, metabolic pathways and signalling proteins have been related to both SIRT1 and mTOR pathways. Herein, we hypothesize that SIRT1 has a role in BC progression and that it interacts with mTOR pathway, modulating bioenergetics and metabolic features.

METHODS



HT-1376
Stage III



TCCSUP
Stage IV

Control
No treatment

YK-3-237
1 μM
SIRT1 activator

EX-527
1 μM
SIRT1 inhibitor

Rapamycin
0.1 μM
mTOR inhibitor

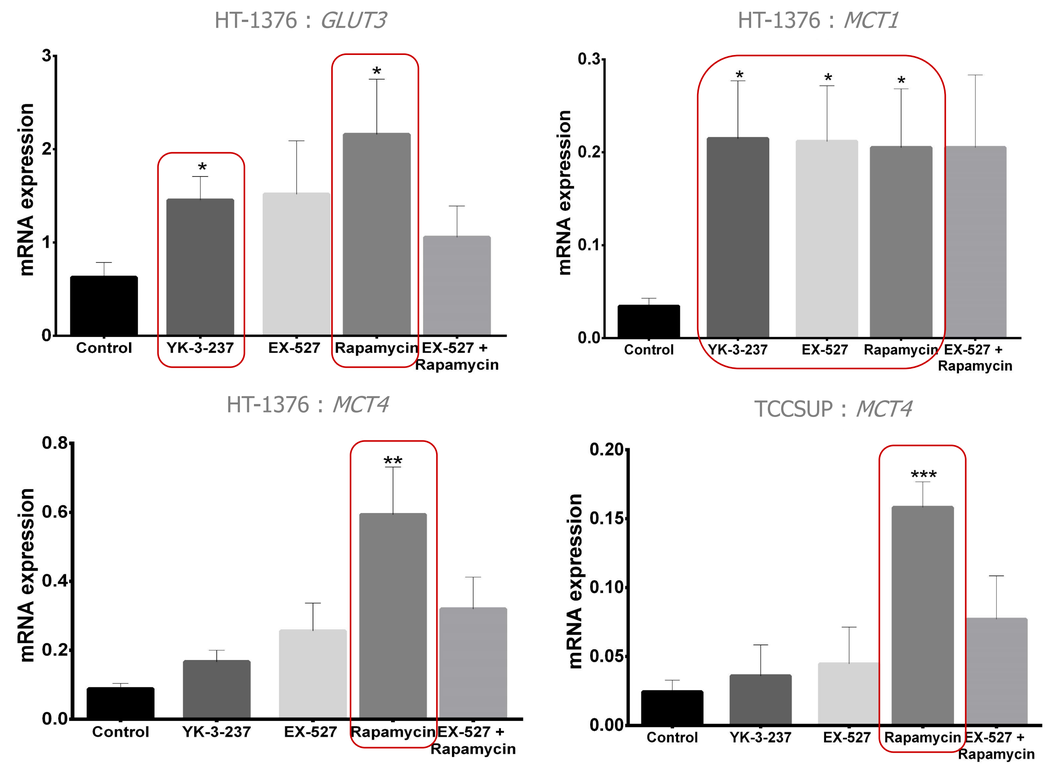
EX-527 + Rapamycin
1 μM + 0.1 μM

24 hours

Metabolism	Mitochondrial potential	Mitochondrial respirometry
• qPCR (genes) • ^{1}H-NMR (metabolites)	• JC-1	• Oxygen consumption

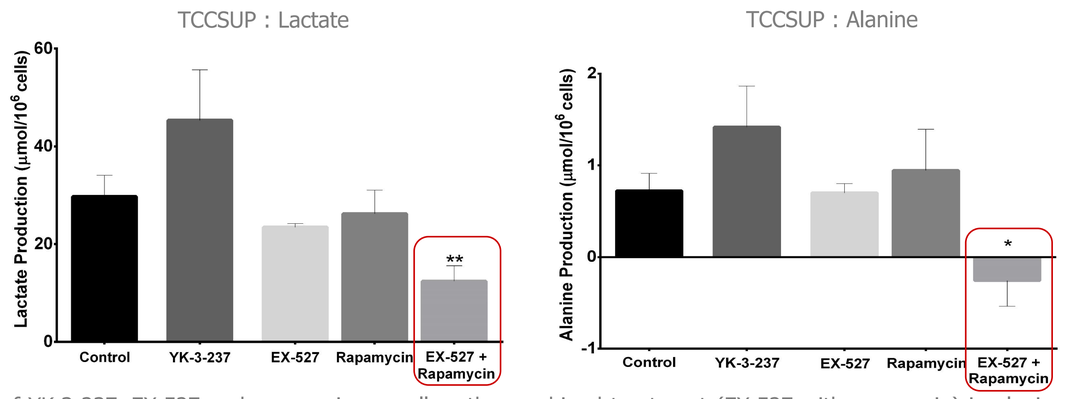
RESULTS

SIRT1 activation upregulated GLUT3 and MCT1 in HT-1376 cells without alteration on metabolites levels. mTOR inhibition alone also upregulated GLUT3, MCT1 and MCT4 expression in HT-1376 cells and increased MCT4 in TCCSUP cells, but with no direct action on metabolites levels.



Effect of YK-3-237, EX-527 and rapamycin, aswell as the combined treatment (EX-527 with rapamycin) in mRNA expression of *GLUT3* and *MCT1* genes of bladder cancer cells HT-1376 and mRNA expression of *MCT4* gene of both cell lines. mRNA expression results are presented in arbitrary units. Results are presented as mean±SEM (n=6). *p<0.05, relatively to control group. **p<0.01, relatively to control group. ***p<0.001, relatively to control group.

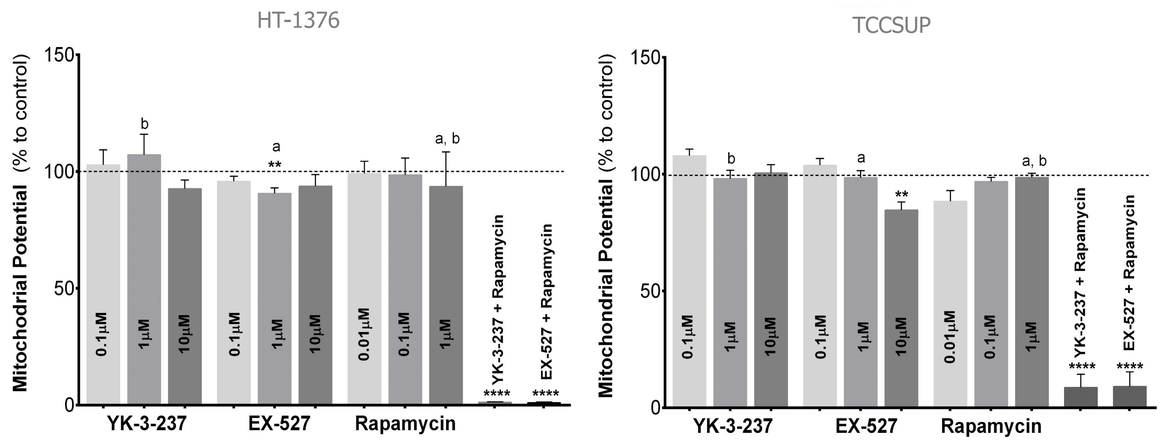
The combined treatment with SIRT1 and mTOR inhibitors significantly decreased lactate production in TCCSUP cells and alanine was consumed instead of produced.



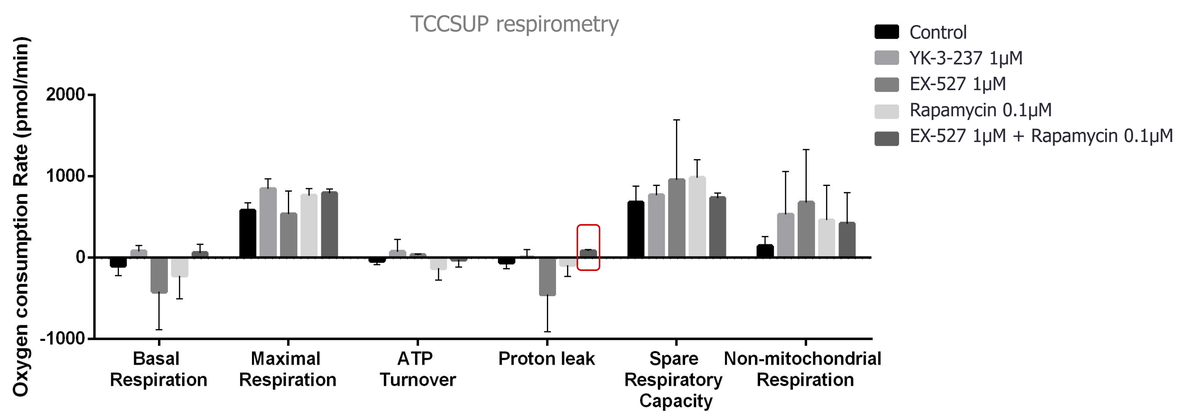
Effect of YK-3-237, EX-527 and rapamycin, aswell as the combined treatment (EX-527 with rapamycin) in alanine and lactate production TCCSUP cells. Results are presented as mean±SEM (n=6). *p<0.05, relatively to control group. **p<0.01, relatively to control group.

RESULTS

Mitochondrial potential was almost nullified after exposure to the combined treatment with both inhibitors in BC cells of both stages and mitochondrial proton leak was slightly increased in TCCSUP cell line respirometry.



Effect of YK-3-237, EX-527 and rapamycin, aswell as the combined treatments in mitochondrial potential of HT-1376 and TCCSUP cells. Results were obtained by JC-1 assay and are presented as mean±SEM (n=6). **p<0.01, relatively to control group. ****p<0.0001, relatively to control group. a – significant result relative to EX-527+rapamycin group. b – significant result relative to YK-3-237+rapamycin group.



Effect of YK-3-237, EX-527 and rapamycin, aswell as the combined treatment (EX-527 with rapamycin) in oxygen consumption rate of bladder cancer cells TCCSUP. Results are presented as mean±SEM (n=3).

CONCLUSION

Our data showed that SIRT1 alteration have differential effects on BC cells of both grades, suggesting a possible therapeutic target for BC progression. These studies highlight the need to preselect patients based on metabolic cancer phenotype, since the cells' behaviour from different stages diverse in metabolic genes, metabolites involved and mitochondrial function. In the context of personalized medicine, these differences in signalling pathways are essential for a specific diagnosis and therapy of each particular tumour. Overall, our data suggest that SIRT1 and mTOR are key players in BC physiology and highlight the need to unveil the interplay of both pathways in BC development.