Targeting the AKT/mTOR pathway attenuates the metastatic potential of colorectal carcinoma CTCs in a murine xenotransplantation model and alters metabolic pathways

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Background & objectives

Cancer-related mortality is mostly associated with metastasis. Circulating tumor cells (CTCs) play an important role in metastasis formation. Aberrant signaling of oncogenic pathways drives tumor progression. One of the most frequently activated signaling pathways in cancer is the PI3K/AKT/mTOR pathway. As the treatment efficacy of single drugs may be compensated by feedback loops within the highly complex signaling pathway network dual targeting may overcome resistance.

In this work the susceptibility of CTC-MCC-41 colon cancer CTCs for AKT and mTOR inhibitors was evaluated. Additionally, the functional role of the three AKT isoforms (*i.e.*, AKT1, AKT2, AKT3) was examined in this cell line.

Methods

The efficacy of AKT inhibitor MK-2206, the mTOR inhibitor RAD001 and the combination of both drugs was examined on CTC-MCC-41 cells in a 2D, 3D as well as in a murine intracardiac xenotransplantation model. Furthermore, stable AKT isoform knockdown (KD)

cells were generated using shRNA transduction. Proliferation was measured by live cell imaging. Differentially regulated proteins were identified using liquid chromatography mass spectrometry-based proteomics and analyzed using *in silico* metabolism analysis.

Results

CTC-MCC-41 cells show a high susceptibility within the nanomolar range for dual targeting of AKT and mTOR using MK-2206 (IC_{50}: 186 nM) and RAD001 (IC_{50}: 2.6 nM) *in vitro*. Combinatory treatment was superior to single agent treatment and showed strong to very strong synergistic effects regarding the inhibition of CTC-MCC-41 proliferation. The efficacy of AKT and mTOR inhibitors was confirmed in a murine intracardiac model with lower overall tumor burden and particularly reduced bone metastasis after treatment with MK-2206 and RAD001. Knockdown of AKT1 and AKT2 reduced proliferation of CTC-MCC-41 cells. Interestingly, an altered metabolism with more pronounced glycolytic properties and reduced fatty acid utilization was associated with the reduced proliferation after AKT1 and AKT2 KD in CTC-MCC-41 cells.

Conclusion

Vertical targeting of AKT and mTOR is superior to single agent treatment and suitable to reduce CTC mediated metastasis in a murine intracardiac xenotransplantation model. KD of AKT1 and AKT2 alters the metabolic profile of CTC-MCC-41 cells. Selective eradication of CTCs may be a suitable target to prevent metastasis and open new treatment methods.