1051 Multiple drug induced feedback loops limit the efficacy of PI3K/AKT/mTOR inhibition as a therapy in bladder cancer

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INTRODUCTION & OBJECTIVES: The PI3K/AKT/mTOR pathway has been identified as an attractive therapeutic target in cancer but to date, only small subsets of patients benefit in clinical trials from inhibitors developed against this pathway. Here, we provide a comprehensive analysis of molecular and functional effects when targeting this pathway in bladder cancer (BLCA) to develop the most effective treatment strategy when targeting the PI3K pathway.

MATERIAL & METHODS: Biochemical effects of small molecule inhibitors against PI3K (PIK-90, BKM-120), AKT (MK-2206), mTORC1 (RAD001), mTOR (INK128, shRNA), PI3K/mTOR (NVPBEZ235), PDK1 (GSK2334470), MEK (U0128) or raptor/rictor (siRNAs) were determined on the expression and phosphorylation level of key molecules in the pathway (AKT, TSC1/2, S6K1, 4E-BP1, ERG) by Western blotting in a panel of ten BLCA cell lines. This was done in short and long term experiments. Functional effects were examined on cell viability (Cell-titer blue), apoptosis (Caspase 3/7-Glo) and cell cycle progression (EdU). Effects on three-dimensional xenografts were analyzed in the chicken chorioallantoic membrane (CAM) model by determining tumor weight and protein expression of Ki67, AKT and S6K1 (immunohistochemistry).

RESULTS: Inhibition of the kinase activity of PI3K, AKT, mTORC1 or PDK1 reduced the viability of only selected cell lines and led to S6K1 but not 4E-BP1 dephosphorylation. Inhibition of PI3K/mTOR or mTORC1/mTORC2 reduced the viability of all examined cell lines and resulted in the dephosphorylation of both 4E-BP1 and S6K1. However, cells were able to survive long-term treatment with these inhibitors, which correlated with molecular feedback loops that led to AKT hyper-phosphorylation an effect that requires PDK1. This could be suppressed by the combination of dual PI3K/mTOR and AKT inhibitors, which led to an increase in apoptosis with a synergistic effect on cell viability. Combination of PI3K, AKT, mTOR inhibitors showed synergy resulting in lower concentrations for treatment and potentially lower toxicity and was also more effective than single inhibitors in reducing tumor growth in the CAM model.

CONCLUSIONS: Complex feedback loops limit the efficacy of inhibitors against molecules within the PI3K/AKT/mTOR pathway in BLCA with AKT being a central molecule that has to be negatively regulated. This can be overcome by a novel combined treatment strategy that inhibits PI3K, AKT and mTOR simultaneously.