A Key Role for ARF in Drug Resistance in Invasive Bladder Cancer

Introduction and Objective: Although most superficial bladder cancers can be removed transurethrally with good prognosis, clinical outcome is more problematic for patients with muscle-invasive disease. Indeed, invasive bladder cancer is a major clinical challenge since it is frequently associated with postoperative recurrence and metastasis. Current treatments for lethal bladder cancer include systemic chemotherapy and molecular targeted therapy; however, survival is poor since most patients eventually develop resistance to the drugs within a short timeframe. Clearly, there is a need to identify novel therapeutic options for invasive bladder cancer as well as a greater understanding of the molecular mechanisms of drug resistance.

Materials and Methods: We investigated mechanisms of drug resistance using genetically-engineered mouse models of invasive bladder cancer based on the combinatorial deletion of *p53* and *Pten* in bladder epithelium. *p53* and *PTEN* are frequently inactivated in human bladder cancers, particularly those with poor prognosis. We treated mouse primary bladder cancers, allograft tumors established from these mice, and bladder cancer cell lines with cisplatin, docetaxel, or rapamycin.

Results: We have observed that these *p53*; *PTEN* deficient tumors express robust levels of $p19^{ARF}$, while targeted deletion of *Arf* retards the acquisition of resistance following drug treatment. The significance of ARF expression was further suggested by analysis of 3 independent cohorts of gene expression profiling and immunostaining of human bladder cancer which revealed that high ARF expression is an independent predictor of poor survival of bladder cancer patients. Furthermore, following drug treatment in the mouse model, the *Arf*-positive, compared to the *Arf*-null, tumors showed significantly higher activation of PI3K-mTOR pathway consistent with significant enrichment in molecular pathways related to the PI3K/AKT pathway and drug resistance, suggesting that a model for drug resistance is via PI3K pathway activation. We have observed similar results in human bladder cancer cell lines either following knock-down of $p14^{ARF}$ in J82 cells that express endogenous $p14^{ARF}$ or following forced expression of $p14^{ARF}$ in ARF-negative UMUC3 human bladder cancer cells, and in both cases it was coincident with deregulated activation of PI3K-mTOR pathway.

Conclusions: ARF contributes to bladder cancer drug resistance by activating PI3K-mTOR pathway, highlighting a potential therapeutic target for advanced invasive bladder cancer patients.