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KGF protects bladder urothelium after cyclophosphamide via AKT

Background:
Cyclophosphamide (CPP), a drug used to treat lymphoma and other diseases, can cause severe acute bladder urothelial injury and chronic problems including fibrosis and cancer. We previously showed that treatment with keratinocyte growth factor (KGF) prior to CPP blocked Basal and Intermediate cell apoptosis. Our objective is to determine the mechanism of cytoprotection and long term benefits of KGF.

Methods:
We treated female mice with 5mg/kg KGF or PBS (vehicle) 24 hours before intraperitoneal (IP) CPP (150mg/kg) or sham injection and harvested mice at various time points. To identify the role of AKT, we administered LY294002 (50mg/kg), a potent AKT inhibitor IP every 12 hours to CPP injured mice ± KGF and harvested mice 12 hours after CPP. We performed H&E staining, TUNEL assays and immunofluorescence (IF) for many proteins on collected samples.

Results:
In PBS treated injured mice, we observed robust urothelial apoptosis 12 hours after CPP in PBS-treated mice and no apoptosis in KGF-treated mice. Addition of LY294002 partially blocked induction of urothelial pAKT staining by KGF and led to breakthrough apoptosis 12 hours after CPP (the inhibitor had no effects in PBS-treated mice). We next interrogated AKT-targets known affect apoptosis such as mammalian target of rapamycin (mTORC1, anti-apoptotic), and BCL2 associated agonist of cell death (BAD, pro-apoptotic). CPP injury alone led to a loss of baseline staining of phospho-P70S6K, a readout of mTORC1 activity. KGF treatment prevented the loss of pS6K staining, consistent with preservation of anti-apoptotic mTORC1 activity. In addition, pretreatment with KGF increased expression of phospho-BAD, which inactivates BAD and thus blocks BAX-triggered apoptosis. To assess long term benefits of KGF, we examined mice 6 months after CPP + KGF/PBS. PBS treated mice had many regions of hyperplasia, ongoing proliferation of $KRT14^+$ Basal cells, and many foci of ectopic $KRT14^+$ cells in the superficial layer. In contrast, KGF treated mice showed few regions of hyperplasia, $KRT14^+$ cell proliferation and no ectopic $KRT14^+$ cells.

Conclusion:
KGF appears to block CPP-induced urothelial cell apoptosis via AKT signaling. KGF-AKT appears to drive cytoprotection by preventing CPP induced loss of mTORC1 activity and by inactivating BAD. KGF treatment appears to improve long term recovery from CPP vs. PBS-treated mice.