Mutagenesis as a result of renal medullary hyperosmolar microenvironment on DNA damage recognition and repair
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Tuberous sclerosis complex is an autosomal dominant proliferative disease in which the functional allele is lost through somatic mutation leading to a renal tumor called an angiomyolipoma. Additional mutagenesis can also occur, which may lead to an aggressive malignancy. Evidence suggests that the mutagenesis may be more common in the renal medulla compared to the renal cortex.
We hypothesized that the renal medullary hyperosmolar microenvironment may dampen the DNA damage response, which leads to the somatic mutagenesis events that cause angiomyolipmas and very aggressive tumors. To test this hypothesis, we gradually adapted immortalized angiomyolipoma cells that contained either an empty expression vector or one containing the recue *TSC2* gene to isoosmolar and hyperosmolar media. The cell lines were adapted to 300, 450 or 600 mOsm using urea or sodium chloride. Double-strand DNA breaks were introduced into the cellular genome using etoposide. The cell lysates were then prepared for both damaged and undamaged cells for analysis by western blot. To detect the initiation on the DNA damage response pathway, we used a monoclonal antibody directed against phospho-S4,S8-RPA32, an integral DNA damage signaling protein. Damage was measured using single cell electrophoresis, also known as the comet assay.
Western blot analysis revealed that, compared to results of cells grow at 300 mOsm, both cell lines exposed to the 450 mOsm urea or sodium chloride identified DNA damage. In contrast, cells grown at 600 mOsm exhibited a marked decrease in the DNA damage response pathway activation. This work may suggest that decreasing the renal medullary concentration gradient may reduce the risk of mutations leading to renal tumors in patients with tuberous sclerosis complex.