

**Osmolalidade e câncer**

**Increased cancer risk of augmentation cystoplasty: possible role for hyperosmolal microenvironment on DNA damage recognition.**


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Patients who have had surgical bladder augmentation have an increased risk of bladder malignancy, though the mechanism for this increased risk is unknown. Hyperosmolal microenvironments such as the bladder may impair DNA damage signaling and repair; this effect may be more pronounced in tissues not normally exposed to such conditions. Comparing gastric and colon epithelial cell lines to transitional epithelial cell lines gradually adapted to an osmolality of 600 mOsm/kg with either sodium chloride or urea, cell lines of gastrointestinal origin were inhibited in their ability to activate ATM and downstream effectors of DNA damage signaling and repair such as p53, Nbs1, replication protein A (RPA), and gammaH2AX following the induction of DNA damage with etoposide. In contrast, bladder cell lines demonstrated a preserved ability to phosphorylate ATM and its effectors under conditions of hyperosmolar urea, and to a lesser extent with sodium chloride. The bladder cell lines' ability to respond to DNA damage under hyperosmolar conditions may be due in part to protective mechanisms such as the accumulation of intracellular organic osmolytes and the uroplakin-containing asymmetric unit membrane as found in transitional epithelial cells, but not in gastrointestinal cells. Failure of such protective adaptations in the tissues used for augmentation cystoplasties may place these tissues at increased risk for malignancy.

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**Hypertonic stress response.**


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Mammalian renal inner medullary cells are normally exposed to extremely high NaCl concentrations. Remarkably, under these normal conditions, the high NaCl causes DNA damage and inhibits its repair, yet the cells survive and function both in cell culture and in vivo. The interstitial NaCl concentration in parts of a normal renal medulla can be 500 mM or more, depending on the species. Studies of how the cells survive and function despite this extreme stress have led to the discovery of protective adaptations, including accumulation of large amounts of organic osmolytes, which normalize cell volume and intracellular ionic strength, despite the hypertonicity of the high NaCl. Those adaptations, however, do not prevent DNA damage. High NaCl induces DNA breaks rapidly, and the DNA breaks persist even after the cells become adapted to the high NaCl. The adapted cells proliferate rapidly in cell culture and function adequately in vivo despite the DNA breaks. Both in cell culture and in vivo the breaks are rapidly repaired if the NaCl concentration is lowered. Although acute elevation of NaCl causes transient cell cycle arrest and, when the elevation is too extreme, apoptosis, proliferation of adapted cells is not arrested in culture and apoptosis is not evident either in culture or in vivo. Further, high NaCl impairs activation of several components of the classical DNA damage response such as Mre11, H2AX and Chk1 leading to inhibition of DNA repair. Nevertheless, other regular participants in the DNA damage response, such as Gadd45α, Gadd153, p53, Hsp70, and ATM are still upregulated by high NaCl. How high NaCl causes the DNA breaks and how the cells survive them is conjectural at this point. We discuss possible answers to these questions, based on current knowledge about induction and processing of DNA breaks.

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**p38alpha- and Dyrk1A-dependent phosphorylation of caspase-9 at an inhibitory site in response to hyperosmotic stress.**


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The cysteine aspartyl protease caspase-9 is a critical component of the intrinsic apoptotic pathway. Activation of caspase-9 is inhibited by phosphorylation at Thr125, which is catalysed by the mitogen-activated protein kinases (MAPks) ERK1/2 in response to growth factors, by the cyclin-dependent protein kinase CDK1-cyclin B1 during mitosis, and at a basal level by the dual-specificity tyrosine-phosphorylation regulated protein kinase Dyrk1A. Here we show that inhibitory phosphorylation of caspase-9 at Thr125 is induced in mammalian cells by hyperosmotic stress. This response does not require ERK1/2 or ERK5, but it is diminished by ablation of Dyrk1A expression by siRNA or chemical inhibition of Dyrk1A by harmine. Phosphorylation of Thr125 in response to hyperosmotic stress is also reduced by chemical inhibition of p38 MAPK and is abolished in p38 alpha(-/-) mouse embryonic fibroblasts. These results show that both Dyrk1A and p38 alpha play roles in the inhibitory phosphorylation of caspase-9 following hyperosmotic stress and suggest a functional interaction between these protein kinases. Phosphorylation of caspase-9 at Thr125 may restrain apoptosis during the acute response to hyperosmotic stress.

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**Hyperosmotic stress up-regulates the expression of major vault protein in SW620 human colon cancer cells.**


The major vault protein (MVP) is the major constituent of the vault particle, the largest ribonuclear protein complex described to date and is identical to lung resistance-related protein (LRP). Although MVP is also expressed in several normal tissues, little is known about its physiological role. MVP played a protective role against some xenobiotics and other stresses. We thus investigated the effect of osmotic stress on MVP expression by treating human colon cancer SW620 cells with sucrose or NaCl. The expression level of both MVP protein and MVP mRNA was increased by the osmotic stress. Sucrose or sodium chloride could also enhance MVP promoter activity. Inhibition of p38 MAPK in SW620 cells by SB203580 inhibited the expression of MVP under hyperosmotic stress. These findings suggested that osmotic stress up-regulated the MVP expression through p38 MAPK pathway. Down-regulation of MVP expression by MVP interfering RNA (RNAi) in SW620 cells increased the sensitivity of the cells to hyperosmotic stress and enhanced apoptosis. Furthermore, MVP RNAi prevented the osmotic stress-induced, time-dependent increase in phosphorylated Akt. These findings suggest that the PI3K/Akt pathway might be implicated in the cytoprotective effect of MVP.

Our data demonstrate that exposure of cells to hyperosmotic stress induces MVP that might play an important role in the protection of the cells from the adverse effects of osmotic stress.

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Hyposmotic stress induces cell growth arrest via proteasome activation and cyclin/cyclin-dependent kinase degradation.

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Ordered cell cycle progression requires the expression and activation of several cyclins and cyclin-dependent kinases (Cdks). Hyposmotic stress causes growth arrest possibly via proteasome-mediated degradation of cyclin D1. We studied the effect of hyposmotic conditions on three colonic (Caco2, HRT18, HT29) and two pancreatic (AsPC-1 and PaCa-2) cell lines. Hyposmotic stress caused reversible growth arrest of the five cell lines in a cell cycle-independent fashion, although some cell lines accumulated at the G1/S interface. Growth arrest was followed by apoptosis or by formation of multinucleated giant cells, which is consistent with cell cycle catastrophe. Hyposmotic dramatically decreased Cdc2, Cdk2, Cdk4, cyclin B1, and cyclin D3 expression in a time-dependent fashion, in association with an overall decrease in cellular protein synthesis. However, some protein levels remained unaltered, including cyclin E and keratin 8. Selective proteasome inhibition prevented Cdk and cyclin degradation and reversed hyposmotic stress-induced growth arrest, whereas calpain and lysosome enzyme inhibitors had no measurable effect on cell cycle protein degradation. Therefore, hyposmotic stress inhibits cell growth and, depending on the cell type, causes cell cycle catastrophe with or without apoptosis. The growth arrest is due to decreased protein synthesis and proteasome activation, with subsequent degradation of several cyclins and Cdks.

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Hyperosmotic stress-induced apoptosis and tau phosphorylation in human neuroblastoma cells.
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A characteristic hallmark of Alzheimer's disease brain is the presence of hyperphosphorylated tau; however, the mechanisms responsible for the aberrant tau phosphorylation are unknown. Recently, it has been shown that apoptotic-like processes may be involved in some of the neuronal loss in Alzheimer's disease. In consideration of these findings, the relationship between tau phosphorylation and apoptosis was examined in human neuroblastoma SH-SY5Y cells that were subjected to hyperosmotic stress. In this model caspase 3 activity, which served as an indicator of apoptosis, was increased by 30 min of osmotic stress and remained elevated through 4 hr. Hyperosmotic stress also resulted in a robust increase in tau phosphorylation at both Ser/Pro and non-Ser/Pro sites. Phosphorylation of Ser262/356 (12E8) and Ser396/404 (PHF-1) increased by 5 min and remained elevated for at least 1 hr. In contrast, phosphorylation within the Tau-1 epitope did not increase (as evidenced by decreased immunoreactivity) until 30 min after treatment but remained elevated for a much greater period of time. Treatment with insulin-like growth factor-1 delayed but did not prevent apoptotic cell death induced by osmotic stress and attenuated the increase in phosphorylation at the Tau-1 epitope. L(+), an inhibitor of glycosyn gen synthase kinase 3 beta, had no effect on osmotic stress-induced caspase activation, but reduced phosphorylation at the Tau-1 epitope. Complete inhibition of osmotic stress-induced caspase activation with DEVD-CHO had no effect on the increases in tau phosphorylation. The results of these studies demonstrate that tau phosphorylation is increased at the specific epitopes during apoptosis. However, the changes in tau phosphorylation likely do not significantly impact the apoptotic process but rather occur concurrently as a result of inappropriate activation of specific protein kinases. Nonetheless, there is increasing evidence of a dysregulation of protein kinases that occurs in Alzheimer's disease brain that may be part of the events of apoptosis, which could contribute to aberrant increases in tau phosphorylation. Copyright 2001 Wiley-Liss, Inc.

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