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Aberrant DNA damage response and DNA repair pathway in high glucose conditions

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Abstract

Background—Higher cancer rates and more aggressive behavior of certain cancers have been reported in populations with diabetes mellitus. This association has been attributed in part to the excessive reactive oxygen species generated in diabetic conditions and to the resulting oxidative DNA damage. It is not known, however, whether oxidative stress is the only contributing factor to genomic instability in patients with diabetes or whether high glucose directly also affects DNA damage and repair pathways.

Results—Normal renal epithelial cells and renal cell carcinoma cells are more chemo- and radiation resistant when cultured in high concentrations of glucose. In high glucose conditions, the CHK1-mediated DNA damage response is not activated properly. Cells in high glucose also have slower DNA repair rates and accumulate more mutations than cells grown in normal glucose concentrations. Ultimately, these cells develop a transforming phenotype.

Conclusions—In high glucose conditions, defective DNA damage responses most likely contribute to the higher mutation rate in renal epithelial cells, in addition to oxidative DNA damage. The DNA damage and repair are normal enzyme dependent mechanisms requiring euglycemic environments. Aberrant DNA damage response and repair in cells grown in high glucose conditions underscore the importance of maintaining good glycemic control in patients with diabetes mellitus and cancer.

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Competing Financial Interests

None.

Authors' contributions

AZ, MC, TY, and RG performed experiments and collected the data. YC, DJR, and PLC designed, analyzed, and interpreted the data. AZ, YC and PLC drafted the manuscript. YC and PLC conceived, coordinated and led the study. All authors read, edited and approved the final manuscript.

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Keywords

Diabetes; DNA damage response; ATR; checkpoint kinase 1; Chemo resistant

Introduction

Diabetes mellitus and cancer are two common diseases that frequently overlap in patients, especially with the dramatically increased prevalence of diabetes in recent years. The relationship between these two diseases has already been suggested through population-based studies from as early as 1959 [1]. In later years, the association of diabetes with an increased risk of several common cancers was established through collected clinical evidence [2]. Most epidemiological data relate to type 2 diabetes mellitus, which accounts for 90% of cases worldwide. Data associating type 1 diabetes mellitus and cancer are less robust. Expert panels are still debating on whether hyperglycemia is the key element in conferring cancer risk in diabetes, or whether diabetes is simply a marker of underlying genetic or metabolic factors (e.g., hyperinsulinemia, tissue insulin resistance) that are more relevant [2]. The molecular pathogenic mechanisms for the direct or indirect association between diabetes mellitus and certain cancers are even less thoroughly explored. In particular, the mechanisms by which diabetes and hyperglycemia cause the accumulation of transforming DNA mutations, the hallmark of cancer, remain undiscovered.

DNA damage has been suggested as a major contributing factor for both diabetes and cancer [2]. Hyperglycemia in vivo and high glucose in cell culture systems are known to induce oxidative stress and to decrease DNA integrity. Due to the complex pathology of diabetes, however, it is unclear whether elevated oxidative stress alone directly causes the DNA damage observed in the collected clinical samples. To date, little is known regarding the relationship between hyperglycemia or high glucose concentrations and DNA damage response and repair mechanisms. Controlled data showing that hyperglycemia or high glucose concentrations alter DNA, or that they adversely affect DNA damage and repair mechanisms, would go a long way in providing reasonable proof that high glucose is pathogenic in cancers associated with diabetes.

DNA damage responses are crucial because the maintenance of genome stability is essential for the survival of an organism. Exogenous agents, such as UV radiation, chemotherapeutic agents, or endogenously-generated reactive oxygen species, such as those known to be generated in diabetes [3], can damage DNA. The integrity of a cell's genome is also challenged during the replication and segregation processes of chromosomes. Multiple surveillance mechanisms, including DNA repair, DNA damage checkpoints, and apoptosis, have evolved to maintain genomic integrity [4, 5]. These pathways are collectively known as the DNA damage response (DDR) pathway. Defects in this pathway can lead to genomic instability and ultimately to cancer [6].

Using cell culture systems and animal models, researchers have studied whether high glucose induces DNA damage [7–10]. Most reports using culture systems have used cells transformed by viral oncoproteins as their sources. Unfortunately, this method of immortalizing cells introduces a major confounding element into the interpretation of

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results: the oncoproteins used to immortalize the cells can affect DNA damage response and repair, independent of glucose conditions. As a result, the DNA damage observed in reports using cells transformed by viral oncoproteins could be due to the direct effect of the oncoprotein on DNA, or to combinations of the effects of the oncoprotein and high glucose. This uncertainty makes interpretation of data complicated.

Experiments with animal models used to address DNA damage as a complication of diabetes mellitus have also been imperfect. The most popular experimental in vivo model of type 1 diabetes is achieved by injecting a mouse or rat with streptozocin (STZ) [11]. STZ is an alkylating agent that damages DNA when it enters cells. STZ is structurally similar to glucose; it enters cell through the GLUT2 glucose transporter and directly damages genomic DNA [12]. High expression levels of GLUT2 in pancreatic beta islet cells allow high levels of STZ to enter these insulin-secreting cells and subject them to severe DNA damage, inducing beta cell apoptosis; as a result, hyperglycemia occurs in animal if a sufficient dose of STZ is administered. GLUT2 expression, however, is not limited to the beta cells in the pancreas [13]. Injection of STZ can induce DNA damage in other organs as well, particularly in the liver and kidneys. Therefore, any DNA damage observed in cell types from reports using STZ-induced diabetes might be caused by the unintended, direct effects of STZ, not just by hyperglycemia. The effects of the high glucose conditions on DNA damage observed in reports using STZ to induce diabetes thus have to be interpreted with uncertainty.

In this report, we use several cell types, including well known human kidney cell lines as well as primary, untransformed renal epithelial cells from wild-type and Big Blue® mice (DNA damage reporter mice) [14, 15], to analyze cellular responses in high glucose conditions. In the setting of high glucose and in appropriate control conditions, we examine cell survival, DNA damage response/repair, spontaneous mutation rate, saturation growth density, and anchorage-independent growth. All the results lead to consistent conclusions: normal and cancer cells become more resistant to DNA damage in high glucose conditions, and this phenomenon results in enhanced survival for cells in high glucose after relatively low doses of DNA damaging treatment. High glucose conditions are also associated with abnormal DNA damage responses and slower, less efficient DNA repair. Moreover, a much higher mutation rate is detected when cells are cultured in high glucose conditions, consistent with the less efficient repair. With defective DNA damage responses and poorer DNA repair, cells in high glucose conditions easily became transformed compared to otherwise identical cells cultured in normal glucose conditions.

Materials and Methods

Cell Culture

Human 786-O renal cell carcinoma cells and HK2 human proximal renal tubular epithelial cells were obtained from American Type Tissue Collection (Rockville, MD) and cultured in 50/50 Ham's F-12/Dulbecco's modified Eagle medium containing 10% fetal bovine serum and antibiotics. Primary murine renal tubular epithelial cells were isolated from a 2-week old kidney as previously described [16] and cultured in DMEM/F12 media (7.8 mM glucose

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with 25mM HEPES). Passage 4 or 5 cells were used in the DNA damage response and comet assays.

Antibodies and chemicals

Anti-Nek1 antibodies have been described [17]. Anti-p48, phospho-ATM-S1987, 53BP1, Rad51, Mre11, and GAPDH antibodies were purchased from GeneTex (Irvine, CA) and horseradish peroxidase-based secondary antibodies from Vector Technologies (Burlingame, CA). Anti-phospho-ATR-S428 and phospho-CHK1-S435 were purchased from Cell Signal Transduction (Danvers, MA). Anti-claspin and phospho-Rad17 antibodies were purchased from Bethyl Laboratories (Montgomery, TX). Etoposide was purchased from Sigma-Aldrich Corporation (St. Louis, MO).

Genotoxic treatment

Cells were treated with etoposide at a concentration of 0.25, 0.5, 1, 2.5 or 5 μM. After one hour of treatment, etoposide was removed; cells were then washed twice with phosphate buffered saline (PBS) and refed with fresh media with one of three different glucose concentrations [low glucose = 7.8 mM, high glucose = 37.8mM, or low glucose (7.8mM) with mannitol (30mM)]. For UV irradiation, cells were first washed with PBS twice and then placed inside a UV cross-linker (Stratagene, La Jolla, CA). The dose of UV radiation was monitored with a UV meter. Fresh medium with different glucose concentrations was then added to the cells. Percentages of cells still surviving 24 hours after different doses of UV or etoposide were determined by counting in triplicate the numbers of cells excluding trypan blue vital dye, divided by total number of cells per plate.

Big Blue® Mutation Assay

Primary Big Blue® renal tubular epithelial cells were isolated from 2-week-old Big Blue® mice and cultured in 50/50 Ham's F-12/Dulbecco's modified Eagle medium containing 10% fetal bovine serum, 7.8 mM glucose, 15 mM HEPES and antibiotics. After the first passage, cells were separately cultured in medium containing either 7.8 mM or 37.8 mM glucose. At indicated passages, genomic DNA was isolated and mutation rate was analyzed according to Big Blue® manufacturer's instructions.

Saturation growth density and soft agar colony formation assays

For the saturation growth density assay, 1×10^5 cells were plated onto 60-mm dishes. Each day thereafter, cell number was determined from one dish of cells until there were no more increases in the cell number for two consecutive days. The experiment was repeated and the results shown represent the means of data from the three experiments. Soft agar colony formation assays were performed as previously described [18–20]. Equal numbers of cells (1×10^5 or 2×10^4) from each of the indicated cell growth conditions at different passages were seeded in 0.367% agar. After 21 days of incubation at 37°C, colonies containing at least 50 cells were counted in each plate.

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Results

Radiation and chemo-resistance in high glucose conditions

To test the effect of glucose on DNA damage, we examined survival following different doses of DNA damage when cells were recovered in different glucose conditions, high glucose (HG, 37.8 mM) or low glucose plus mannitol (MG. 7.8 mM glucose plus 30 mM mannitol). Mannitol was added to the low glucose media to control for the effects of hyperosmolarity (i.e., to keep osmolarity equivalent in HG and MG media). Primary, wild-type mouse renal epithelial cells and established human renal cell carcinoma (RCC) cells were used for this experiment. Wild-type mouse renal epithelial cells were more resistant to low doses of UV irradiation when they were recovered in the HG media. With high dose UV irradiation, there was no significant difference between MG and HG conditions (Fig. 1A). Human RCC is well known for its chemo- and radiation resistance [21–23]. To know whether glucose concentration plays a role in chemo-resistance, we tested RCC cell survival after treatment with genotoxic agents and recovery in high or low glucose conditions. We also compared HK2 normal human tubular epithelial cells to 786-O RCC cells treated identically. Both human cell types were more resistant to the etoposide treatment when grown in HG conditions compared to MG conditions (Fig. 1B). The RCC cells were more resistant to etoposide compared to HK2 cells, in both MG and HG conditions (Fig. 1B). In HG, there was no significant killing of 786-O cells even at the highest drug dose tested.

Improper DNA damage response in high glucose

One potential explanation for the radiation and chemo-resistance observed in high glucose conditions might be improper DNA damage response signal transduction in the high glucose environment. To test this possibility, we used Western blot analysis to examine the relative expression, abundance, or activation of early DNA damage response proteins (Nek1, 53BP1, claspin, Rad51, Mre11, γH2AX). Nek1 protein was upregulated or more stably expressed in both hyperosmolar conditions, HG and MG (Fig. 2B). This result suggests that Nek1, in addition to being involved specifically in the DNA damage response, is also a more general stress response protein. Even before any specific damaging treatment, the DNA damage response protein γH2AX, which marks damaged DNA sites, was more abundant in cells recovered in HG medium, but not in those recovered in MG medium (Fig. 2A, lanes 1, 8, and 15; and Fig. 2C). The activation of γH2AX in the HG condition suggests that high glucose indirectly damages DNA or that it creates a condition similar to DNA damage in cells. With low dose UV irradiation, γH2AX is upregulated in all conditions, and the activation is sustained during the recovery phase. The upregulation of claspin and Rad51 proteins after low dose UV irradiation, however, was not sustained in the HG condition.

To further investigate the γH2AX-dependent signaling pathway in cells grown in high glucose, we examined how ATR and ATM kinases are activated when cells are exposed to HG or MG. ATR activation (phosphorylation at its serine 473 residue) was observed only to a slight degree in the HG condition (Fig. 3A, C), despite the previously demonstrated upregulation of γH2AX in the same condition. UV-induced ATR activation was intact in both HG and MG, but sustained ATR activation was not observed when damaged cells were recovered in HG medium. Rad17, a checkpoint response protein downstream of ATM and

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ATR, was upregulated in all three conditions (LG, MG, HG) following irradiation. Another key checkpoint control protein downstream of ATR, activated CHK1 (phosphorylated at its serine 345 residue), however, was not observed in HG conditions following irradiation (Fig. 3A, D). The expected activation of ATM after irradiation was intact in both HG and MG conditions (Fig. 3B). Culturing cells in HG medium, without any UV irradiation, did not activate ATM at all (Fig. 3B, lanes 1, 5, and 9, and E). Taken together, these results suggest that DNA damage response checkpoint signaling is faulty in high glucose conditions.

High glucose increases mutation rate

The observation of failure of the proper response of the checkpoint protein CHK1 following low dose UV irradiation in high glucose conditions suggests that cells in HG may fail to correct the mutations that arise from errors during mitotic replication. Defective checkpoint control in HG conditions could then potentially lead to the accumulation of growth-promoting mutations over time, a hallmark of transformation to cancer. To test whether cells grown in HG conditions would have increased mutation frequency, we employed Big Blue® cells for analysis. Primary renal tubular epithelial (RTE) cells were isolated from Big Blue® mice. These cells provide an excellent tool for examining mutation frequency because of the integrated λ*cII* locus and utilization of lacZ expression as a reporter [13]. Genomic DNA was extracted from the RTE cells at different passages, and then packaged into infectious bacteriophages. After infection of *E. coli* hosts with these phages, mutations in the *cII* gene prevent transcription from the *cI* gene, and force λ phages into their lytic cycle. Mutation frequencies in the λ*cII* gene are determined by quantifying plaques, which stain blue when they express lacZ (Fig. 4). As expected, higher passage cells, which have cumulatively undergone many more replication cycles, accumulated more mutations; this observation represents a good internal control for the integrity of the assay and supports its utility in looking at the effect of high glucose on mutation rate. Most importantly for our study, mutation rates were significantly higher in the cells cultured in HG compared to the cells cultured from the outset in LG. The HG conditions increased mutation rate for both low and high passage cells. This increased mutation rate in the cells cultured in high glucose conditions suggests that the cells have functionally impaired DNA damage/repair responses.

High glucose impairs DNA repair

To test whether DNA damage repair was also impaired in HG conditions, in the setting of the demonstrated accumulation of more mutations, we employed comet assays to examine the ability of cells to prepare their damaged DNA. We treated cells with low dose UV irradiation (0.1 mJ/cm^2) and allowed them to recover in LG, MG, or HG conditions. After gel electrophoresis in alkaline conditions, the nuclear “comet” tail length (a measure of the severity of DNA damage/breaks) and tail moment (a measure of the amount of damaged DNA) were quantified (Fig. 5A). The untreated cells did not accumulate more DNA breaks when cultured for 24 hours in MG or HG compared to culture in LG (Fig. 5B). After 0.1 mJ/cm^2 UV irradiation, however, all cells showed significant DNA breaks in all three culture conditions. Some of these same cells were allowed to recover and repair the damaged DNA in MG or HG (Fig 5C, D). The repair status of damage DNA was measured again by the comet assay (Fig. 5C, D). After one hour of recovery, a significant amount of damaged DNA was repaired in the MG media, as is evident by shortened “comet” tail lengths and smaller

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tail moments. For the cells recovered in HG conditions, in contrast, there was only a slight decrease in tail lengths and moments. After 24 hours of recovery, cells in HG still had significant amounts of damaged, unrepaired DNA when compared to cells recovered in MG. These results strongly suggest that DNA damage repair after low dose DNA damage is impaired in high glucose conditions.

High glucose induces transformation potential

The impaired DNA damage response/repair ability in high glucose conditions and resulting accumulation of mutations suggest that cells in HG have an increased potential to gain growth advantages and to become transformed. Again, primary renal epithelial cells from wild-type or Big Blue® mice were used to test the effect of glucose on cell growth behavior and on the ability of the cells to transform. After cells started to grow primarily from the kidney pieces in low glucose (7.8 mM) DMEM/F12 media with 25mM HEPES, they were allowed to grow to confluence (passage 0, P0) before subculture using a 3T3 protocol in media containing either LG (7.8 mM) or HG (37.8 mM) concentrations. At different passages thereafter, the cells were analyzed for their ability to grow into higher density in a dish as a monolayer (Fig. 6A) or in an anchorage-independent manner in soft-agar (Fig. 6B). Cells began to grow into higher saturation density after only a few passages in HG, while a much higher passage was needed to achieve the same saturation density in LG conditions (Fig. 6A). The cells also differentially demonstrated a transforming phenotype: anchorage-independent growth was observed after only a few passages in HG, but not at all in LG (Fig. 6B). Although cells in LG showed the ability to grow into higher saturation densities in monolayers at higher passages, they still failed to form colonies in soft agar. Higher passage cells therefore grew more readily than low passage cells, but only those primary cells maintained in HG transformed by passages 7 to 11. These results suggest that cells easily gain growth advantages and the ability to transform when maintained in high glucose conditions.

Discussion

In this communication, we have shown that cells in high glucose growth conditions have higher mutation rates and slower repair ability, that they acquire a transformed phenotype, and that they are more resistant to chemo-therapeutic agents. The DNA damage/repair signaling pathway after sublethal treatment with DNA damaging agents is also impaired in high glucose conditions. Our report is not the first to investigate the influence of glucose in the DNA damage repair pathway. It is, however, to our knowledge the first to demonstrate the effect of high glucose on mutation rate, DNA damage repair rate, the DNA damage response signaling pathway, and transforming ability using the primary cells that were never immortalized or transformed by viral oncoproteins.

It is known that high glucose increases oxidative stress [24–26]. This exaggerated oxidative stress also can lead to genomic instability. A proper response to a genomic insult is required for effective repair of damaged DNA in order to avoid the accumulation of detrimental mutations that will cause immediate, programmed cell death. Canonical DNA damage responses have been well characterized [4]. Upon a genomic insult, several protein kinases

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are activated quickly; these are the sensors ATM, ATR, and Nek1. In the experiments we show here, ATR and Nek1 are both activated when cells are cultured in high glucose. Nek1, an early DNA damage response protein [16, 18, 27, 28], is activated in both high glucose and low glucose/hyperosmolar mannitol conditions, which suggests that Nek1 is a general stress response protein, the action of which is not limited to the DNA damage response. Unlike Nek1, the activation of ATR is more specifically evident in high glucose conditions, not in the low glucose/mannitol conditions. ATR apparently is a more selective stress response protein, one that is activated by high glucose and DNA damage, but not primarily by hyperosmotic stress. ATM is activated in neither high glucose nor low glucose/hyperosmolar mannitol conditions, suggesting that ATM is specifically a DNA damage response kinase, not a stress response protein.

During the DNA damage response, γH2AX can be detected at DNA double-strand break sites and can activate downstream signaling. Using cells transformed with the SV40 large T-antigen, researchers have reported that high glucose induces DNA damage in nutrient deprived cells [9, 29]. DNA breaks were shown to be increased in high glucose conditions. Since the cells were nutrient deprived, it is hard to discern whether the DNA breaks were due to the high glucose, to the nutrient deprivation, or maybe even in part to the SV40 T-antigen. We exposed cells to high glucose conditions, with otherwise normal nutrients and without the confounding influence of any oncoprotein. With these conditions, we observed that expression of γH2AX protein, which corresponds to DNA double-strand breaks, was increased. This implies that cells grown in high glucose conditions, like those of untreated diabetic patients, could have more DNA double-strand breaks than those maintained in normal glucose conditions. It is possible, even likely, that oxidative stress leads to oxidative injury and to DNA double-strand breaks. Alternatively, there may be defects in the DDR pathways when cells are exposed to high glucose conditions. The DDR pathway consists of a cascade of kinase activations, which induce cell cycle arrest for proper DNA repair or elicit cell death through apoptosis if repair is not effective [5, 30, 31]. Our survey of key components in the DDR pathway uncovered a critical defect in the DDR pathway when cells are grown in high glucose conditions—namely a defect involving the CHK1 kinase. ATM and Rad3-related kinase (ATR) and CDK have been shown to be involved in the CHK1 kinase activation [32, 33]. ATR is upregulated in high glucose conditions, as we have shown here. Significantly, however, the downstream checkpoint control protein, CHK1, was not activated in high glucose conditions, meaning that there is a defect somewhere between ATR and CHK1 in the DDR pathway. As we have shown, this defect leads to an accumulation of mutations, which is consistent with high glucose being at least in part pathogenic in a mechanism to explain the correlation between diabetes and cancer. Identifying the critical cellular and molecular factors affected by the high glucose condition warrants further investigation.

DNA damaging agents have been used in combating cancer for decades. The execution of the DDR pathway is vital in successfully killing cancer cells while sparing normal cells. In this report, we showed that RTE, HK2, and 786-O cells are all more resistant to cancer therapeutic agents, namely UV radiation and etoposide, when cultured in high glucose conditions. The resistance was more evident when a low dose of a DNA damaging agent, compared to a high dose, was used to treat the cells. These results have potentially important

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implications in the treatment of cancer in patients with diabetes mellitus. Hyperglycemia may make cells more resistant to chemo- and radiation therapy. In addition to affecting the choice of therapeutic anti-cancer options and mortality for individual cancer types, high blood glucose concentrations may be a manipulatable factor for improving response to therapy. Just as tight glycemic control has been shown to improve outcomes in post-surgical and intensive care settings, it may also be important in patients receiving cancer treatment. Epidemiologic studies to date have shown higher incidences and/or poorer outcomes only for certain cancers, in particular those arising from the liver, pancreas, and endometrium [2, 29]; but accumulating data is also beginning to show correlations between diabetes and cancers of the colon, bladder, breast, and kidneys. Our studies using kidney cells should open new avenues of research in other cell types, as well as in exploring the details of how in high glucose conditions CHK1 signaling malfunctions downstream from DNA damage and stress response sensors.

Conclusions

In the report, we examined the effect of high glucose on the DNA damage response. Chemo- and radiation resistance was observed in high glucose conditions. CHK1 activation failed and a DNA repair rate were slower or less efficient. Mutations also accumulated, and a transforming phenotype was observed after relatively few passages of cells cultured in high glucose conditions. The aberrant response to chemotherapeutics found for cells grown in high glucose conditions suggests the importance of maintaining euglycemia in cancer patients, especially those treated with DNA-damaging therapies.

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List of abbreviations

ATR	Ataxia telangiectasia and Rad3 related
ATM	Ataxia telangiectasia mutated
Nek1	Never-in-mitosis A related protein kinase
CHK1	checkpoint kinase 1
CDK	cell cycle dependent kinase
UV	Ultraviolet
γH2AX	histone 2A family X protein phosphorylated on S193
Mre11	Double-strand break repair protein MRE11
Rad51	radiation sensitive mutant 51
Glut2	glucose transporter 2

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STZ	streptozocin
DDR	DNA damage response

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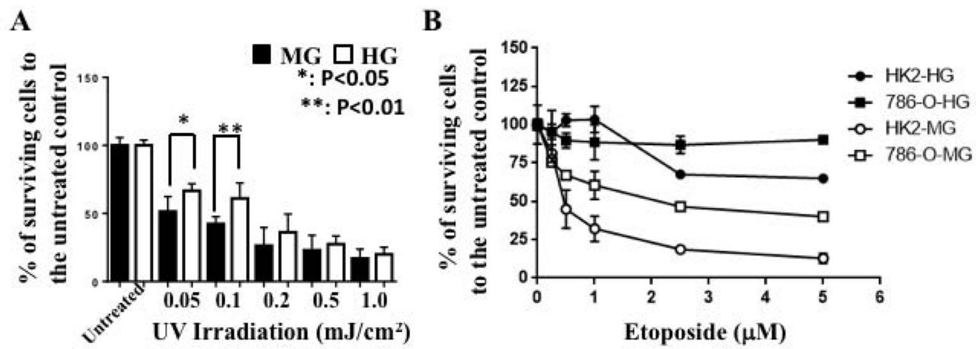

Figure 1.

Radiation and chemo-resistance in high glucose conditions. Mouse renal tubular epithelial cells (A) and human renal cells (HK2) and renal cell carcinoma cells (RCC) (786-O) (B) were subjected to different doses of UV radiation or treated with the topoisomerase inhibitor etoposide. Cell survival was examined 24 hours later. After UV radiation, cells were recovered in either 7.8 mM glucose with 30 mM mannitol (MG) or 37.8 mM glucose (HG). For etoposide treatment, cells were maintained in MG or HG media. Human and mouse cells become more resistant to DNA damaging agents when cultured in high glucose conditions. Greater chemo-resistance was observed in 786-O RCC cells when compared to HK2 normal renal cells in the low glucose conditions. While in the high glucose, HK2 cells also showed chemo-resistance, similar to 786-O RCC cells.

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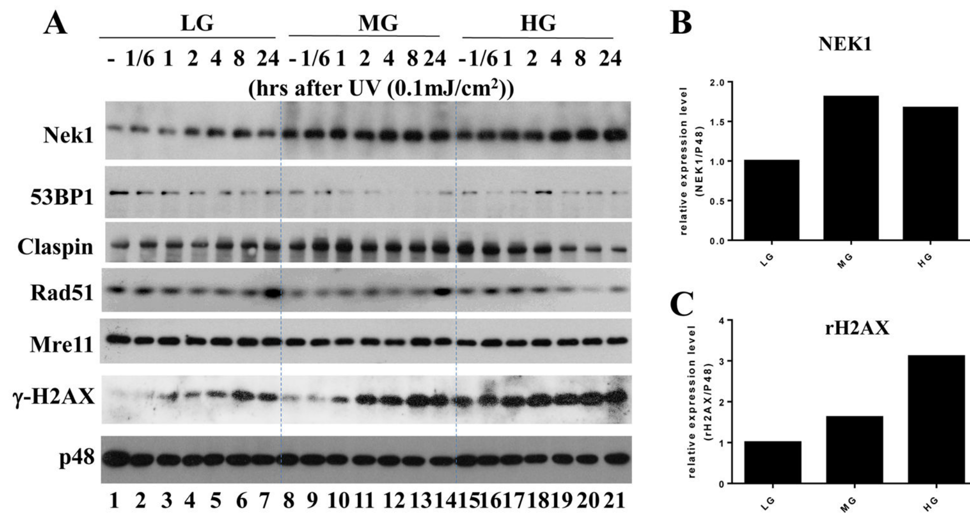

Figure 2.

DNA damage response in cells in high glucose conditions. Mouse renal tubular epithelial cells (passage 3) were treated with UV irradiation (0.1 mJ/cm^2). The cells were thereafter collected at different time points, and in media containing different concentrations of glucose [low glucose (LG, 7.8 mM); high glucose (HG, 37.8 mM)] or in low glucose plus 30 mM mannitol (MG). Total proteins were extracted from cell lysates and subjected to SDS-PAGE analysis (A). The DNA damage response proteins, Nek1, 53BP1, claspin, Rad51, Mre11, and γH2AX, were analyzed. Analysis of p48 expression was included to serve as a protein loading control. Nek1 abundance was increased in both MG and HG low conditions, even before any UV irradiation (A, B). The abundance of claspin and Rad51 wanes over the 24 hours after UV irradiation, specifically in the high glucose condition (A). In the LG and MG conditions, γH2AX, which accumulates at sites of damaged DNA, increased in the hours after UV irradiation, as expected (A). Only in the cells recovered in the HG condition, however, was γH2AX expression significantly upregulated even before any UV treatment (A, lanes 1, 8, and 15, and C).

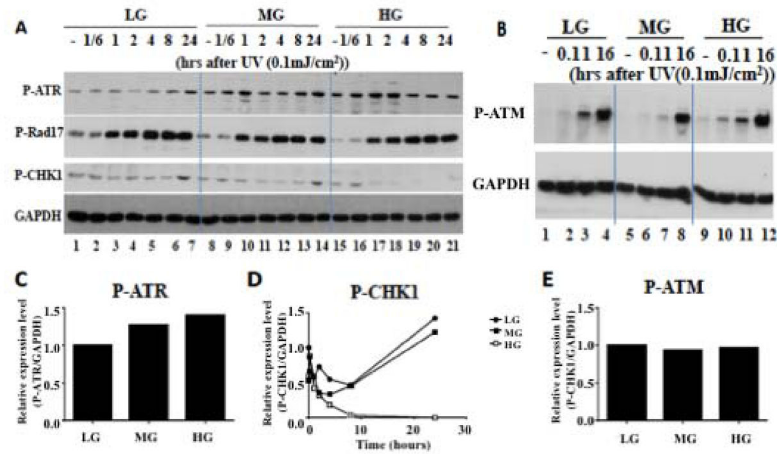

Figure 3.

CHK1 signaling is impaired in high glucose conditions. Mouse renal tubular epithelial cells (passage 3) were treated with UV irradiation (0.1 mJ/cm^2); cells were collected and proteins from lysates were subjected to SDS-PAGE analysis in a manner identical to that described for Fig. 2. Activation by specific phosphorylation of the DNA damage response proteins, ATR, Rad17, CHK1, and ATM, was examined after UV irradiation and after the cells were the maintained in different glucose or glucose plus mannitol concentrations. Here GAPDH was included as the protein loading control. The timing of ATR activation (P-ATR) is normal, irrespective of the glucose condition, as is the timing of Rad17 activation (P-Rad17) (A). Activation of ATM (P-ATM) is also intact in LG, MG, and HG conditions after UV irradiation (B). Histograms show that P-ATR (activated ATR) expression is high at baseline (before UV irradiation) in both high glucose (HG) and low glucose plus 30 mM mannitol (MG) conditions (C). A graph of the expression level of P-CHK1 (activated CHK1) versus time after UV irradiation shows that CHK1 fails to be activated at all, specifically in the HG condition (D). Histograms quantitating relative P-ATR expression show that culturing cells in HG or MG medium, without any UV irradiation, did not activate ATM compared to culture in LG medium (E).

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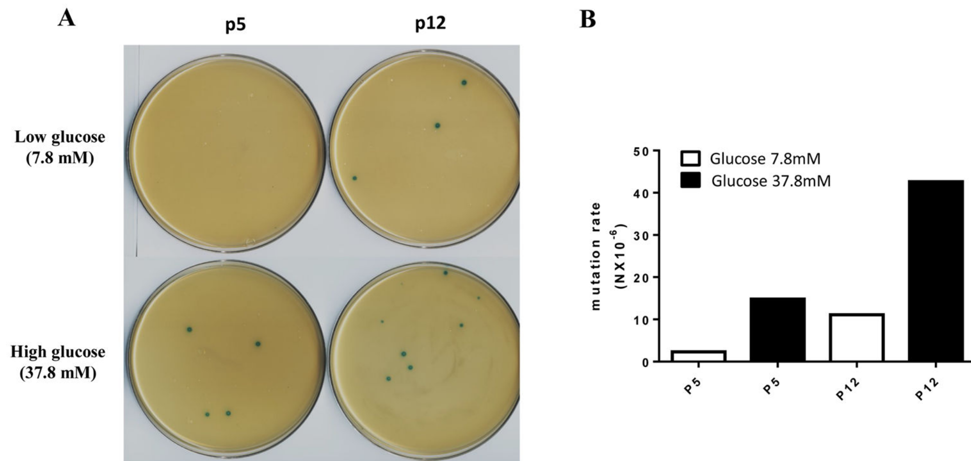

Figure 4.

Increased mutation rate in high glucose conditions. Renal tubular epithelial cells from Big Blue® mice were cultured in DMEM/F12 media containing low (7.8 mM) or high (37.8mM) glucose concentrations. At different passages, cells were harvested and mutation rates were analyzed by Big Blue® assay (counting blue colonies). The mutation rate was significantly higher in the cells maintained from primary culture in medium containing a high glucose concentration. Representative plates (A) and histograms quantitating blue colonies (B) are shown.

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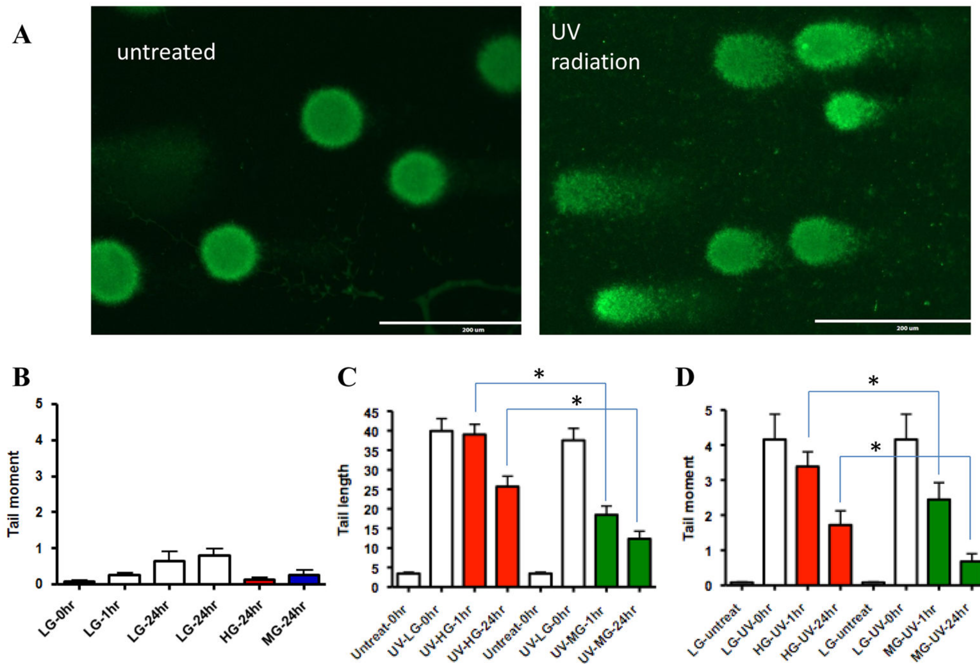

Figure 5.

Reduced DNA repair ability in high glucose conditions after UV irradiation. Renal tubular epithelial cells were cultured in media containing a low (7.8 mM) concentration of glucose. After they were washed three times with PBS, the cells were UV irradiated at low dose (0.1 mJ/cm^2) and refed with 7.8 mM glucose (LG), 7.8mM glucose plus 30 mM mannitol (MG), or 37.8mM glucose (HG). At the indicated times, cells were harvested and subjected to comet assays (A). Cells cultured for 24 hours in different concentrations of glucose, but not treated with UV were also analyzed by comet assays; in these control conditions, no significant differences were observed between cells in different cultured in LG, HG, or MG, either at 0 or 24 hour time points (B). DNA repair ability as assessed by comet tail length (C) and tail moment (D), however, was significantly different in cells maintained in the various glucose concentrations. Based on the comet assays, cells cultured in low glucose repair their DNA faster or better than those cultured in the high glucose. * $p<0.001$

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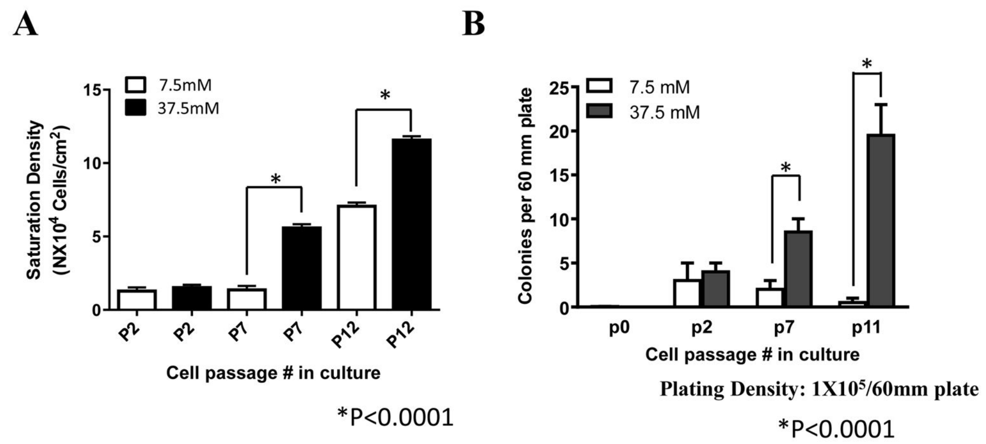

Figure 6.

High glucose increases cell growth rate. Mouse renal epithelial cells (2×10^5) were cultured from primary conditions by plating them onto 60-mm dishes, and then maintained and passed using a 3T3 protocol. Cells at different passages were examined for the saturation density (A) and colony formation in soft agar (B). The cells in the high glucose condition grew to have higher saturation densities and became transformed after only a few passages, as demonstrated by growth as colonies in soft agar.