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NAD(P)H:Quinone oxidoreductase 1 (NQO1) protects bladder epithelium against painful bladder syndrome in mice

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Abstract

Painful Bladder Syndrome (PBS) or interstitial cystitis (IC) is a poorly understood chronic disease, which is characterized by thinning of bladder epithelium and intense pain. Here we demonstrate that NAD(P)H:quinone oxidoreductase 1^{−/−} (NQO1^{−/−}) mice developed in our laboratory represent a new animal model of PBS. NQO1 is known to protect against physiological stress as well as transcription factors against proteasomal degradation. In the present study we demonstrate that NQO1 is necessary for bladder epithelium integrity, and to prevent the development/progression of PBS. We observed down regulation of energy metabolism, adhesion, and apoptotic signaling cascades, which led to mitochondrial aberrations and profound alterations in energy metabolism, increased susceptibility to ROS generation and apoptosis in luminal epithelium in NQO1^{−/−} mice that were absent in wild-type mice. These pathophysiological changes led to incidence of PBS in NQO1^{−/−} mice. The results together demonstrate for the first time that NQO1 is an endogenous factor in protection against PBS.

Keywords

NQO1; bladder; Interstitial Cystitis; Painful Bladder Syndrome

Introduction

NQO1 is a ubiquitous cytosolic phase II biotransformation enzyme whose primary physiological role is catalysis of two-electron reduction of quinones and thereby their detoxification [1]. NQO1 is induced downstream exposure to radiation and chemical stress [1–3]. Epidemiological studies have shown a correlation between NQO1P187S mutation leading to loss of enzyme with bladder cancers in smokers [2,4].

Painful Bladder Syndrome (PBS), also known as interstitial cystitis (IC), is a disease usually associated with advanced age and it primarily affects women more than men [5–6]. The pathology of PBS develops sequentially with the formation of lesions on the interior of the bladder wall, which is lined with a layer of cuboidal luminal epithelium. This is followed by

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an eventual degradation of the contact between the luminal epithelium and the basal myoepithelial layers which form the bulk of the bladder wall [7]. This loss of contact leads to intense pain and impairment of bladder function, for which effective diagnostic tools and treatment has not been developed. The molecular causes of PBS have yet to be elucidated, although there is increasing evidence which suggests the centrality of damage to luminal epithelial cell layer in the development of this disease [8].

There are several key features of PBS that are relevant for investigating the pathophysiological features including, but not limited to, alterations in genes regulating apoptosis and cell proliferation [8], defects in luminal epithelium [7], mast cell invasion [9], and possibly autoimmune involvement [10–11]. However, genetic biomarker(s) of susceptibility to PBS remain unknown. In the present study we investigated these features of PBS development and underlying signaling changes which connect loss of NQO1 in bladder tissue with PBS incidence in mice.

Materials and Methods

Mice

C57/BL6 mice containing or lacking NQO1 were characterized previously [12] and housed in our Animal Housing facility using chow and water ad libitum. Mice were sacrificed according to IACUC-approved protocols.

Morphological examination of bladders

Whole bladders from age-matched mice of both sexes, ages 6, 12, and 24 weeks, wild-type, NQO1^{-/-}, and NQO1^{+/-} heterozygous genotypes were collected from isoflurane-euthanized mice and examined for absence and presence of PBS using dissecting microscopic, histological, and electron microscopic techniques.

Histochemistry and Immunohistochemistry

Whole bladders from age-matched mice were fixed in 10% formalin and embedded in paraffin. Sections were cut and mounted to charged slides, and Hematoxylin/Eosin stained slides were prepared. Histochemistry slides were prepared: TUNEL assay performed on paraffin-embedded tissue sections, and trichrome staining performed. Immunohistochemistry was performed on remaining slides, using antibodies against Tryptase.

Cell culture and Western blots

Bladder cells were cultured in DMEM+1% p/s+10% FBS. Tissue homogenates were prepared by removing whole bladders from freshly-sacrificed mice and lysing tissue as a 10% homogenate in ice-cold RIPA buffer (50mM Tris-Cl, pH 7.4, 150mM NaCl, 1% NP-40, 1mM EDTA, 0.5% Na Deoxycholate, and 1% Triton X100 with 1mM PMSF and 1x Protease Inhibitor Cocktail (Complete, Roche)) using glass and Teflon pestle system, and further clarified via centrifugation at 14000rpm at 4°C for 10 minutes. Western blotting was performed essentially according to Towbin *et al.* [13].

Immunoprecipitation

Human bladder carcinoma T24 cells were cultured in DMEM+10% FBS+1% p/s at 37°C in 5% carbon dioxide. Cells were incubated with chemicals as necessary and washed 3x in PBS on ice. Cells were lysed in RIPA buffer and protein A/G beads (Santa Cruz, CA) were equilibrated with 1mg aliquots for 1h. After equilibration, beads were removed and lysates

were incubated o/n with 1:100 of relevant antibodies, and next day incubated with 30 microliters A/G beads for 1h before 3x RIPA buffer wash and boiling 5 mins.

Gene microarray studies

Microarray studies were performed using freshly-excised bladder tissue from age-matched 24 week old female wild-type and NQO1^{-/-} mice. Bladders in groups of 3 were homogenized in glass pestles on ice to form a pool of total RNA using the RNEasy kit and QiaShredder (Qiagen). Quality of purified RNA was verified using ABI Bioanalyzer, and pools meeting quality criteria were selected for microarray studies. Samples were annealed to Mouse Genome 430 2.0 ST array (Affymetrix) with 3 chips used per sample group, according to manufacturer instructions. Statistical and pathways analysis of results of microarray studies were performed by Dr. Conover Talbot at Johns Hopkins University and by Brad Patrick, using Ingenuity software (Redwood City, CA) to perform statistical analysis of results using Spotfire software (Tibco, Somerville, MA). Results were grouped according to physiological process and fold-change.

Reverse-transcriptase PCR

Primers for RT-PCR of MUP1 gene were designed using NCBI database. NQO1 primers were used as genotype markers and GAPDH primers were used as loading control. Reactions were performed using Superscript One-step RT-PCR kit (Invitrogen) as per manufacturer recommendation, and separated on 1% agarose gel, and photographed.

Ex-vivo cell culture and chemical treatment

Whole bladders from age-matched female wild-type and NQO1^{-/-} 24 weeks-old mice were excised and minced in a 10cm dish with a razorblade. Pieces were suspended in microfuge tubes containing 500 microliters of 1% Collagen reconstituted in PBS and incubated at 37°C with 700rpm shaking for 45 minutes to dissociate cells from tissue. Tubes were centrifuged at 500g for 3 minutes to pellet cells, and resuspended in full cell culture media. Cells were plated in 8-well tissue culture chamber slides (Lab-Tek) with cells from one bladder equally divided into one 8-well slide, into 1mL media total volume. After 2–4 days of growth with daily media changes, cells were treated with various concentrations of benzo[a]pyrene or acetone control in fresh media for 18h, followed by removal and addition of DCF (Sigma) at 5 micromolar for 1h. DCF was then removed and full media added for 1h, followed by immediate mounting of cover slips in VectaShield (Vector Labs) and viewing under FITC-viewing filter to determine fluorescence of ROS-modified DCF within cells.

Electron microscopy

Tunneling electron microscopy studies were performed on fixed whole bladder tissues at the University of Maryland Electron Microscopy Core Facility. Whole bladders were fixed in a solution of 4% Paraformaldehyde/2.5% Glutaraldehyde overnight at 4°C from freshly-sacrificed mice. Bladders were embedded in successive concentrations of epoxy resin until encased and then sections trimmed until appropriate orientation and size was achieved. Sections were embedded on grids and stained with Uranyl Acetate/Lead and photographs were taken at 4400x and 30000x magnifications, using samples of 24 week old age-matched female wild-type and NQO1^{-/-} (samples from 2 separate animals from each genotype). Black bars on photographs indicate size references.

Statistical analysis

All experiments were performed at least 3 replicates and data collected were statistically analyzed where noted using Student's T test.

Results

PBS dependence on NQO1 gene copy number

We observed that while in wild-type bladders luminal epithelium was intact at both 6 and 24 weeks, luminal epithelium in bladders from NQO1^{-/-} mice had profound loss of integrity from 6 to 24 weeks of age, as visualized in H&E staining (Fig. 1A). Similarly, in trichrome-stained tissue sections we observed a detachment of the luminal epithelium from the underlying basolateral layer in NQO1^{-/-} mice at 24 weeks of age, which was not present at 6 weeks, or at any age in wild-type mice (Fig. 1B). To investigate whether there was an immune response present in affected bladders tissue sections were stained for Tryptase, a well-characterized marker for mast cell invasion of tissues [14]. We found that there was a significant increase in mast cell invasion of bladder tissues in NQO1^{-/-} bladders at 24 weeks of age, compared to wild-type (Fig. 1C). In morphological studies of whole bladders excised from 6-, 12- and 24-weeks old female and male mice of wild-type and NQO1^{-/-} genotypes there was a profound increase of PBS observed in female NQO1^{-/-} mice that were at least 12 weeks of age, which was not present in juvenile NQO1^{-/-} mice (6 weeks old) (Fig. 1D). Twelve-weeks and older male NQO1^{-/-} mice also showed PBS, but at lower incidence (fewer mice positive for PBS-like disease) than female mice (Fig. 1D). PBS was not observed in any mice from the wild-type population (Fig. 1D). These results suggest that PBS is both age and sex driven, which is regulated by the presence of NQO1 in bladder. Further, we observed female and male NQO1^{+/-} heterozygous mice at similar ages and found that they exhibited more or less similar morphology of PBS to homozygous NQO1^{-/-} mice, suggesting that NQO1 must be present in both alleles to prevent this pathology (Fig. 1D). Supplementary Figure S1 illustrates the lack of morphological changes present in NQO2^{-/-} mice which served as the criteria for PBS-like disease in NQO1^{-/-} mice.

Increased apoptotic signaling in NQO1-null, PBS-positive bladders

Subsequent to these observations, we investigated the biological effects of the PBS morphology on bladder tissues. Sections of bladder from 6- and 24-weeks old wild-type and NQO1^{-/-} mice were stained for terminal deoxynucleotidyl transferase dUDP nick-end labeling (TUNEL), a well-characterized marker for DNA fragmentation in cells. We observed that while there was a slight increase in DNA damage at 6 weeks in NQO1^{-/-} over wild-type bladder epithelium, there was a much larger difference in TUNEL-positive cells along the luminal epithelium at 24 weeks (Fig. 2A). The analysis also showed an increase in caspase 3 activity (Fig. 2B) and tumor suppressor p53 (data not shown) in 24-weeks old NQO1^{-/-} mice as compared to age matched wild-type mice. Additionally, stressors have been shown to play a role in bladder pathology [15] and PBS patients may be more likely to accrue further environmental stress-related tissue damage due to weaker nociception [16]. Therefore, we investigated the role of environmental stressors on bladder stress signaling. In ex vivo studies where cells from wild-type and NQO1^{-/-} bladders were cultured and subjected to environmental insult via the well-characterized hydrocarbon benzo[α]pyrene (B[α]P), we observed a markedly increased susceptibility in NQO1^{-/-} bladder cells to B[α]P-induced generation of reactive oxygen species (ROS), relative to wild-type bladder cells (Fig. 2C). These results suggest that NQO1^{-/-} bladder tissue exhibits a tendency towards significantly stronger pro-apoptotic signaling than does wild-type, which might explain the profound loss of adhesion of the luminal epithelial layer, exposed to the harsh environment of the bladder lumen, in morphological examinations.

Disruption of several regulatory cascades in PBS-positive bladder tissue

We performed microarray analysis to discover possible molecular explanations for these observed morphological and apoptotic changes. Downregulation of several cellular signaling pathways, including mitochondrial function, cellular growth/proliferation/differentiation,

anti-apoptosis, and extracellular matrix/adhesion was observed (Fig 3A). Interestingly, NQO1^{-/-} mice bladder showed a significant down regulation of PGC1 α , a well-characterized strong regulator of energy metabolism [17] and major urinary proteins (MUP1 and MUP2), a class of proteins which have as yet been poorly characterized, but have been shown to regulate energy metabolism in cells (Fig. 3A) [18–19]. Microarray data also showed down regulation of anti-apoptotic and up regulation of apoptotic factors in NQO1^{-/-} mice, as compared to wild type mice bladder tissues (Fig. 3A). We followed these microarray studies up with RT PCR and/or Western blotting to verify changes in most relevant genes and determined that there was, in fact, a significant down regulation of MUPs, PGC1 α , and anti-apoptotic/growth and differentiation factors such as Bcl-2, p21, and p27 (fig. 3, B and C). Western analysis also showed significant up-regulation in pro-apoptotic factors such as p53, Bad, and Bax (Fig. 3C). Additionally, there was a significant downregulation in extracellular matrix/adhesion-related factors such as Keratins 4 and 23 (Fig. 3C). These results suggest a set of molecular signaling cascades, including energy metabolism and mitochondrial function, which may lead directly to apoptosis and ECM disruption, thus explaining the observed morphological aberrations in PBS-positive NQO1^{-/-} mouse bladder tissues.

Ultrastructural changes in NQO1-null bladder mitochondria

After determining that the loss of NQO1 in the bladder leads to problems in energy metabolism signaling we investigated possible changes in mitochondria, which may explain a decrease in mitochondrial function. We used tunneling electron microscopy techniques to photograph the full thickness of bladders from 24 week old wild-type and NQO1^{-/-} female mice and observed the environment in the luminal epithelium where the previously-observed lesions were present. In the luminal epithelium we measured the mitochondria and recorded differences between wild-type and NQO1^{-/-} tissues. There were striking ultrastructural differences in the mitochondria of NQO1^{-/-} bladders, such as a significant decrease in average size (Fig. 4A), as compared to wild-type. Additionally, we observed that the mitochondria of bladders lacking NQO1 expression tended to be rounded, as opposed to the typical elliptical/bacillus shape found in most tissues and observed in wild-type bladder mitochondria, although the wide variance in counts across various fields led to p-values too high to achieve statistical relevance (Fig. 4B). The most striking observation, however, was that in many NQO1^{-/-} mitochondria, we observed both an increase in fragmented outer mitochondrial membranes and a noticeable decrease or lack of discernable cristae structure within the mitochondrions (Fig. 4B and C), all of which may explain observed changes in energy metabolism and may be explained by upstream loss of gene expression.

NQO1 regulation of PGC1 α via direct interaction

Earlier, we have found that NQO1 directly interacts with p53 and p63 and protect these factors against 20S proteasomal degradation [20–21]. Therefore, we investigated whether signs of mitochondrial energy metabolism which were previously observed could be due to a lack of protection of metabolism-signaling factors by NQO1. We selected PGC1 α for further studies on mechanism of NQO1 control of factors because of its established role in control of energy metabolism [17]. We also included two established regulators of PGC1 α expression, cAMP response element-binding (CREB) and AMP-activated protein kinase (AMPK) [22] in our studies since PGC1 α RNA was down regulated in bladders from NQO1^{-/-} mice in microarray analysis (Fig. 3A). Western analysis demonstrated significant decrease not only in PGC1 α but also its regulators CREB and AMPK in bladders from NQO1^{-/-} mice, as compared to wild type mice (Fig. 3C and 5A). We also used human bladder carcinoma cell line T24 for NQO1 control of PGC1 α studies since it expresses detectable amounts of NQO1, PGC1 α , CREB and AMPK. We used siRNA targeted NQO1 knockdown studies to determine that CREB, AMPK and PGC1 α are all significantly

regulated by NQO1 (Fig 5B). siRNA mediated inhibition of NQO1 led to significant decreases in CREB, AMPK and PGC1 α (Fig. 5B). Furthermore we showed that CREB and AMPK but not PGC1 α proteins were rescued from down regulation when proteasome activity was inhibited by MG-132 (Fig. 5C), suggesting that proteasomal degradation is a significant route of down regulation of CREB and AMPK proteins and that NQO1 expression is necessary to prevent that degradation. Next we performed immunoprecipitation studies to determine whether NQO1 can directly interact with CREB and AMPK, or PGC1 α and whether that interaction is affected by known NQO1 inducer and stress agent benzo(a)pyrene. While we found no interaction with PGC1 α directly (data not shown), we did find that NQO1 is capable of binding to both CREB and AMPK in T24 and that such interaction appears to be increased during stress conditions leading to stabilization of factors (Fig. 5D and E). Immunoprecipitation studies also showed 20S proteasome interaction with CREB and AMPK that more or less did not change with benzo(a)pyrene stress (Fig. 5D and E).

Based on the above results we constructed a model for NQO1 protection against PBS (Fig. 6, left panel) and NQO1 control of energy metabolism regulation of PGC1 α via protection of AMPK/CREB from proteasomal degradation (Fig. 6, right panel).

DISCUSSION

Previously, animal models have been generated that mimic certain aspects of PBS pathophysiology [23–25]. These included autoimmune cystitis model [23], cathelicidin peptide induced bladder inflammation [24] and more recently APF based inhibition of bladder epithelial repair model [25]. These models temporarily mimic some aspects of PBS and are not based on knocking down of a susceptible marker as developed by us in the current report. The published models [23–25] are unrelated to NQO1 $^{-/-}$ model of PBS characterized in the present report.

It seems apparent from the studies in the present report that NQO1 can strongly regulate several processes in the mouse bladder, which are relevant to the pathophysiology of PBS. The observed morphological changes in NQO1 $^{-/-}$ mouse bladder, including loss of adhesion of luminal epithelium and mast cell invasion, were characteristic of PBS. These changes suggested significant increases in apoptotic signaling, which was verified in studies involving DNA fragmentation and ROS-generation susceptibility. Investigation of modulation in gene expression in NQO1 $^{-/-}$ bladder tissue led to the observation of increased expression of several pro-apoptotic factors and downregulation in anti-apoptotic factors, as well as downregulation of several key regulators of both proliferation and ECM adhesion. Ultrastructural studies of wild-type and NQO1 $^{-/-}$ bladders revealed that mitochondrial morphology was indeed significantly altered in NQO1 $^{-/-}$ tissue from that of wild-type tissue, findings which suggest that downstream effects of observed down regulation of energy metabolism regulators directly targets mitochondria in the luminal epithelium. Additionally these mitochondrial changes may serve as a middle step in the pathophysiological pathway towards loss of overall energy metabolic function in luminal epithelium and eventual apoptosis and/or loss of adhesion to underlying basolateral structures of the affected bladder. In addition, B cell/immune deficiency in NQO1 $^{-/-}$ mice as reported earlier [26] might also have contributed to development of PBS in mice (Fig. 6). It is noteworthy that immune deficiency has been identified as one of the factors contributing to development of PBS in human [10,11]. Overall, the above findings suggest a strong role of NQO1 in the pathology of Painful Bladder Syndrome (Fig. 6).

The mechanism of protection exerted by NQO1 against PBS was investigated by exploration of the two known physiological activities of this enzyme. Firstly, we determined that

antioxidant NQO1 protect bladder epithelial cells against accumulation of high levels of ROS, as is evidenced by such accumulation after environmental chemical insult. We hypothesize that it is this protection against redox imbalance afforded by constitutive expression of NQO1 in bladder epithelium which preserves this layer from the progression of PBS in adult mice.

Alternately, NQO1 may prevent the onset of PBS via the second well-characterized mechanism of protection of various signaling molecules against degradation by the 20S proteasomal complex. This activity of NQO1 has been shown to play a significant role in preservation of signaling for several proteins, including p53 and p63 [20,21], and may act directly to prevent degradation of a large number of the targets including energy metabolism regulator PGC1 α already identified in these studies. Future directions include identifying the scope of this unique mechanism of protection and determining its precise implications for the pathophysiology of PBS. This mechanism might work alone or in conjunction with antioxidant property of NQO1.

The human NQO1 gene is localized on chromosome 16q22 and NQO2 on 6p25 [27]. A C-->T polymorphism of human NQO1 gene produces a proline to serine (P187S) substitution that leads to degradation of the protein [28,29]. Individuals carrying both mutated genomic alleles are completely deficient in NQO1 activity, whereas heterozygous individuals have intermediate NQO1 activity compared with normal individuals [29,30]. Approximately 2–4% individuals are homozygous and 20–25% are heterozygous for this mutation [31,32]. The current studies suggest that human individuals that carry NQO1P187S homo- or heterozygous mutation are susceptible to develop PBS and are a subject of future investigation. It is noteworthy that disruption of NQO2, the only other member of the NQO gene family, in mice did not demonstrate signs of PBS in younger/older female/male mice. Therefore, NQO1, and not NQO2, is the endogenous factor in protection against PBS. It is also noteworthy that the effect of NQO1 loss on PBS-like disease onset appears to be a sex-specific phenomenon, with far lower penetrance in male mice than in female mice. This disparity may be related to the disparity in the incidence of PBS/IC in humans, with females being affected far more than males. Further studies of PBS-positive NQO1-null mice with a comparison of males vs. females using a genomic approach may be necessary to determine the mechanisms behind this.

CONCLUSIONS

In conclusion, we generated a mouse model of PBS, which showed that NQO1 is an endogenous factor in protection against PBS in female mice and possibly in male mice. Both NQO1 $-/-$ and NQO1 $+/-$ mice demonstrated symptoms of PBS. Disruption of NQO1 gene in mice led to increased reactive oxygen species, alterations in factors regulating energy metabolism and adhesion, and loss of mitochondrial structures including cristae structures. These together resulted in increased apoptotic cell death and loss of bladder urothelium. Mechanistically, NQO1 through its antioxidant property and its control of stability of energy metabolism and other factors against 20S proteasomal degradation contributed to protection against PBS. The results also suggested that human individuals that carry NQO1P187S homo- or heterozygous mutation might be susceptible to develop PBS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

PBS	Painful Bladder Syndrome
NQO1	NAD(P)H: Quinone Oxidoreductase 1
ROS	Reactive Oxygen Species
DCF	2',7'-Dichlorofluorescein
ECM	Extracellular matrix
PGC1α	Peroxisome proliferator activated receptor gamma coactivator 1-alpha
AMPK	AMP activated kinase
CREB	cAMP response element binding

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Research highlights

- Mice model for Painful Bladder Syndrome in aged female NQO1^{-/-} mice is developed.
- NQO1^{-/-} mice bladders showed Painful Bladder Syndrome-like ulcerations.
- NQO1 loss led to alteration in bladder mitochondrial structure and energy metabolism.
- Loss of NQO1 also increased apoptosis that led to Painful Bladder Syndrome.
- We conclude that NQO1 protects against Painful Bladder Syndrome.

FIG. 1.

Painful Bladder Syndrome in NQO1^{-/-} mice. (A), Damage to luminal epithelium in NQO1^{-/-} mice. Bladders from wild-type and NQO1^{-/-} mice (both 6 and 24 week old, age-matched female) were fixed in formalin and embedded in paraffin, and sections were stained with hematoxylin & eosin. (B), Separation of bladder luminal epithelium from basolateral layers in NQO1^{-/-} mice. Paraffin-embedded bladder sections (6 and 24 week old female) were stained with Masson's Trichrome staining. (C), Mast cell invasion in NQO1^{-/-} bladders. Paraffin-embedded sections (6 and 24 week old female) were stained with anti-Tryptase antibodies and stained with Vector Red with hematoxylin counterstain. To right is a bar graph showing tryptase (red)-positive cells per microscopic field, averaged from 10 fields, at ages 6 and 24 weeks of age. (D), Incidence of Painful Bladder Syndrome in mouse bladders according to genotype, age, and sex. Twenty mice in each group were harvested; bladders were dissected and examined visually and via microscopy to determine bladder health and incidence of PBS. Bladders showing symptoms consistent with PBS were counted as PBS-positive. Error bars are SD.

FIG. 2.

Increased apoptotic signaling in NQO1^{-/-} mice with PBS. (A), Increased DNA damage in NQO1^{-/-} mice bladders. Paraffin-embedded sections from bladders (6 and 24 weeks old) were stained for terminal deoxynucleotidyl dUTP nick-end labeling (TUNEL) stain to detect fragmentation of genomic DNA. TUNEL-positive nuclei are stained dark brown (blue staining is nonspecific background). Beneath photographs is bar graph counting TUNEL-positive nuclei at 6 and 24 weeks. (B), Increased caspase expression and activity in bladders from NQO1^{-/-} mice. Bladders from 24 week old female wild-type and NQO1^{-/-} mice were excised and lysed in either RIPA buffer (for Western blots) or Promega Passive Lysis buffer (caspase 3 activity assay) and analyzed. Bar graph measures relative caspase 3 activity, normalized to wild-type values. (C), Increased susceptibility to benzo[α]pyrene (B[α]P)-induced ROS production in cells derived from NQO1^{-/-} bladders. Bladders cells from 24 week-old female wild-type and NQO1^{-/-} mice were incubated with 100 μ M B[α]P in full media for 18h followed by 1h DCF (10 μ M) and 1h media recovery, and then visualized under coverslips via microscopy. ROS-positive cells fluoresce green. Beneath photographs is bar graph counting ROS-positive cells at for control (acetone) and B[α]P-treated cells. Error bars are SD.

FIG. 3.

Modulation of signaling cascades in NQO1^{-/-} mice with PBS. (A), Changes in genes regulating mitochondrial function, growth/differentiation/proliferation, apoptosis, and extracellular matrix adhesion. Bladders from 24 week old female wild-type and NQO1^{-/-} mice were excised and total RNA was purified using Qiagen RNEasy kit with DNase treatment. Aliquots were analyzed via gene microarray and results were analyzed with statistical and pathways analysis software. (B), Decrease in major urinary protein expression in NQO1^{-/-} bladder tissue. Total RNA from 24 week old bladders was analyzed via RT-PCR using primers against MUP1, NQO1, and GAPDH control. (C), Decrease in protein expression of key regulating factors in NQO1^{-/-} bladder. Bladders from 24 week old mice were excised and homogenized in RIPA buffer, and aliquots were separated via SDS-PAGE gel and probed with antibodies against MDM2, p53, Bcl-2, Bad, Bax, PARP, p21, p27, PGC1- α , Keratins 4 and 23, NQO1, and GAPDH control. Error bars are SD.

FIG. 4.

Electron microscopic analysis of mitochondrial aberrations in NQO1^{-/-} bladders with PBS. (A), Decrease in mitochondrial size in NQO1^{-/-} bladder epithelium. Bladders from 24 week old female wild-type and NQO1^{-/-} mice were fixed in glutaraldehyde/formaldehyde, embedded in epoxy resin, and sections containing luminal epithelium were photographed via tunneling electron microscopy (TEM) at 4400x magnification. Bar graph measures average size of mitochondria present in at least 10 fields. (B), Increased roundedness of mitochondria in NQO1^{-/-} bladder epithelium. Resin-embedded bladder sections were photographed via TEM at 30000x magnification. (C), Measurements of mitochondrial aberrations in NQO1^{-/-} bladders. TEM photographs of resin-embedded bladder sections were measured for shape (rounded vs elongated) and fragmentation of inner cristae. Measurements are averages for mitochondria found in at least 10 visual fields and error bars are SD.

FIG. 5.

NQO1 regulation of energy metabolic regulator factor PGC1 α in bladder. (A), Bladders from female wild type and NQO1 $^{-/-}$ mice were immunoblotted and probed with antibodies as indicated. (B), T24 human bladder carcinoma cells were transfected with control or NQO1-specific siRNA for 24h and then immunoblotted with indicated antibodies. (C), T24 human bladder carcinoma cells were transfected with control or NQO1-specific siRNA for 24h and then incubated for 6h with vehicle or proteasome inhibitor MG-132 before lysis and separation via SDS-PAGE with probing with antibodies listed. (D/E), T24 cells were incubated for 16h with B[a]P and then immunoprecipitated with antibodies against CREB (D) and AMPK (E) overnight. Samples were separated via SDS-PAGE and probed with antibodies listed.

*PGC1 α , MDM2, Bcl2, etc.

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FIG. 6.

Hypothetical pathway of Painful Bladder Syndrome pathology in NQO1 $^{-/-}$ mice. Left panel. Loss of NQO1 expression in bladders leads to down regulation of various regulating factors, which leads to mitochondrial damage, affecting energy metabolism. That coupled with already-observed B-cell deficiency leading to immune system attack of the luminal epithelium causes apoptosis and damage to extracellular matrix, which ultimately results in Painful Bladder Syndrome, which has as a feature mast cell invasion of tissue. Right panel. Hypothetical model for NQO1 regulation of PGC1 α via direct interaction with and protection of known regulators AMPK and CREB.