Metabolic State Determines Sensitivity to Cellular Stress in Huntington Disease: Normalization by Activation of PPARγ

Youngnam N. Jin1, Woong Y. Hwang1, Chulman Jo2, Gail V. W. Johnson1,2*

1 Department of Pharmacology and Physiology, University of Rochester, Rochester, New York, United States of America, 2 Department of Anesthesiology, University of Rochester, Rochester, New York, United States of America

Abstract

Impairments in mitochondria and transcription are important factors in the pathogenesis of Huntington disease (HD), a neurodegenerative disease caused by a polyglutamine expansion in the huntingtin protein. This study investigated the effect of different metabolic states and peroxisome proliferator-activated receptor γ (PPARγ) activation on sensitivity to cellular stressors such as H2O2 or thapsigargin in HD. Striatal precursor cells expressing wild type (STHdhQ7) or mutant huntingtin (STHdhQ111) were prepared in different metabolic conditions (glucose vs. pyruvate). Due to the fact that STHdhQ111 cells exhibit mitochondrial deficits, we expected that in the pyruvate condition, where ATP is generated primarily by the mitochondria, there would be greater differences in cell death between the two cell types compared to the glucose condition. Intriguingly, it was the glucose condition that gave rise to greater differences in cell death. In the glucose condition, thapsigargin treatment resulted in a more rapid loss of mitochondrial membrane potential (ΔΨm), a greater activation of caspases (3, 8, and 9), and a significant increase in superoxide/reactive oxygen species (ROS) in STHdhQ111 compared to STHdhQ7, while both cell types showed similar kinetics of ΔΨm-loss and similar levels of superoxide/ROS in the pyruvate condition. This suggests that bioenergetic deficiencies are not the primary contributor to the enhanced sensitivity of STHdhQ111 cells to stressors compared to the STHdhQ7 cells. PPARγ activation significantly attenuated thapsigargin-induced cell death, concomitant with an inhibition of caspase activation, a delay in ΔΨm loss, and a reduction of superoxide/ROS generation in STHdhQ111 cells. Expression of mutant huntingtin in primary neurons induced superoxide/ROS, an effect that was significantly reduced by constitutively active PPARγ. These results provide significant insight into the bioenergetic disturbances in HD with PPARγ being a potential therapeutic target for HD.

Introduction

Huntington disease (HD) is an inherited neurodegenerative disease caused by an abnormal expansion of polyglutamine in the huntingtin (Htt) protein. Neuronal degeneration in HD patients begins in the striatum, especially GABAergic medium size spiny neurons, followed by involvement of the cerebral cortex as the disease progresses [1]. Despite the discovery of the unique causative genetic mutation of Htt almost two decades ago [2] there is still no satisfactorily effective treatment, and the underlying pathogenic mechanisms of HD are still elusive. Bioenergetic deficits manifested as weight loss, muscle wasting, reduced glucose uptake in cortex and striatum, and increased incidence of diabetes have been implicated in the pathogenic progression of HD [3,4,5]. Importantly, an increasing number of studies have shown that mutant Htt (mHtt) results in mitochondrial impairment such as deficits in the electron transport chain, Ca2+ handling defects, and increased sensitivity of mitochondria to permeability transition pore (mPTP) opening [4,5,6,7]. Furthermore, numerous studies have demonstrated that oxidative stress plays a pivotal role in the pathogenesis of HD [8,9,10].

Transcriptional dysregulation has been considered a crucial pathogenic mechanism in HD [5,11]. Many studies have reported that the nuclear localization of mHtt leads to dysregulation of transcriptional factors/cofactors including peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1α (PGC-1α) [12]. PGC-1α is a master regulator of mitochondrial functions as it regulates the expression of genes involved in mitochondrial bioenergetics and respiration, detoxification of ROS, and thermogenesis. PGC-1α is repressed in models of HD and PGC-1α expression significantly protects striatal neurons from mHtt-induced toxicity [12]. PGC-1α acts as a transcriptional coactivator via interaction with a variety of transcription factors including PPARγ of the PPAR family. PPARγ is an important regulator in adipogenesis, fatty acid oxidation, and mitochondrial function. PPARγ hetero-dimerizes with retinoid X receptor (RXR) [13]. Upon ligand binding, PPARγ transactivates the target genes with the support of coactivators including PGC-1α. Thiazolidinediones (e.g., rosiglitazone (RSO), pioglitazone, troglitazone) are exogenous PPARγ agonists which have been clinically used to treat type 2 diabetes. PPARγ activation is beneficial in the R6/2 mouse model of HD [14] as well as other models of neurological diseases.
Our previous study showed that PPARγ activity was severely compromised in STHdhQ111 cells [6]. Further, thapsigargin (TG) induced a loss of mitochondrial membrane potential (ΔΨm) in STHdhQ111 but not STHdhQ7 cells (striatal cells expressing Htt) and RSG treatment attenuated TG-induced ΔΨm loss in STHdhQ111 cells [6].

Figure 1. Mutant huntingtin expression sensitizes striatal cells to stressors in the glucose condition. A, H2O2 treatment in the glucose condition results in significantly greater cell death in STHdhQ111 than STHdhQ7 cells, while both cell types show similar cell death responses to H2O2 in pyruvate condition. n = 4. B, RSG treatment does not protect striatal cells from H2O2 toxicity. n = 3–4. C, TG treatment in the glucose condition results in much greater cell death in STHdhQ111 than STHdhQ7 cells, while both cell types show similar cell death responses to TG in the pyruvate condition. n = 4–6. D, RSG significantly attenuates TG-induced cell death in the glucose and pyruvate conditions. n = 3–4. RP, rolipram. Data shown are mean ± SE. * P<0.05, ** P<0.01.
studies suggest that transcriptional dysregulation is tightly linked with mitochondria defects and that activating the impaired transcriptional pathways is likely to have beneficial effects in HD. Given that bioenergetic disturbance has emerged as a key component in the pathogenesis of HD, in the present study we hypothesized that different metabolic conditions (glucose vs. pyruvate) would differentially impact cell death induced by stressors such as H2O2 or TG in HD and wild type models. In addition, although we previously showed that TG-induced ΔΨm loss in STHdh Q111 cells was attenuated by PPARγ activation, whether PPARγ activation protects striatal cells from stress-induced cell death remained untested. Therefore, we investigated further whether the pathological changes induced by stresses can be rescued by PPARγ activation. We expected that STHdh Q111 cells would show greater cell death compared to STHdh Q7 cells, and that the pyruvate condition would exacerbate the differences in cell death between the two cell types compared to what was observed in the glucose condition because there are numerous studies showing mitochondrial impairment in HD models, including deficits in ATP production [20,21]. Unexpectedly but intriguingly, the glucose condition resulted in much greater differences in stress-induced cell death between the two cell types. These findings suggest that the bioenergetic status of the STHdh Q111 cells is not a major contributor to the enhanced sensitivity to cell death stressors, and that other variables likely play a more important role. Further, PPARγ activation protected STHdh Q111 cells against stresses and significantly reduced superoxide/ROS generation in STHdh Q111 cells and primary cortical neurons expressing mHtt. These results provide important insight into the pathogenesis of HD involving transcriptional dysregulation, oxidative stress, and metabolic impairment, and suggest that PPARγ may be a potential therapeutic target for HD.

**Results**

**Mutant Huntingtin Sensitizes Striatal Cells to Stressors in the Glucose Condition**

To investigate if metabolic conditions differentially affect the susceptibility of striatal cells expressing Htt or mHtt to different stressors, striatal cells were maintained in media containing glucose which supports both glycolysis and oxidative phosphorylation (Oxphos) or pyruvate which predominantly supports Oxphos [22,23]. Striatal cells were treated with different concentrations of H2O2 or TG for 12 h and cell death was assessed by measuring LDH release. Unexpectedly, in the glucose condition STHdh Q111 cells showed much greater cell death in response to H2O2 or TG compared to STHdh Q7 cells, while in the pyruvate condition both cell types showed a similar cell death response (Fig. 1, A and C). It should be noted that LDH release may be underestimated in the pyruvate condition because pyruvate acts as a competitive inhibitor in the LDH assay [24]. Hence, it is not appropriate to compare the extent of LDH release between the glucose and pyruvate conditions. To further confirm the results from the LDH assay, cell viability was also measured using the resazurin assay (Fig. 2) [23]. As with the LDH assay (Fig. 1), greater differences in response to stressors between the two cell types were observed in the glucose condition as compared to the pyruvate condition when viability was measured with the resazurin assay (Fig. 2). Together these results demonstrate that mHtt sensitizes striatal cells to stressors in the glucose condition, while striatal cells expressing either Htt or mHtt show similar susceptibility to stressors in the pyruvate condition.

Since cells in the glucose condition utilize glycolysis as well as Oxphos, cells were treated with Oxphos inhibitors in the glucose medium to further understand how the different metabolic conditions affect the susceptibility to cellular stress [Fig. S1]. STHdh Q111 cells in the presence of either oligomycin, a complex V inhibitor, or rotenone, a complex I inhibitor, showed greater cell death in response to H2O2 or TG compared to STHdh Q7 cells, suggesting that glycolysis may be a critical factor in rendering striatal cells expressing mHtt more sensitive to stressors. Since the concentration of glucose in media was high (25 mM), we also measured cell death in low (5 mM) glucose to determine if the greater difference in cell death between the two cell types in the glucose condition compared to the pyruvate condition was due to the effect of high glucose or the metabolic state. In 5 mM glucose, STHdh Q111 cells exhibited a similar cell death profile in response to H2O2 or TG as observed in 25 mM glucose (Fig. S2), indicating that it is the metabolic condition that contributes to the differential cell death. Next, we examined whether the PPARγ agonist, RSG, attenuated cell death in response to H2O2 or TG. Striatal cells were pretreated with RSG for 24 h prior to treatment with H2O2 or TG. RSG treatment significantly reduced cell death in response to TG but not H2O2 in both glucose and pyruvate conditions (Fig. 1, B and D). We also tested whether treatment with rolipram (RP), a phosphodiesterase IV (PDE4) inhibitor, attenuated TG-induced cell death, since intracellular cAMP levels are decreased in HD models and RP treatment has beneficial effects [26,27]. However, RP did not attenuate TG-induced cell death (Fig. 1D). In addition, an endogenous PPARγ agonist 15-d-PGJ2 also protected STHdh Q111 cells from TG-induced death (data not shown).

**Repressed PPARγ Activity Is Independent of the Protein Level**

We previously showed that PPARγ signaling is impaired in STHdh Q111 cells [6]. To confirm that disturbance of PPARγ signaling was due to the presence of mHtt we measured PPARγ activity in additional striatal cell lines. Two new striatal cell lines expressing mHtt (1A and 6L) exhibited a substantial reduction in the basal activity of PPARγ compared to two new striatal cell lines expressing Htt (B3 and E4) (Fig. 3A and D). This result suggests that disturbance of PPARγ signaling is impaired in HD models and RP treatment has beneficial effects [26,27]. Furthermore, CRE basal activity and PGC-1α promoter activity were also significantly compromised in 1A and 6L STHdh Q111 cells, which is in agreement with previous reports [Fig. S2, B and C] [6,12]. However, we found that PPARγ protein levels were variable in B3 and E4 while the original STHdh Q111 cells exhibited lower levels of PPARγ than the original STHdh Q7 cells (Fig. 3D). This result indicates that the repressed activity of PPARγ is independent of its expression level.

**Protective Effect of Rosiglitazone Is Due to the Specific Activation of PPARγ**

To determine if the protective effects of RSG on TG-induced cell death were specifically due to PPARγ activation, we co-administered GW9662, a PPARγ antagonist, with RSG for 24 h prior to TG treatment. GW9662 completely abolished the protective effects of RSG on TG-induced cell death, suggesting that the protective effect of RSG stems from PPARγ activation (Fig. 4D). To test whether the reduced activity of PPARγ in striatal cells is sufficient to sensitize striatal cells to stressors in the glucose condition, shRNA for PPARγ was stably expressed in STHdh Q7 cells (Fig. S3). The stable expression of shRNA- PPARγ significantly reduced the activity of PPARγ in STHdh Q7 cells (Fig. S3A). However, cell death induced by treatment with H2O2 or TG was not significantly increased by the stable expression of shRNA- PPARγ in STHdh Q7 cells (Fig. S3B). This result suggests
that reduced PPARγ activity is not the only causative factor in the sensitization of STHdhQ111 cells to stressors in the glucose condition.

We next examined how RSG treatment affected the expression of specific genes in STHdhQ7 and STHdhQ111 cells. Striatal cells were incubated with or without 24 h of RSG treatment in the glucose condition prior to collection. Quantitative RT-PCR was performed as described in materials and methods (Fig. 4B). STHdhQ111 cells show a significantly reduced expression of genes related to mitochondrial function [PPARγ, cytochrome C (CytC), uncoupling proteins (UCP4, UCP5)], calcium regulation [sarco(endoplasmic reticulum Ca2+-ATPase 2 (SERCA2)], and ROS response [superoxide dismutase 1 (SOD1)]. SOD2 appears to be decreased in STHdhQ111 cells. Retinoid X receptor α (RXRα), an obligatory signaling partner of PPARγ, appears to be increased in STHdhQ111 cells, suggesting that the reduced activity of PPARγ is not attributable to RXRα expression. RSG significantly upregulated CytC, UCP4, UCP5, SOD1, and SERCA2 in STHdhQ111 but not STHdhQ7 cells. PPARγ, RXRα, and SIRT1 showed no change after RSG treatment in both cell types. UCP2 was significantly increased and appeared to be further induced by RSG treatment in STHdhQ111 cells. PGC-1α trended higher in both cell types after RSG treatment but the increase was not statistically significant. PGC-1α gene regulation in STHdhQ111 cells was further investigated using PGC-1α promoter luciferase reporter assay (Fig. 4C). RSG induced a slight but significant increase of PGC-1α promoter activity and mutation of the PPAR-response element (PPRE), but not the cAMP response element (CRE) site, in the PGC-1α promoter completely abolished RSG-induced activation. These results suggest that PGC-1α expression is likely upregulated by RSG treatment in STHdhQ111 cells. Immunoblot of CytC showed a significantly reduced level in STHdhQ111 cells compared to STHdhQ7 cells. RSG increased CytC expression in STHdhQ111 but not STHdhQ7 cells, confirming the quantitative RT-PCR data (Fig. 4, B and D).

Metabolic Conditions Differentially Impact Mitochondrial Membrane Potential Loss (ΔΨm) in Response to Thapsigargin

ΔΨm is tightly linked with mitochondrial functions such as Ca2+ buffering, ATP synthesis, and cell death processes [28]. In order to understand how different metabolic conditions result in different patterns of TG-induced cell death, we monitored ΔΨm using JC-1 dye [29,30]. Treatment with FCCP, a mitochondrial uncoupler, markedly reduced the JC-1 ratio, validating the response of JC-1 dye to ΔΨm (data not shown). TG treatment increased the ΔΨm at early time points in both cell types in both metabolic conditions. ΔΨm in STHdhQ111 cells began to drop 2 h after TG treatment in the glucose condition, while STHdhQ7 cells maintained ΔΨm above the baseline until 6 h. TG treatment in the pyruvate condition resulted in similar kinetic responses in both cell types with the ΔΨm dropping below the baseline after 4 h (Fig. 5A). These results suggest that the distinct kinetic responses in TG-induced ΔΨm-loss in the different metabolic conditions may be a contributing factor to the different cell death profiles. We next tested whether RSG affects TG-induced ΔΨm loss. ΔΨm was measured 5 h after adding TG (Fig. 5B). Treatment of STHdhQ7, but not STHdhQ111 cells, with RSG dramatically increased the basal level of ΔΨm in both glucose and pyruvate conditions. RSG treatment slightly but significantly delayed TG-induced ΔΨm-loss of STHdhQ111 cells, an effect that was completely abrogated by GW9662 treatment, indicating the specific activation of PPARγ by RSG treatment.
PPARγ Activation Reduces Caspase Activation Induced by Thapsigargin

Next, since caspase activation plays a pivotal role in cell death processes, we tested whether caspase activation is involved in TG-induced cell death and if RSG attenuates caspase activation. TG treatment resulted in the formation of cleaved caspase 3, an active form of caspase 3, after 3 h in both cell types in the glucose condition (Fig. 6A). RSG dramatically reduced the level of cleaved caspase 3 in both cell types. Interestingly, the basal level of cleaved caspase 3 in STHdhQ111 cells is much higher compared to STHdhQ7 cells and substantially decreased by RSG treatment, while the total level of caspase 3 is similar between two cell types. The basal level of caspase 9 in STHdhQ111 cells was higher than in STHdhQ7 cells (Fig. 6E). The total levels of caspase 3 and 9 were not changed in response to TG or by RSG treatment. TG strongly induced cleaved PARP (poly ADP-ribose polymerase), another indicator of caspase 3 activation, and RSG reduced the level of cleaved PARP in both cell types. Although these results indicate that RSG ameliorates TG-induced cell death in part by attenuating caspase activation, these results do not explain why TG-induced cell death is significantly greater in STHdhQ111 cells.

Because immunoblot based assays do not represent the real activity of caspases, we next measured the activity of three different caspases in response to TG (Fig. 6B-D). TG significantly increased the activities of caspase 3 and 9 in STHdhQ111 cells, while STHdhQ7 cells only exhibited increased activity of caspase 3 and to a much lesser extent than STHdhQ111 cells. In addition, caspase 8 activity was measured since TG induces the activity of caspase 8 [31,32] and caspase 8 is implicated in HD [33,34,35]. Caspase 8 activity in STHdhQ111 cells, but not STHdhQ7 cells, was significantly increased by TG treatment. RSG significantly diminished TG-induced activation of these caspases in STHdhQ111 cells. These results suggest that the greater cell death in STHdhQ111 cells by TG treatment may be due to the greater activation of caspases which is reduced by RSG. Furthermore, STHdhQ111 cells display higher basal activities of caspase 3, 8, and 9, which is in line with the immunoblot results. The higher basal activities of caspases may also contribute to the greater sensitivity of STHdhQ111 cells to the stressors.

Stable Expression of Constitutively Active PPARγ Significantly Attenuates Cell Death Induced by H2O2 or Thapsigargin

It has been shown that RSG may affect signaling pathways independent of PPARγ pathway [36]. Therefore, we tested whether the protective effect of RSG mainly results from PPARγ activation. We established STHdhQ111 cells stably expressing constitutively active PPARγ, V16-PPARγ2 (Fig. 7A). Stable expression of VP16-PPARγ2 significantly increased PPARγ activity at the basal level (Fig. 7B). #41 cell line exhibited greater PPARγ activity than #12 cell line, although the expression levels of VP16-PPARγ2 were similar between two cell lines. Importantly, both #12 and #41 cell lines exhibited the greater activity of PPARγ compared to STHdhQ7 cells and showed significantly reduced cell death in response to H2O2 or TG in a PPARγ activity dependent manner (Fig. 7, C and D). These results
indicate that PPARγ activation plays a pivotal role in the protective effect of RSG.

Metabolic Condition Differentiates Superoxide/ROS Generation in Response to Thapsigargin

We previously showed that TG induced a greater generation of ROS in STHdh Q111 cells compared to STHdh Q7 cells [6]. Superoxide can be generated in mitochondria and cytosol and contribute to cell death/stress signaling pathways. Hence we measured superoxide levels in different metabolic conditions using dihydroethidium (DHE), a commonly used dye for superoxide detection, although it has been suggested that DHE may be oxidized by other ROS [37]. Interestingly, different metabolic conditions resulted in distinct patterns of superoxide/ROS production (Fig. 8A). STHdh Q111 cells show higher basal levels...
of superoxide/ROS in both glucose and pyruvate conditions. TG induced a substantial amount of superoxide/ROS in STHdhQ111 but not STHdhQ7 cells in the glucose condition, while in the pyruvate condition TG significantly increased superoxide/ROS in both cell types to a similar extent (Fig. 8, A and B). This result suggests that the distinct cell death patterns between the two different metabolic conditions in response to TG maybe in part related to superoxide/ROS generation.

PPARγ Activation Significantly Reduces Superoxide/ROS Generation

PPARγ plays an important role in regulating defense mechanisms against oxidative stress [38]. Therefore, we investigated whether PPARγ activation ameliorates superoxide/ROS production in HD cell models. We tested STHdhQ111 cells stably expressing VP16-PPARγ2. The stable expression of VP16-PPARγ2 led to pronounced reductions in TG-induced superoxide/ROS generation in STHdhQ111 cells in the glucose condition (Fig. 9A). The quantitative data shows that TG-induced superoxide/ROS production is significantly attenuated in two stable cell lines expressing VP16-PPARγ2 (Fig. 9B), suggesting that the protective effect of PPARγ activation on TG-induced cell death involves the regulation of oxidative responses including superoxide. We next investigated whether mHtt expression enhances superoxide/ROS production in primary neurons. Rat primary cortical neurons were transfected with vector, Htt (Htt568Q23), or mHtt (Htt568Q145) on DIV 7 and superoxide/ROS generation was measured 6 days later (Fig. 9C–F). The expression of Htt568Q23 and Htt568Q145 were verified by immunoblot of HEK cells after transfection (Fig. 9D). Primary neurons expressing Htt568Q145 exhibited a higher frequency of DHE positive cells than those transfected with vector or Htt568Q23 (Fig. 9, C and E). Next, we tested whether cotransfection of VP16-PPARγ2 with Htt568Q145 decreases the percentage of DHE positive neurons. The frequency of DHE positive neurons was significantly reduced by VP16-PPARγ2 expression compared to cotransfection of an empty vector with Htt568Q145 (Fig. 9F). For comparison, PGC-1α was cotransfected with Htt568Q145, which also resulted in a significant reduction in the percentage of DHE positive neurons (Fig. 9F). These results suggest that mHtt increases oxidative stress, presumably including superoxide, and that the activation of PPARγ/PGC-1α may be a promising target to protect neurons from increased oxidative stress.

Discussion

Numerous studies have suggested that bioenergetic impairment is an important contributing factor to HD pathogenesis [3,4,5]. These include the finding that the activity of the pyruvate dehydrogenase complex (PDH), which links glycolysis and TCA cycle/Oxphos, is significantly reduced in brain of HD patients [39,40]. These studies led us to hypothesize that different metabolic conditions may result in alterations in the susceptibility of neuronal cells expressing mHtt to different stressors. Our initial prediction was that in the pyruvate condition, where cellular bioenergetics is predominantly dependent on mitochondrial Oxphos, greater differences in cell death between STHdhQ111 and STHdhQ7 cells would be observed. Unexpectedly, it was in the glucose condition in which the greater differences between two cell types were observed, with the extent of cell death induced by H2O2 or TG being significantly greater in STHdhQ111 cells. Our initial hypothesis was that due to the mitochondrial deficits in the
STHdhQ111 cells, specifically deficiencies in ATP production [20,21], greater differences in stress-induced cell death would be observed in the pyruvate condition. However in retrospect, it is not surprising that the greater differences were observed in the glucose condition. First, deficits in PDH have been reported in HD cases [39,40], as well as in HD mouse models [41], which in combination with other defects in mitochondria bioenergetics, may make STHdhQ111 cells more reliant on glycolysis for ATP production, and thus more vulnerable to stress in the glucose condition. Second, we assumed that a subtle energy deficiency would be a pivotal contributor to cell death in response to H2O2 or TG, however it is not unreasonable that other variables are more important in determining cell death outcomes. Taken together, our study provides new insight for understanding the interrelationship between bioenergetic disturbances and the pathogenesis in HD.

Increased caspase activation has been associated with HD pathogenesis [33,34,35]. Our results demonstrate that the protective mechanisms of PPARγ activation against TG-induced cell death involve a slight delay of ΔΨm loss (Fig. 5) and inhibition of caspase activation (Fig. 6). The basal activities of three caspases (3, 8, and 9) were higher in STHdhQ111 cells compared to STHdhQ7 cells, rendering mHtt-expressing striatal cells more vulnerable to various stressors. A delay of ΔΨm-loss could be a contributing factor to the inhibitory effect of RSG on caspase activation. Since in the glucose condition STHdhQ111 cells exhibited much faster kinetics of TG-induced ΔΨm-loss than STHdhQ7 cells, we also examined major components of mPTP such as cyclophilin D (CypD) and voltage-
dependent anion channel (VDAC) (Fig. S4). In general, the higher expression of CypD supposedly increases the probability of mPTP opening [42,43,44]. However, the basal expression of CypD was lower at both the mRNA and protein level in STHdhQ111 cells than STHdhQ7 cells, while the expression of VDAC appeared to be equivalent in the two cell types. In addition, RSG treatment did not alter the expression of CypD or VDAC (Fig. S4C). These results suggest that the protective effect of RSG and the higher susceptibility of STHdhQ111 cells to TG may not be related to mPTP. Similarly, a recent study using R6/2 mice crossed with CypD knockout mice demonstrated that the deletion of CypD in the R6/2 mice resulted in enhanced mitochondrial Ca2+ buffering but did not show any improvement in the pathogenic symptoms or a delay in disease progression [45]. Further, RSG has been reported to protect cells against stresses by upregulation of PPARγ and Bcl-2, an anti-apoptotic protein that has been shown to be implicated with HD [14,46]. To test whether the protective effect of RSG could be due to an increase of Bcl-2 and/or PPARγ, we monitored the expression of Bcl-2 and PPARγ in both cell types in the presence or absence of RSG. STHdhQ111 cells show significantly lower levels of Bcl-2 and PPARγ in the basal condition (Fig. S4C). These results suggest that the protective effect of RSG is not due to the upregulation of Bcl-2 or PPARγ.

TG-induced cell death can be mediated by endoplasmic reticulum (ER) stress and unfolded protein response (UPR) [47,48]. UPR induces the expression of the molecular chaperone BiP/GRP78 and activates the ER-resident caspase-12 through processing by calpain and caspase. TG did not induce the expression of BiP/GRP78 or increase cleaved form of caspase 12 in two cell types. RSG did not alter BiP/GRP78 expression or caspase 12 activation in the presence or absence of TG (Fig. S5). This result suggests that the UPR is probably not a major contributor to TG-induced cell death in these experimental conditions, and the protective effect of RSG does not result from modulation of UPR. However, interestingly, the pro and active forms of caspase 12 are much higher in STHdhQ111 cells compared to STHdhQ7 cells. Taken together with the higher basal activity of caspase 3, 8, and 9, this result suggests that the higher levels of the activity and/or expression of caspases may render STHdhQ111 cells more susceptible to cellular or exogenous stresses.

mHtt has been proposed to impair mitochondrial function by direct interaction [49,50] as well as indirectly by transcriptional dysregulation including PGC-1α signaling [12]. PGC-1α and PPARγ play important roles in mitochondrial biogenesis and detoxification of ROS. We previously demonstrated that PPARγ signaling was severely compromised in STHdhQ111 cells [6].
the levels of SOD1 and SOD2 were reduced in STHdhQ111 cells in the glucose condition but not in the pyruvate condition, we investigated if superoxide/ROS generation in two cell types may differ in different metabolic conditions. STHdhQ211 cells produced more superoxide/ROS at the basal level and in response to TG in the glucose condition compared to STHdhQ7 cells, while in the pyruvate condition similar profiles of superoxide/ROS generation were observed in both cell types (Fig. 8). The profiles of TG-induced superoxide production in the two cell types in different metabolic conditions are similar to those of TG-induced cell death, suggesting that superoxide/ROS is likely to be a crucial mediator of TG-induced cell death. In addition, a large body of evidence provides evidence that PPARγ activation reduces superoxide/ROS generation in various models [38,58,59].

Oxidative stress has been proposed as one of key components in the pathogenesis of HD [8,9,10]. Recent studies using proteomic approaches identified proteins modified by oxidative stress in human HD samples and R6/2 mice and the activities of oxidized proteins were severely compromised [9,10]. Interestingly, many identified proteins are involved in glycolysis or mitochondrial metabolism, suggesting that oxidative stress could lead to metabolic disturbances and neuronal dysfunction [9,10]. We found that in the glucose condition STHdhQ211 cells showed reduced expression of genes involved in ROS response at least in part due to the repressed signaling pathways of PPARγ/PGC-1α and produced higher levels of superoxide/ROS in basal condition than STHdhQ7 cells. We postulate that mHtt interferes with transcriptional processes, leading to disruption in the expression of genes involved in bioenergetics and ROS response, in turn resulting in impaired metabolism and enhanced ROS. Increased oxidative stress may have an impact on transcriptional processes. For example, the promoter of SQSTM1/p62 exhibited oxidative damage in samples from HD samples, resulting in decreased expression of p62 [60].

In summary, we demonstrate for the first time that different metabolic states result in surprisingly differential cellular sensitivities to stressors in the context of HD. We also directly demonstrate that PPARγ activation significantly attenuates superoxide/ROS production and cell death in response to stressors in striatal precursor cells and primary cortical neurons expressing mHtt. This study provides new important insights into a cycling feed forward mechanism in HD involving transcriptional dysregulation, oxidative stress, and metabolic impairment and proposes PPARγ as a potential target for a therapeutic strategy in HD.

Materials and Methods

Ethics Statement

All animal protocols have been approved by the UCAR at the University of Rochester (UCAR#2007-023R).

Materials

Rosiglitazone (RSG), GW9662, rolipram (RP), thapsigargin (TG), and caspase substrates were purchased from Alexis. dihydroethidium (DHE) was purchased from Invitrogen. All other chemicals were purchased from Sigma, if not otherwise indicated.

Plasmid Constructs

PPRE×3-TK-Luc and human PGC-1α promoter-Luc were obtained from Addgene [61,62]. The human PPARγ and PGC-1α constructs were purchased from OriGene. mutPPRE- PGC-1α promoter-Luc and mutCRE- PGC-1α promoter-Luc were kindly provided from Dr. Francesc Villarroya [63]. pCRE-Luc was
VP16-PPARc2 construct was a gift from Dr. Mitchell Lazar. To make VP16-PPARc2-pHM6/PUR, VP16-PPARc2 was amplified by PCR with primers containing MfeI/NotI sites and subcloned into EcoRI/NotI sites of pHM6/PUR. pHM6/PUR was made by inserting the blunt ended puromycin resistance gene at the PsiI site of pHM6 (Roche). The full length wild type human Htt DNA with 23 polyQ, pRc/CMV-HDFLQ23, was a gift from Dr. Christopher Ross and the full length mutant human Htt DNA with 145 polyQ was obtained from CHDI. To make constructs of truncated huntingtin with 568 amino acids, Htt568Q23 and Htt568Q145, each fragment of the huntingtin cDNA of Htt568Q23 and Htt568Q145 was generated by PCR with primers including BamHI/EcoRI sites and the respective full length huntingtin DNA was used as a template. PCR products were digested with BamHI and EcoRI and then subcloned into FIGB vector derived from FG12 vector [64]. FIGB contains IRES-GFP so that transfected cells can be identified.

**Cell Culture and Different Metabolic Media**

The immortalized striatal precursor cell lines, STHdhQ111 (the original one, B3, E4) and STHdhQ111 (the original one, 1A, 6L), made from striatal primordia of E14 mouse embryos expressing Htt with 7 polyQ or mHtt with 111 polyQ were kindly provided by Dr. Marcy MacDonald [65]. Cells were cultured in DMEM containing 25 mM glucose and 4 mM glutamine (Invitrogen) supplemented with 4% fetal bovine serum (FBS, HyClone) and 4% bovine growth serum (BGS, HyClone), and 100 units/ml penicillin and 100 µg/ml streptomycin (Invitrogen) in the incubator at 33°C containing 5% CO2. For the two different metabolic conditions, 24 h after plating, the medium was purchased from Clontech. VP16-PPARγ2 construct was a gift from Dr. Mitchell Lazar. To make VP16-PPARγ2-pHM6/PUR, VP16-PPARγ2 was amplified by PCR with primers containing MfeI/NotI sites and subcloned into EcoRI/NotI sites of pHM6/PUR. pHM6/PUR was made by inserting the blunt ended puromycin resistance gene at the PsiI site of pHM6 (Roche). The full length wild type human Htt DNA with 23 polyQ, pRc/CMV-HDFLQ23, was a gift from Dr. Christopher Ross and the full length mutant human Htt DNA with 145 polyQ was obtained from CHDI. To make constructs of truncated huntingtin with 568 amino acids, Htt568Q23 and Htt568Q145, each fragment of the huntingtin cDNA of Htt568Q23 and Htt568Q145 was generated by PCR with primers including BamHI/EcoRI sites and the respective full length huntingtin DNA was used as a template. PCR products were digested with BamHI and EcoRI and then subcloned into FIGB vector derived from FG12 vector [64]. FIGB contains IRES-GFP so that transfected cells can be identified.

**Cell Culture and Different Metabolic Media**

The immortalized striatal precursor cell lines, STHdhQ111 (the original one, B3, E4) and STHdhQ111 (the original one, 1A, 6L), made from striatal primordia of E14 mouse embryos expressing Htt with 7 polyQ or mHtt with 111 polyQ were kindly provided by Dr. Marcy MacDonald [65]. Cells were cultured in DMEM containing 25 mM glucose and 4 mM glutamine (Invitrogen) supplemented with 4% fetal bovine serum (FBS, HyClone) and 4% bovine growth serum (BGS, HyClone), and 100 units/ml penicillin and 100 µg/ml streptomycin (Invitrogen) in the incubator at 33°C containing 5% CO2. For the two different metabolic conditions, 24 h after plating, the medium was purchased from Clontech. VP16-PPARγ2 construct was a gift from Dr. Mitchell Lazar. To make VP16-PPARγ2-pHM6/PUR, VP16-PPARγ2 was amplified by PCR with primers containing MfeI/NotI sites and subcloned into EcoRI/NotI sites of pHM6/PUR. pHM6/PUR was made by inserting the blunt ended puromycin resistance gene at the PsiI site of pHM6 (Roche). The full length wild type human Htt DNA with 23 polyQ, pRc/CMV-HDFLQ23, was a gift from Dr. Christopher Ross and the full length mutant human Htt DNA with 145 polyQ was obtained from CHDI. To make constructs of truncated huntingtin with 568 amino acids, Htt568Q23 and Htt568Q145, each fragment of the huntingtin cDNA of Htt568Q23 and Htt568Q145 was generated by PCR with primers including BamHI/EcoRI sites and the respective full length huntingtin DNA was used as a template. PCR products were digested with BamHI and EcoRI and then subcloned into FIGB vector derived from FG12 vector [64]. FIGB contains IRES-GFP so that transfected cells can be identified.

**Cell Culture and Different Metabolic Media**

The immortalized striatal precursor cell lines, STHdhQ111 (the original one, B3, E4) and STHdhQ111 (the original one, 1A, 6L), made from striatal primordia of E14 mouse embryos expressing Htt with 7 polyQ or mHtt with 111 polyQ were kindly provided by Dr. Marcy MacDonald [65]. Cells were cultured in DMEM containing 25 mM glucose and 4 mM glutamine (Invitrogen) supplemented with 4% fetal bovine serum (FBS, HyClone) and 4% bovine growth serum (BGS, HyClone), and 100 units/ml penicillin and 100 µg/ml streptomycin (Invitrogen) in the incubator at 33°C containing 5% CO2. For the two different metabolic conditions, 24 h after plating, the medium was purchased from Clontech. VP16-PPARγ2 construct was a gift from Dr. Mitchell Lazar. To make VP16-PPARγ2-pHM6/PUR, VP16-PPARγ2 was amplified by PCR with primers containing MfeI/NotI sites and subcloned into EcoRI/NotI sites of pHM6/PUR. pHM6/PUR was made by inserting the blunt ended puromycin resistance gene at the PsiI site of pHM6 (Roche). The full length wild type human Htt DNA with 23 polyQ, pRc/CMV-HDFLQ23, was a gift from Dr. Christopher Ross and the full length mutant human Htt DNA with 145 polyQ was obtained from CHDI. To make constructs of truncated huntingtin with 568 amino acids, Htt568Q23 and Htt568Q145, each fragment of the huntingtin cDNA of Htt568Q23 and Htt568Q145 was generated by PCR with primers including BamHI/EcoRI sites and the respective full length huntingtin DNA was used as a template. PCR products were digested with BamHI and EcoRI and then subcloned into FIGB vector derived from FG12 vector [64]. FIGB contains IRES-GFP so that transfected cells can be identified.
completely changed as previously described with modification [23]. Glucose medium consists of DMEM containing 25 mM glucose and 4 mM glutamine supplemented with 10 mM Hepes and 2% dialyzed FBS (HyClone). Pyruvate medium consists of DMEM containing 4 mM glutamine without glucose (Invitrogen) supplemented with 5 mM galactose, 10 mM Hepes, 4 mM sodium pyruvate, and 2% dialyzed FBS. The dialyzed FBS is necessary to rule out any exogenous metabolic contributions.

Generation of Stable Cell Lines

STHdhQ111 cells were transfected with VP16-PPARγ2-phM6/PUR using Lipofectamine 2000 (Invitrogen). STHdhQ111 cells stably expressing VP16-PPARγ2 were selected by treatment with 2.5 μg/ml puromycin. Approximately 3 weeks later, individual colonies were picked, amplified, and tested for the expression and the activity of VP16-PPARγ2. #12 and #41 clonal cells were selected and used for study.

Primary Cortical Neuronal Culture

Primary cortical neuronal culture from rat embryos was prepared as described previously with modification [60]. In brief, whole brains were removed from E17-18 rats. The cortices were then dissected, treated with 0.05% trypsin at 37°C for 30 min, and gently triturated with a fire polished glass Pasteur pipette. Dissociated cells were plated onto glass coverslips coated with 40 μg/ml poly-D-lysine (Millipore) with Minimum Essential Media (MEM, Invitrogen) containing 25 mM Hepes and GlutaMAX equivalent to 2 mM glutamine supplemented with 5% FBS in the incubator at 37°C containing 5% CO2. Five hours after plating, medium was replaced with Neurobasal medium (NBMI, Invitrogen) supplemented with 0.4 mM glutamine and B27 (Invitrogen). Every 3 days, half of the medium was removed, collected as conditioned NBM, and replenished with the complete NBMI.

Lactate Dehydrogenase (LDH) Release Assay

LDH release was measured using LDH release assay kit (Roche) as an assessment of cell death. Cells were plated in 48 well plates and were ~80-90% confluent after 24 h. Media were replaced with different metabolic media in the absence or presence of 20 μM RSG for 24 h prior to TG or H2O2 treatment. Twelve hours after TG or H2O2 treatment, LDH release was measured following the manufacturer’s instructions.

Resazurin Assay

The cell viability was determined by monitoring conversion of resazurin into a fluorescent product resorufin using CellTiter-Blue Cell Viability Assay kit (Promega). Twenty four hours after plating cells in 24 well plates, media were replaced with different metabolic media. Next day cells were treated with TG or H2O2. Resazurin solution was added to the media after 6 h according to manufacturer’s instructions. Two hour after incubation, resorufin fluorescence was measured using Synergy HT plate reader (BioTek) with excitation at 540 nm and emission at 590 nm. Results are presented as a percent of control cells.

RNA Isolation, Reverse Transcription, and Real-time PCR

Cells were plated followed by incubation in either glucose or pyruvate media. After 24 h, total RNA was extracted using TRIzol (Invitrogen) according to the manufacturer’s instruction. Extracted total RNA was treated with RNase free- DNaseI Amplification Grade (Invitrogen) to remove contaminating DNA. Two micrograms of total RNA was reverse transcribed using SuperScriptIII reverse transcriptase and random hexamers (Invitrogen). The reaction mixture was diluted with 600 μl of DEPC-treated H2O. The PCR reaction was prepared in triplicate containing 10 μl of diluted cDNA, 2.5 μl of 2.5 μM primer mixture (forward and reverse), and 12.5 μl of SYBR GreenER qPCR SuperMix (Invitrogen) in 96 well optical PCR reaction plate (Bio-Rad). PCR reactions were performed in MyiQ real-time PCR system (BioRad). Amplification conditions consisted of an initial hot start at 95°C for 10 min followed by amplification of 45 cycles (95°C for 15 s, 60°C for 20 s, and 72°C for 40 s). Melting curve analysis was performed immediately after amplification. The relative amount of mRNAs was calculated by using the ΔΔCt (Ct threshold cycle) method. The Ct value of TATA binding protein (TBP) was used for normalization. The sequences of primers are shown in Table 1.

Western Blot Analysis

Cells were washed with ice-cold phosphate-buffered saline (PBS) and lysed with modified RIPA buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.4% SDS, 0.2% sodium deoxycholate, 5% glycerol, 1 mM EDTA, 20 mM NaF, 2 mM Na3VO4) containing protease inhibitors (1 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml pepstatin). The lysates were sonicated, cleared by centrifugation, and assayed to determine protein concentration using BCA assay (Pierce Biotechnology). Proteins (10–100 μg) were separated by SDS-PAGE and transferred to nitrocellulose membrane. The membrane was blocked with 5% skim milk in Tris-buffered saline containing 0.05% Tween 20 (TBST), and incubated with the specific antibodies in TBST containing 2% BSA or skim milk at 4°C overnight. Antibodies for PPARγ, caspase 3, cleaved caspase 3, cleaved caspase 9, cleaved caspase 12, PARP, and VDAC were obtained from Cell Signaling Technology, antibodies for huntingtin actin (Bip/GRP78) from Chemicon, the antibody for cytochrome C from BD Biosciences Pharmingen, the antibody for Bcl-2 from Sigma. After washing three times, HRP-conjugated secondary antibody (1:3000) was applied and the blot was visualized by chemiluminescence. The intensity of immunoreactive bands was quantified by using Image J software.

Dual Luciferase Reporter Assays

Cells were plated in 24 well plate. The next day, a reporter plasmid (3×PPRE-Luc, PGC-1α promoter-Luc, mutPPRE-PGC-1α promoter-Luc, or CRE-Luc) and a normalizing plasmid pRL-TK (Promega) were transfected using Lipofectamine 2000. The next day, cells were treated with vehicle control or drug. After 16 h, cells were lysed with Passive lysis buffer (Promega), and the reporter activity was measured using the Dual-Luciferase Reporter Assay System (Promega). The reporter activity from Firefly luciferase was normalized with the Renilla luciferase activity.

Caspase Activity Assay

Cells were treated with 20 μM RSG for 24 h prior to TG treatment in the glucose condition and then 12 μM TG was added for 5 h. Cells were rinsed with cold PBS and harvested in 200 μl of NP40 lysis buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.5% NP40, 1 mM PMSF, 10 μg/ml of
protease inhibitors). Twenty microgram of each cell lysate was transferred into 96 well black wall plates, 200 μl of caspase assay solution (20 mM Hepes, pH 7.5, 10% glycerol, 2 mM DTT) containing 45 μM each caspase substrate [caspase-3 substrate (Ac-DEVD-AMC), caspase-8 substrate (Ac-IETD-AMC), or caspase-9 substrate (Ac-LEHD-AMC), or caspase-9 substrate (Ac-VEID-AMC)] was added. Plates were wrapped with aluminum foil and returned to the setup of green fluorescence (excitation at 485 nm and emission at 528 nm) and red fluorescence (excitation at 540 nm and emission, while lower DY m was measured using 5,5′,6,6′-tetrachloro-1,1′,3,3-tetra-ethyl-benzimidazolyl-carboxyline iodide (JC-1, Invitrogen) dye. Higher DY m leads JC-1 to form aggregates that exhibit a red emission, while lower DY m was transferred into 96 well black wall plates, 200 μl of caspase assay solution (20 mM Hepes, pH 7.5, 10% glycerol, 2 mM DTT) containing 45 μM each caspase substrate [caspase-3 substrate (Ac-DEVD-AMC), caspase-8 substrate (Ac-IETD-AMC), or caspase-9 substrate (Ac-LEHD-AMC), or caspase-9 substrate (Ac-VEID-AMC)] was added. Plates were wrapped with aluminum foil and returned to the setup of green fluorescence (excitation at 485 nm and emission at 528 nm) and red fluorescence (excitation at 540 nm and emission at 390 nm). The ratio of red fluorescence to green fluorescence was calculated as ΔΨm and then JC-1 ratio from each treatment was normalized by JC-1 ratio from the control of each cell type.

### Intracellular Superoxide/ROS Measurement

Superoxide/ROS generation was measured using DHE, a cell-permeable reduced form of ethidium bromide which is not cell-permeable. DHE itself exhibits a blue fluorescence in cytoplasm. Oxidation especially by superoxide transforms DHE into oxidized DHE products which exhibit a red fluorescence. The red fluorescence of oxidized DHE products becomes much brighter after DNA intercalation. Twenty four hours after plating striatal cells, the media was replaced with the different metabolic media. Imaging experiments for DHE were performed after 24 h. Glucose medium was changed with G-HBSS consisting of HBSS (20 Hepes, pH 7.4, 137 NaCl, 5 KCl, 0.5 KH2PO4, 0.5 Na2HPO4, 10 NaHCO3, 0.01 glycerol, in mM); 2 mM CaCl2, 0.6 mM MgCl2, and 10 mM glucose, and pyruvate medium was changed with Ox-HBSS consisting of HBSS, 2 mM CaCl2, 0.6 mM MgCl2, 5 mM galactose, 2 mM glutamine, and 1 mM pyruvate. 5 μM DHE was included in G-HBSS or Ox-HBSS. TG was added 10 min later. Plates were wrapped with aluminum foil and replaced in the incubator. 30 min later, oxidized DHE was imaged at 20× magnification on an Observer D1 microscope (Zeiss) coupled with a digital CCD camera (ORCA-ER, Hamamatsu Photonics). The red fluorescence from oxidized DHE was detected by using a 545/40 excitation filter and a 630/75 emission filter with the same exposure time among each experimental groups. DHE positive cells were counted using Image J software. Primary cortical neurons were transfected with 0.8 μg of plasmids as indicated using Lipofectamine 2000 on DIV 7. Five hours after transfection, the media was replaced with half conditioned NBM and half complete NBM. On DIV 13, neurons were rinsed with G-HBSS and maintained in G-HBSS containing 2.5 μM DHE. Plates were wrapped with aluminum foil and replaced in the incubator. One hour later, all GFP-positive neurons were examined for DHE staining and photographed with a 40× oil objective on the microscope system as described above. DHE positive neurons were counted when the red fluorescence occurred in the nucleus and its intensity was at least 50% higher than that in cytoplasm.

### Statistical Analysis

Data were expressed as mean ± SE (standard error) and analyzed using Student’s t test except where noted. Statistical significance was considered when a P value was <0.05.

### Supporting Information

**Figure S1** Glycolysis may contribute to the increased sensitivity of STHdhQ111 cells to stressors. Striatal cells were treated with either 10 μg/ml oligomycin or 10 μM rotenone to inhibit Oxphos in the glucose condition for 2 h prior to

| GENES | FORWARD PRIMER | REVERSE PRIMER | ACCESSION No. |
|-------|----------------|----------------|---------------|
| PGC-1α | GAAGTGGTGATGCACTCCAAC | AATAGGGGCAATCGCTCTTC | NM_008904 |
| PPARγ | GGAACGACCTCAGCGATTCT | TCCGAATTTGGAATCTGAGG | NM_011146 |
| SIRT1 | GCAGATGACAGATGGACAAGAT | ATGGAGCGCAATCGTCTTCT | NM_019812 |
| CyR | CCAACTTCCACGTGTCGTC | ATACCGGTTATCCTCTCCCC | NM_007808 |
| RXRα | TGATACGCAAGATGCAAGTGG | TGGATCATCGTCAGTGGCAT | NM_011305 |
| UCPCR | AAGCATAAGCTGACCTGACGA | ATAGGTCTGACTGCTGATG | NM_011671 |
| UCP4 | TGCAATGGCGAAGACGCGAG | AGCGTTGCTCCTGCTAGGG | AB106930 |
| UCP5 | ACCCCTGGGATGGTGAGAAC | TGATGTTTCAATCGCTACGAA | AF155812 |
| SOD1 | GGTTGCGCAATGGTCTGACG | TACTGGGCAATCTCACTCCA | NM_011434 |
| SOD2 | TTAAGGAGAATGGCAGCGGCTG | TGTGTCTCCGTGAAAGGTCTG | NM_013671 |
| SERCA2 | TGGCAAAATGGATGCAACCAGCA | GCAGGAACTTGTCAGCAACAGGA | AJ223584 |
| CypD | AATGGAAGACGCGGAGAGA | CACTGGTTCCTCCAGGATGA | NM_026352 |
| TBP | AGGTTTCTAGCGTCTGCTTGTG | NM_013684 |

PGC-1α, peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1α; SIRT1, sirtuin 1; CyR, cytochrome C; RXRα, retinoid X receptor α; UCPCR, uncoupling protein; SOD, superoxide dismutase; SERCA2, sarco(endo)plasmic reticulum Ca2+ ATPase 2; CypD, cyclophilin D; TBP, TATA binding protein.

doi:10.1371/journal.pone.0030406.t001
treatment with stressors. Cell viability was measured 8 h after treatment with H2O2 (A) or TG (B) using the resazurin assay. Oligomycin or rotenone treatment in the glucose condition led to significantly reduced viability of STHdhQ111 cells in response to H2O2 or TG compared to STHdhQ7 cells. n = 5. Data shown are mean ± SE. * P<0.05, ** P<0.01.

(TIF)

Figure S2 STHdhQ111 cells in the low glucose (5 mM) condition still exhibit much greater cell death in response to stressors. STHdhQ7 and STHdhQ111 cells were maintained in the low glucose condition for 24 h prior to treatment with stressors for 12 h. A, H2O2 treatment resulted in much greater cell death in STHdhQ111 than STHdhQ7 cells. B, TG treatment also resulted in significant cell death in STHdhQ111 cells while STHdhQ7 cells were resistant to given treatment, n = 2. Data shown are mean ± SD (standard deviation).

(TIF)

Figure S3 Knockdown of PPARγ in STHdhQ7 cells does not increase sensitivity to stressors. A, STHdhQ7 cells stably expressing shRNA for PPARγ were generated and show significantly reduced PPRE activity compared to naïve STHdhQ7 cells. n = 3–4. B, Reduced PPARγ activity in STHdhQ7 cells does not aggravate cell death in response to H2O2 or TG in the glucose condition. n = 3–6. Data shown are mean ± SE.

(TIF)

Figure S4 STHdhQ111 cells show a significant reduction in the expression of Bcl-2 and cyclophilin D compared to STHdhQ7 cells. Cells were maintained for 24 h in the glucose condition and harvested for either western blot or real-time PCR analysis. A, Immunoblot results show significantly reduced protein expression of Bcl-2 and cyclophilin D (CypD) in STHdhQ111 cells compared to STHdhQ7 cells. n = 4. B, The mRNA level of CypD is significantly reduced in STHdhQ111 cells. n = 4. C, STHdhQ7 and STHdhQ111 cells were incubated in the presence or absence of 20 μM RSG for 24 h. RSG treatment does not change the protein expression of PPARγ, Bcl-2, CypD, or VADC. Data shown are mean ± SE. * P<0.05, *** P<0.001 vs. STHdhQ7.

(TIF)

Figure S5 TG-induced cell death may not involve ER stress or UPR response in the given condition. STHdhQ7 and STHdhQ111 cells were treated with and without 20 μM RSG for 24 h in the glucose condition, and then 12 μM TG was treated for 3 h or 5 h. Cells were harvested and prepared for western blot analysis. TG treatment does not induce activation of caspase 12 or increase expression of BiP/GRP78 in both cell types in the given period of time. Interestingly, STHdhQ111 cells exhibit higher level of pro- and active caspase 12 compared to STHdhQ7 cells. RSG treatment does not have impact on either caspase 12 activation or BiP/GRP78 induction.

(TIF)

Figure S6 The pyruvate condition results in a slight change in gene expression profile as compared to the glucose condition. STHdhQ7 and STHdhQ111 cells were maintained in the pyruvate condition for 24 h. Real-time PCR was performed as described in materials and methods. n = 4. The relative mRNA levels of STHdhQ111 cells to the corresponding each gene of STHdhQ7 cells were plotted. mRNA levels of PPARγ and UCP4 are decreased and mRNA level of UCP2 is highly increased in STHdhQ111 cells as shown in the glucose condition. Similarly, mRNA levels of PGC-1α and SIRT1 are not different between two cell types as shown in the glucose condition. However, there is no difference in mRNA levels of Cytc, SOD1, SOD2, and SERCA2 between two cell types, which were shown to be decreased in STHdhQ111 cells. Data shown are mean ± SE.

(TIF)

Acknowledgments

We thank Dr. Marc E. MacDonald (Harvard University) for the striatal precursor cell lines, Dr. Bruce M. Spiegelman (Harvard University) for PPRE-B-Lac/Zac and PGC-1α promoter constructs, Dr. Francesc Villarroya (University of Barcelona) for PGC-1α promoter constructs, Dr. Mitchell Lazar (University of Pennsylvania) for a VP16- PPARγ2 construct, Dr. Christopher Proschel (University of Rochester) for the Fig B vector and Dr. Christopher A. Ross (Johns Hopkins University) for the pRe/CMV-HDFLQ23 construct.

Author Contributions

Conceived and designed the experiments: YNJ GVW J. Performed the experiments: YNJ WYH CJ. Analyzed the data: YNJ GVWJ. Wrote the paper: YNJ GVWJ.

References

1. Ferrante RJ, Kowall NW, Richardson EP, Jr. (1991) Proliferative and degenerative changes in striatal spiny neurons in Huntington’s disease: a combined study using the Golgi method and calbindin D28k immunocytochemistry. J Neurosci 11: 3177–3187.
2. The Huntington’s Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington’s disease chromosomes. Cell 72: 971–983.
3. Heneka MT, Sastre M, Dumitrescu-Ozimek L, Hanke A, Dewachter I, et al. (2006) Acute treatment with the PPARγ agonist pioglitazone and lipopolysaccharide increases pro-inflammatory cytokines in Huntington’s disease via activation of the peroxisome proliferator-activated receptor gamma (PPARγ) and selective inhibition of central inflammatory cytokines in mice. Brain 129: 1442–1453.
4. Heneka MT, Castres M, Duret-Ozimek L, Hanke A, Dewachter I, et al. (2005) Acute treatment with the PPARγ agonist pioglitazone and lipopolysaccharide reduces glial inflammation and astroglial activation in the same region of Huntington’s disease via activation of the peroxisome proliferator-activated receptor gamma. J Biol Chem 280: 25620–25627.
5. Milakovitch T, Quintanilla RA, Johnson GV (2006) Mutant huntingtin expression prevents mitochondrial dysfunction in mutant huntingtin-expressing cells: possible role of PPARγ-activator-induced receptor-gamma (PPARγ/PPARγ) in the pathogenesis of Huntington disease. J Biol Chem 281: 25620–25627.
6.russo MA, Rodriguez-Colman MJ, Tamat J, Tamat J, Ortega Z, et al. (2010) Protein oxidation in Huntington disease affects energy production and vitamin B6 metabolism. Free Radical Biol Med 49: 612–621.
7. Perhiagi M, Poon HF, Maragz W, Pierce WM, Klein JB, et al. (2005) Proteomic analysis of protein expression and oxidative modification in r6/2 transgenic mice: a model of Huntington disease. Mol Cell Proteomics 4: 1849–1861.
8. Ross CA, Thompson LM (2006) Transcription meets metabolism in neurodegeneration. Nat Rev Mol Cell Biol 7: 1235–1241.
9. Cui L, Jeong H, Borovecki F, Parkhurst CN, Tanese N, et al. (2006) Transcriptional repression of PGC-1α by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. Cell 127: 39–69.
10. Glass CK, Ogawa S (2006) Combinatorial roles of nuclear receptors in inflammation and immunity. Nat Rev Immunol 6: 44–55.
11. Chiang MC, Chen CM, Lee MR, Chen HW, Chen HM, et al. (2010) Modulation of energy deficiency in Huntington’s disease via activation of the peroxisome proliferator-activated receptor gamma. Hum Mol Genet 19: 4043–4050.
12. Benen MT, Sastre M, Duret-Ozimek L, Hanke A, Dewachter I, et al. (2005) Acute treatment with the PPARγ agonist pioglitazone and lipopolysaccharide reduces glial inflammation and astroglial activation in the same region of Huntington’s disease via activation of the peroxisome proliferator-activated receptor gamma. J Biol Chem 280: 25620–25627.
13. Heinke MT, Castres M, Duret-Ozimek L, Hanke A, Dewachter I, et al. (2005) Acute treatment with the PPARγ agonist pioglitazone and lipopolysaccharide reduces glial inflammation and astroglial activation in the same region of Huntington’s disease via activation of the peroxisome proliferator-activated receptor gamma. J Biol Chem 281: 34783–34795.
14. Breiden T, Callebert J, Heinke MT, Landrath G, Laumann JM, et al. (2002) Protective action of the peroxisome proliferator-activated receptor-gamma agonist pioglitazone in a mouse model of Parkinson’s disease. J Neurochem 82: 615–624.
21. Kaisi M, Kipiani K, Chen J, Calingasan NY, Real MF (2005) Peroxisome proliferator-activated receptor-gamma agonist extends survival in transgenic mouse model of amyotrophic lateral sclerosis. Exp Neurol 191: 331–336.

22. Liao Y, Yin W, Signore AP, Zhang F, Hong Z, et al. (2006) Neuroprotection against focal ischemic brain injury by the peroxisome proliferator-activated receptor-gamma agonist rosiglitazone. J Neurochem 97: 435–448.

23. Nino M, Iwabuchi K, Kikuchi S, Ato M, Morohashi T, et al. (2001) Amelioration of experimental autoimmune encephalomyelitis in C57BL/6 mice by an agonist of peroxisome proliferator-activated receptor-gamma. J Neuroimmunol 116: 40–48.

24. Milakovic T, Johnson GV (2005) Mitochondrial respiration and ATP production are significantly impaired in striatal cells expressing mutant huntingtin. J Biol Chem 280: 30773–30782.

25. Seong IS, Ivanova E, Lee JM, Choo YS, Fossale E, et al. (2005) HD CAG repeat impairs a domain of huntingtin in mitochondrial energy metabolism. Human Molecular Genetics 14: 2071–2080.

26. Oliviaer JM, Chen S, Almeida S, Riley R, Goncalves J, et al. (2006) Mitochondrial-dependent Ca2+-handling in Huntington’s disease striatal cells: effect of histone deacetylase inhibitors. J Neurosci 26: 11174–11186.

27. Rossignol R, Glikerson R, Agegger R, Yamagata K, Remington SJ, et al. (2004) Energy homeostasis: modulation of mitochondrial structure and oxidative capacity in cancer cells. Cancer Res 64: 983–993.

28. Murthy VV (1995) Use of pyruvate oxidase to overcome pyruvate inhibition during the lactate to pyruvate reaction for assaying lactate dehydrogenase in human red blood cells. Clin Chem Lab Med 33: 225–229.

29. Gundemir S, Johnson GV (2010) Intracellular localization and conformational state of transglutaminase 2: implications for cell death. PLoS ONE 4: e6123.

30. DeMarch Z, Giampa C, Patassini S, Bernardi G, Fusco FR (2008) Beneficial effects of roslipam in the R6/2 mouse model of Huntington’s disease. Neurobiol Dis 30: 373–387.

31. Gines S, Seong IS, Fossale E, Ivanova E, Trettel F, et al. (2003) Specific progressive CAMP reduction impairs energy deficit in presymptomatic Huntington’s disease knock-in mice. Hum Mol Genet 12: 497–508.

32. Shoshan-Barmatz V, De Pinto V, Zocchi-Ambrosini M, Rasiv Z, Kneissn L, et al. (2010) VDAC, a multi-functional mitochondrial protein regulating cell life and death. Mol Aspects Med 31: 227–285.

33. Zheng Z, Chen H, Wang H, Ke B, Zheng B, et al. (2010) Improvement of retinal vascular injury in diabetic rats by statins is associated with the inhibition of mitochondrial reactive oxygen species pathway mediated by peroxisome proliferator-activated receptor-gamma coactivator 1alpha. Diabetes 59: 2315–2325.

34. Troiano L, Ferrari R, Laugi E, Nemes E, Roat E, et al. (2007) Multispectral analysis of cells with different mitochondrial membrane potential during apoptosis by polychromatic flow cytometry. Nat Protoc 2: 2719–2727.

35. Ji DG, Jun JH, Chang JW, Hong YM, Song S, et al. (2004) Calcium binding of ARC mediates regulation of caspase 8 and cell death. Mol Cell Biol 24: 9763–9770.

36. Yamaguchi H, Bhalla K, Wang HG (2003) Bax plays a pivotal role in recruitment and activation of caspase-8 by the Huntingtin-interacting protein1 in thapsigargin-induced apoptosis of human colon cancer HCT116 cells. Am J Physiol Renal Physiol 298: 1105–1112.

37. Zielonka J, Kalyanaraman B (2010) Hydroethidine- and MitoSOX-derived red fluorescence through uncoupling proteins. Antioxid Redox Signal 11: 1805–1816.

38. King AL, Swain TM, Dickinson DA, Lesort MJ, Bailey SM (2010) Chronic ethanol consumption enhances sensitivity to Ca2+-mediated opening of the mitochondrial permeability transition pore and increases cyclophilin D in liver. Am J Physiol Gastrointest Liver Physiol 299: G54–G66.

39. Nasrallah R, Clark J, Corinaldi J, Paris G, Miura P, et al. (2010) Recruitment and activation of caspase-8 by the Huntingtin-interacting protein1 in thapsigargin-induced apoptosis of human colon cancer HCT116 cells. Am J Physiol Gastrointest Liver Physiol 299: G54–G66.

40. Choo YS, Johnson GV, MacDonald M, Detloff PJ, Lesort M (2004) Mutant huntingtin directly increases permeabilization of mitochondria to the calcium-induced permeability transition and cytochrome c release. Hum Mol Genet 13: 1407–1420.

41. Panov AV, Gutekunst CA, Leavitt BR, Hayden MR, Burke JR, et al. (2002) Early mouse hippocampal and cortical cell death in Huntington’s disease are a direct effect of polynucleotides. Nat Neurosci 5: 731–736.

42. Zhang X, Li X, Prabhakaran K, Zhang I, Leavesley HB, et al. (2007) Uncoating protein-2-mediated opening of the mitochondrial permeability transition pore. J Biol Chem 282: 9105–9117.

43. King AL, Swain TM, Dickinson DA, Lesort MJ, Bailey SM (2010) Chronic ethanol consumption enhances sensitivity to Ca2+-mediated opening of the mitochondrial permeability transition pore and increases cyclophilin D in liver. Am J Physiol Gastrointest Liver Physiol 299: G54–G66.

44. Rasola A, Sciacovelli M, Pantic B, Bernardi P (2010) Signal transduction to the permeability transition pore. FEBS Lett 580: 1989–1996.

45. Perry GM, Tallaksen-Greene S, Kumar A, Heng MY, Kneynsberg A, et al. (2010) Mitochondrial calcium uptake capacity as a therapeutic target in the R6/2 mouse model of Huntington’s disease. Hum Mol Genet 19: 3354–3371.

46. Fuenzalida K, Quintanilla R, Ramos P, Piderit D, Fuentralba RA, et al. (2007) Peroxisome proliferator-activated receptor gamma up-regulates the Bcl-2 anti-apoptotic protein in neurons and induces mitochondrial stabilization and protection against oxidative stress and apoptosis. J Biol Chem 282: 37006–37015.

47. Martinez-JA, Zhang Z, Swelw SI, Hayes RL, Wang KK, et al. (2010) Calpain and caspase processing of caspase-12 contribute to the ER stress-induced cell death pathway in differentiated PC12 cells. Apoptosis 15: 1480–1493.

48. Rusu H, Umsani T, Shimoke K, Nakayama H, Matsumura Y, et al. (2008) Nicotinamide suppresses tumour necrosis-inhibited, but not dexamethasoin-inhibited, expression of cGMP during ER-stress-mediated apoptosis in PC12 cells. J Biochem 144: 251–257.

49. Choo YS, Johnson GV, MacDonald M, Detloff PJ, Lesort M (2004) Mutant huntingtin directly increases permeabilization of mitochondria to the calcium-induced permeability transition and cytochrome c release. Hum Mol Genet 13: 1407–1420.

50. Panov AV, Gutekunst CA, Leavitt BR, Hayden MR, Burke JR, et al. (2002) Early mouse hippocampal and cortical cell death in Huntington’s disease are a direct effect of polynucleotides. Nat Neurosci 5: 731–736.

51. Zhang X, Li X, Prabhakaran K, Zhang I, Leavesley HB, et al. (2007) Uncoating protein-2-mediated opening of the mitochondrial permeability transition pore. J Biol Chem 282: 9105–9117.

52. Nashraa R, Clark J, Cornilard J, Paris G, Miura P, et al. (2010) Thiazolidinediones alter growth and epithelial cell integrity, independent of PPAR[gamma] and MAPK activation, in mouse M1 Cortical Collecting Duct Cells. Am J Physiol Renal Physiol 298: 1105–1112.

53. Zielonka J, Kalyanaraman B (2010) Hydroethidine- and MitoSOX-derived red fluorescence is not a reliable indicator of intracellular superoxide formation: another inconvenient truth. Free Radic Biol Med 48: 1003–1001.

54. Doman F, Wallace DM, O’Driscoll C, Cotter TG (2009) Rosiglitazone acts as a neuroprotectant in retinal cells via up-regulation of sestrin-1 and SOD-2. J Neurobiol 109: 631–643.

55. Ashworth C, Singh J, Corral-L Portions of this work were supported by grants from the National Institutes of Health (R01CA136639). The authors performed the work at the Department of Neurology, Washington University School of Medicine, St. Louis, MO, USA (CA) and the Department of Neurology, University of California, San Francisco, San Francisco, CA, USA (R01CA136639).

56. Handschin C, Rhee J, Lin J, Perricelli MA, Spiegelman BM (2006) Metabolic State and Cell Sensitivity to ROS in HD