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Colon tumour cells increase PGE_2 by regulating COX-2 and 15-PGDH to promote survival during the microenvironmental stress of glucose deprivation

Heather R.Roberts, Helena J.M.Smartt, Alexander Greenhough, Amy E.Moore, Ann C.Williams and Christos Paraskeva*

School of Cellular and Molecular Medicine, Cancer Research UK Colorectal Tumour Biology Research Group, University of Bristol, Bristol, BS8 1TD, UK

*To whom correspondence should be addressed. Tel: +01173312072; Fax: +01779287896;
Email: c.paraskeva@bristol.ac.uk

Due to poor tumour-associated vasculature, tumour cells are subjected to a fluctuating microenvironment with periods of limited oxygen and glucose availability. Adaptive mechanisms to adverse microenvironments are important for tumour cell survival. The cyclooxygenase (COX)-2/prostaglandin E_2 (PGE_2) pathway has key roles in colorectal tumorigenesis. Although glucose is important as an energy source and in maintaining endoplasmic reticulum homeostasis, relatively little is known regarding how tumour cells adapt to the microenvironmental stress of reduced glucose availability. Here, we report the novel findings that glucose deprivation of colorectal tumour cells not only increases COX-2 expression but also decreases 15-hydroxyprostaglandin dehydrogenase (15-PGDH) expression, resulting in increased extracellular PGE_2. Furthermore, we have shown that PGE_2 promotes tumour cell survival during glucose deprivation. Glucose deprivation enhances phosphoinositide 3-kinase/Akt activity, which has a role in both the up-regulation of COX-2 and down-regulation of 15-PGDH. Glucose deprivation also activates the unfolded protein response (UPR) resulting in elevated C/EBP-homologous protein (CHOP) expression. Interestingly, inhibiting CHOP expression by small interfering RNA during glucose deprivation attenuates the reduction in 15-PGDH expression. This is the first report linking activation of the UPR with a reduction in expression of tumour-suppressive 15-PGDH and may have implications for tumour cells' ability to survive exposure to therapeutic agents that activate the UPR. Our data suggest that diverse microenvironmental stresses converge to regulate PGE_2 as a common and crucial mediator of cell survival during adaptation to the tumour microenvironment and may lead to novel chemopreventive and therapeutic strategies.

Introduction

Colorectal cancer is one of the most common causes of cancer-related deaths in industrialized countries. There has been considerable progress in recent years in identifying genetic and epigenetic events that drive colorectal tumorigenesis. However, there is increasing awareness that the tumour microenvironment also has significant roles in tumour development and progression and may thus be an important target for chemoprevention and therapy (1,2). Due to inadequacies of the tumour-associated vasculature, regions of low glucose and oxygen availability can develop within a growing tumour. Adaptation to adverse fluctuating microenvironments will enable increased tumour cell survival. Furthermore, exposure to a restricted microenvironment can lead to clonal selection of cancer cells and progression to a more malignant phenotype. For instance, hypoxia can select for tumour cells with lower apoptotic potential such as those with loss of p53 function (3), and depriving colon cancer cell lines of glucose has been shown to drive the selection of cells with activating mutations in the *KRAS* oncogene (4). There has been considerable progress in understanding adaptive responses to hypoxia (5). However, although recognized to be key, not only as an energy and biosynthetic source for tumour cells but also in maintaining endoplasmic reticulum (ER) homeostasis via its role in asparagine (N)-linked glycosylation of proteins, much less is known regarding the means by which tumour cells can adapt to the microenvironmental stress of reduced glucose availability.

The cyclooxygenase (COX)-2/prostaglandin E_2 (PGE_2) signalling pathway is important in colorectal tumorigenesis. COX-2 catalyses the conversion of arachidonic acid to a number of prostaglandins including PGE_2, which is thought to be the main prostaglandin responsible for the pro-tumorigenic effects of elevated COX-2 expression in tumours (6,7). Colorectal tumours express increasing amounts of COX-2 with progression from adenoma to carcinoma (8,9). A wealth of genetic and pharmacological data has demonstrated that COX-2 activity and elevated PGE_2 levels can promote colorectal tumorigenesis (10–13). Although inhibiting COX-2 is considered an attractive means to inhibit tumour development, this approach is limited due to the side effects associated with systemic inhibition of COX-2 activity.

Most efforts to reduce PGE_2 to date have focused on inhibiting cyclooxygenase activity. However, recent evidence has indicated an important tumour suppressor role for 15-hydroxyprostaglandin dehydrogenase (15-PGDH) in a number of cancers including colon (14), breast (15) and lung (16,17). 15-PGDH is the key enzyme in the degradation of PGE_2, catalysing the conversion of PGE_2 into a significantly less biologically active 15-keto form, and reduced 15-PGDH expression will elevate intra-tumoral PGE_2. Given the importance of PGE_2 in colorectal tumorigenesis, a greater understanding of how both COX-2 and 15-PGDH are regulated may be critical in order to develop more effective target-based approaches to replace or complement current chemopreventive and/or therapeutic strategies.

Interestingly, we have previously reported that the microenvironmental stress of hypoxia increases extracellular PGE_2 through a hypoxia inducible factor-1-dependent up-regulation of COX-2, and this increase in PGE_2 is pivotal in promoting cell survival under hypoxic conditions (18). However, whether glucose deprivation increases the levels of PGE_2 through regulating the expression of key genes that control PGE_2 levels has not previously been addressed.

We report here for the first time that depriving colon tumour cells of glucose both elevates COX-2 expression and represses 15-PGDH expression, leading to an increase in extracellular PGE_2 which can increase tumour cell viability during glucose deprivation. We further show that enhanced activation of the phosphoinositide 3-kinase (PI3K)/Akt signalling pathway by glucose deprivation leads to both increased COX-2 and decreased 15-PGDH expression. We also show that glucose deprivation leads to activation of the unfolded protein response (UPR) and report the novel finding that, through increased levels of C/EBP-homologous protein (CHOP), the UPR can lead to the suppression of 15-PGDH expression. Combined with our previous findings in hypoxic conditions (18), this study suggests that elevating PGE_2 may be a common mechanism by which colorectal tumour cells adapt to diverse tumour microenvironments with implications for developing novel chemopreventive and therapeutic strategies against colorectal cancer.

Materials and methods

Cell culture

The colon carcinoma cell lines HT29 and SW480 (American Type Culture Collection, Rockville, MD), the RG/C2 adenoma cell line and the AA/C1/SB/10C cell line (both derived in this laboratory) were maintained in standard high glucose (25 mM) Dulbecco's modified Eagle's medium (Autogen Bioclear, Wiltshire, UK), as described previously (19). The RG/C2 and AA/C1/SB/10C cell lines have been described in detail previously (20,21). Briefly, AA/

Abbreviations: CHOP, C/EBP-homologous protein; COX, cyclooxygenase; ER, endoplasmic reticulum; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; PGE_2, prostaglandin E_2; siRNA, small interfering RNA; UPR, unfolded protein response.

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C1/SB/10C is a tumorigenic *in vitro* transformed variant of a non-tumorigenic adenoma cell line (AA/C1). RG/C2 is a non-tumorigenic anchorage-dependent adenoma-derived cell line. For glucose deprivation, cells were incubated with a low energy Dulbecco's modified Eagle's medium manufactured without glucose or pyruvate (Autogen Bioclear), which throughout this paper is referred to as glucose deprivation. The time in hours in the figures refers to when media was changed to either glucose-deprived medium or standard high glucose Dulbecco's modified Eagle's medium. Hypoxia treatment was performed as described previously (22). Cells were either grown in standard growth condition in normoxia (21% O_2) or hypoxia (1% O_2) where indicated. NS-398, PGE_2 and tunicamycin were from Sigma-Aldrich (Poole, UK), LY294002 and thapsigargin from Calbiochem (La Jolla, CA).

Western blotting

Western blotting was performed as described previously (19) using COX-2, COX-1 (Santa Cruz Biotechnology, Santa Cruz, CA), 15-PGDH (Abcam, Cambridge, UK), t-Akt, t-ERK1/2, p-Akt (Ser473), p-ERK1/2 (Thr202/Tyr204), CHOP (Cell Signaling Technology, Danvers, MA), hypoxia inducible factor-1α (BD Biosciences, Oxford, UK), β-actin and α-tubulin (Sigma-Aldrich) antibodies. Anti-15-PGDH and anti-CHOP antibodies were validated by small interfering RNA (siRNA).

PGE_2 quantification

PGE_2 levels were determined using a competitive enzyme immunoassay (Cayman Chemical (Ann Arbor, MI), as described previously (18,19).

3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue assay

Relative cell viability was determined using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, as described previously (19).

siRNA transfections

siRNAs were obtained from Ambion (Cambridgeshire, UK). Transfections were carried out according to the reverse transfection method recommended by Ambion, using Lipofectamine-2000 (Invitrogen, Carlsbad, CA).

RNA extraction and quantitative real-time polymerase chain reaction analysis

Total RNA was extracted from cells using an RNeasy kit (Qiagen, Crawley, UK). RNA samples were treated with DNase I (Ambion) and complementary DNA prepared using M-MLV reverse transcriptase (Promega, Madison, WI). Comparative quantitative real-time polymerase chain reactions were performed as described previously (19). Transcript levels were normalized to the housekeeping gene TATA box-binding protein.

Statistical analysis

Statistical analyses were performed using either analysis of variance followed by Tukey's post hoc test or Student's *t*-test and expressed as: $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

Results

Glucose deprivation of colon tumour cell lines increases COX-2 and decreases 15-PGDH expression

Glucose is an important energy source and biosynthetic precursor for tumour cells. Yet, tumour cells are subjected to fluctuations in glucose availability *in vivo*, and to survive, tumour cells must be able to adapt to restricted glucose availability. To investigate the potential role of PGE_2 in the adaptation of tumour cells to glucose deprivation, we examined whether glucose deprivation increased the levels of PGE_2 through the regulation of two key enzymes central to the control of PGE_2 levels: COX-2 and 15-PGDH. The colon carcinoma cell line HT29 was cultured in glucose-deprived media. As expected, glucose deprivation caused a significant time-dependent growth inhibition and an increase in cell death in all colon tumour cell lines examined compared with cells grown in standard conditions with supplemented glucose. For example, after 24 h in glucose-deprived medium cell death had increased in HT29 by 1.94-fold, in HCA7 cells by 1.95-fold and in SW480 by 1.6-fold compared with standard medium. By 48 h in glucose-deprived medium, cell death had increased in HT29 by 3.14-fold, in HCA7 by 2.44-fold and in SW480 by 2.38-fold compared with standard growth medium.

Interestingly, glucose deprivation of HT29 cells caused an increase in COX-2 protein and messenger RNA expression after 8 and 16 h (Figure 1A and C). The increase in COX-2 protein levels was maintained until at least 72 h of glucose deprivation (Figure 1A).

A reduction in the molecular mass of COX-2 protein was apparent during glucose deprivation, as demonstrated by a downward shift in the COX-2 protein detected by western blotting (Figure 1A). Since COX-2 is glycosylated at asparagine residues (23), we hypothesized that this decrease in molecular mass was due to a reduction in N-linked glycosylation. In standard culture conditions, COX-2 is glycosylated at three asparagine residues, and a further asparagine site is glycosylated in almost 50% of COX-2 molecules, accounting for the doublet which can be observed during immunoblotting for COX-2 (23). Treatment of HT29 cells with tunicamycin (an N-linked glycosylation inhibitor) decreased the mass of COX-2 to the same size as the smallest COX-2 protein expressed during glucose deprivation (Figure 1B). Combining tunicamycin treatment with glucose deprivation did not further decrease COX-2 protein mass, confirming that the reduction in COX-2 protein mass by glucose deprivation was due to a reduction in N-linked glycosylation.

Having shown that COX-2 expression was increased by glucose deprivation, we next examined whether 15-PGDH expression was also regulated by glucose deprivation. Interestingly, 15-PGDH protein and messenger RNA expression in HT29 cells were repressed after 16 h of glucose deprivation (Figure 1A and D) and, like COX-2, the change in 15-PGDH protein expression persisted until at least 72 h of glucose deprivation (Figure 1A).

COX-2 and 15-PGDH protein expression were also assessed in other colon tumour cell lines subjected to glucose deprivation (Figure 1E–G). COX-2 expression increased during glucose deprivation in other cell lines examined. In addition, glucose deprivation decreased 15-PGDH expression in a number of cell lines. Notably, in the RG/C2 adenoma and SW480 carcinoma cell lines, which express very low to undetectable levels of COX-2, 15-PGDH expression was reduced by glucose deprivation, suggesting that 15-PGDH can be regulated independently of COX-2. In summary, we have shown that an increase in COX-2 and/or a decrease in 15-PGDH protein expression are a common response of colonic tumour cell lines to glucose deprivation.

Hypoxia induces COX-2 expression but does not alter 15-PGDH expression

We previously reported that hypoxia up-regulates COX-2 expression and extracellular PGE_2 levels in colon carcinoma cells (18) but did not examine whether hypoxia regulates 15-PGDH expression. Since both 15-PGDH and COX-2 can influence the levels of PGE_2, it was therefore of interest to determine if 15-PGDH expression is also regulated by hypoxia. 15-PGDH expression in HT29 cells was not altered by exposure to hypoxic conditions, despite increases in hypoxia inducible factor-1α and COX-2 protein expression (Figure 1H). 15-PGDH expression did not change in response to hypoxia in several other cell lines examined, including SW480 and HCT15 cell lines (data not shown). Therefore, colon tumour cells respond differently to two important microenvironmental stresses. As with hypoxia, glucose deprivation increases COX-2 protein expression, but in contrast to hypoxia, glucose deprivation also down-regulates 15-PGDH expression, including in tumour cell lines in which COX-2 protein is low/undetectable.

Glucose deprivation increases extracellular PGE_2, which increases cell viability in glucose-deprived conditions

The tumour-promoting effects of COX-2 are thought to be primarily due to the production of PGE_2 (6,7). Having shown that glucose deprivation increases COX-2 (but not COX-1) and decreases 15-PGDH protein expression in HT29 cells (Figure 2), we determined the effect of glucose deprivation on extracellular PGE_2 levels. Depriving HT29 cells of glucose resulted in a 4.6-fold increase in extracellular PGE_2 (Figure 2A). The COX-2-selective inhibitor NS-398 significantly inhibited production of PGE_2 synthesized during glucose deprivation (Figure 2A), suggesting that COX-2 plays an important role in the increase of PGE_2 during glucose deprivation. The repression of 15-PGDH expression by glucose deprivation is also likely to contribute to the increase in extracellular PGE_2 during glucose

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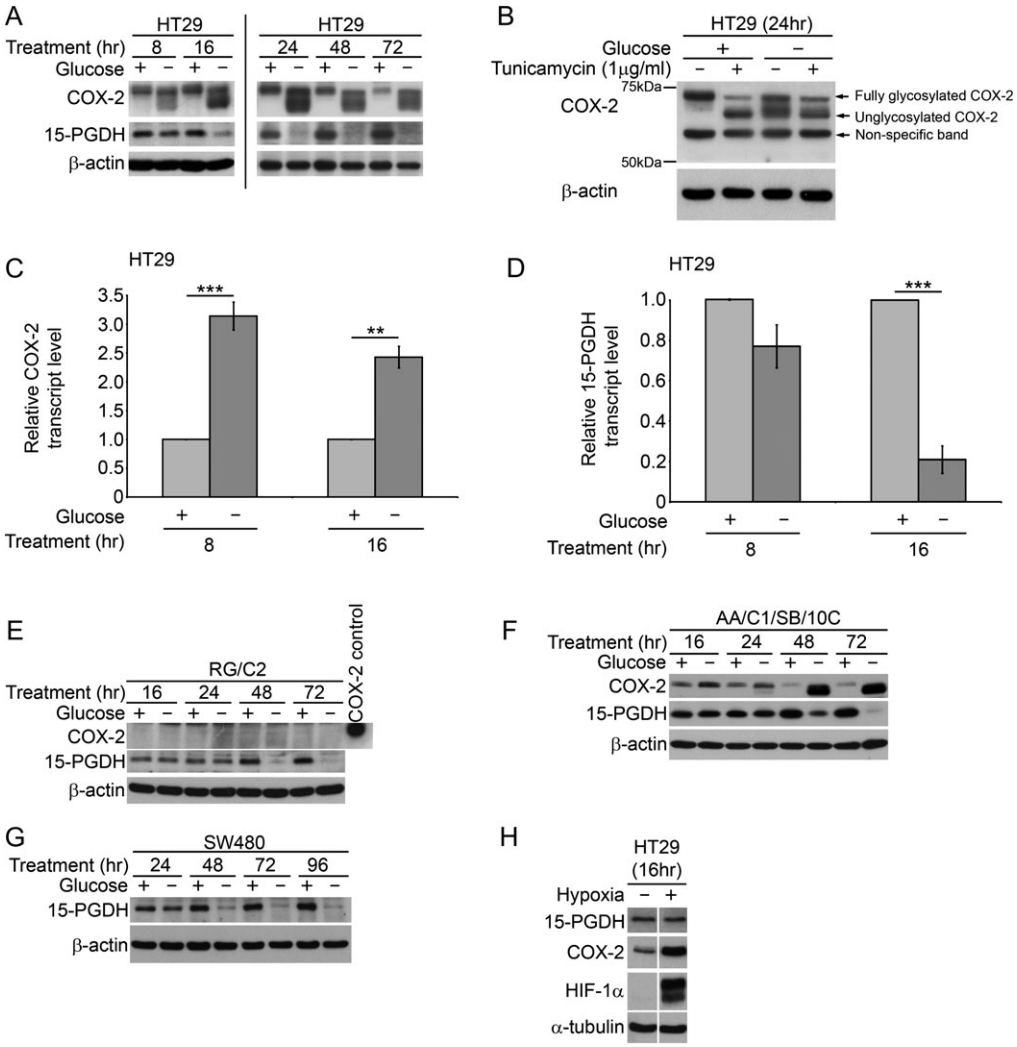

Fig. 1. Glucose deprivation increases COX-2 and decreases 15-PGDH expression, while hypoxia up-regulates COX-2 but not 15-PGDH. (**A**) Glucose deprivation causes an increase in COX-2 and a reduction in 15-PGDH protein expression in HT29 cells. Cells were treated with Dulbecco's modified Eagle's medium containing or deficient in glucose. (**B**) Reduction in molecular mass of COX-2 is due to a reduction in N-linked glycosylation. Tunicamycin treatment in glucose-containing medium reduces the mass of COX-2 protein to the same size as the smallest COX-2 protein expressed. Tunicamycin treatment during glucose deprivation does not further reduce COX-2 protein mass. (**C** and **D**) Early changes in COX-2 and 15-PGDH protein expression during glucose deprivation are mirrored at the messenger RNA level. Quantitative real-time polymerase chain reaction data are normalized to the expression of the housekeeping gene TATA box-binding protein and expressed as a fold of that expressed in cells in glucose-containing Dulbecco's modified Eagle's medium. Columns indicate mean data from three independent experiments carried out in triplicate. Bars indicate standard error of the mean. Statistical analysis performed using Student's *t*-test; $^{**}P < 0.01$ and $^{***}P < 0.001$. (**E–G**) Changes in protein expression of COX-2 and 15-PGDH are also observed with glucose deprivation of other colon tumour cell lines, including RG/C2, AA/C1/SB/10C and SW480. (**H**) Hypoxia treatment of HT29 cells increases COX-2 but does not alter 15-PGDH protein expression. HT29 cells were treated with normoxia (21% O_2) or hypoxia (1% O_2). Cells were lysed after 16 h and examined for COX-2, hypoxia inducible factor-1α (previously presented in reference (19)) and 15-PGDH expression. hypoxia inducible factor-1α expression confirmed hypoxic culture conditions. β-actin and α-tubulin were used as loading controls.

deprivation, potentially through prolonging its activity; we (19) and others (24,25) have previously reported that reducing 15-PGDH expression by siRNA increases extracellular PGE_2 in carcinoma cell lines.

PGE_2 has been implicated in a number of tumour-promoting events in cancer cells, including promotion of cell survival. Thus, whether PGE_2 could promote cell survival during glucose deprivation was examined. Treatment of the RG/C2 cell line [a colon adenoma-derived cell line which has previously been shown to be growth stimulated by PGE_2 in standard culture conditions (26,27)] with PGE_2 resulted in a significant increase in viable cell number, as determined by MTT assay, during glucose deprivation (Figure 2C). These findings suggest that colon tumour cells subjected to glucose deprivation will increase extracellular PGE_2 and that this increase in PGE_2 has a potentially important role in promoting survival and adaptation to these adverse conditions.

Increased PI3K/Akt activity during glucose deprivation leads to both COX-2 up-regulation and 15-PGDH down-regulation

Having shown that glucose deprivation regulates two key enzymes involved in controlling the level of PGE_2, it was of interest to determine the mechanism of regulation of these two enzymes. Two pathways that are frequently deregulated in colorectal cancer and have been associated with stimulating COX-2 expression and repressing 15-PGDH expression are the PI3K/Akt and the Ras-Raf-MEK-ERK1/2 pathways. ERK1/2 (28) and Akt (29,30) signalling can increase COX-2 expression, and ERK1/2 signalling has been implicated in the repression of 15-PGDH expression (31–33). Recently, we reported that the PI3K/Akt pathway can be involved in the repression of 15-PGDH expression in colorectal carcinoma cells (19). The effect of glucose deprivation on the activity of the PI3K/Akt and Ras-Raf-MEK-ERK1/2 pathways in HT29 cells was therefore examined by analysis of Akt and ERK1/2

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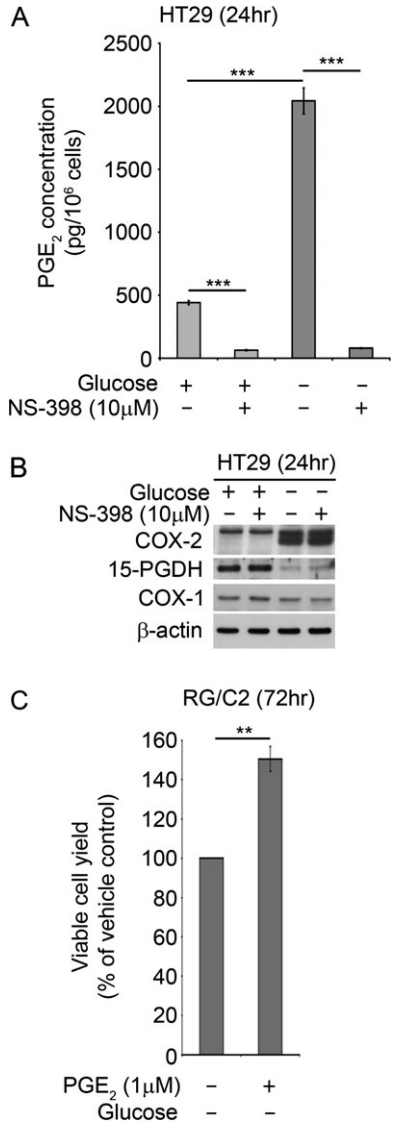

Fig. 2. Glucose deprivation increases extracellular PGE_2 and PGE_2 can promote viability. (**A**) Glucose deprivation of HT29 cells increases extracellular PGE_2, which is inhibited by the COX-2-selective inhibitor NS-398. Cells were pre-incubated with NS-398 or vehicle for 4 h prior to treatment with Dulbecco's modified Eagle's medium containing or deficient in glucose ± NS-398 for 24 h. PGE_2 content in culture media standardized to cell number. Columns indicate data from one experiment, representative of three individual experiments carried out in triplicate. Bars indicate standard deviation. Data analysed by analysis of variance followed by Tukey's post hoc test. (**B**) Glucose deprivation increases COX-2 and decreases 15-PGDH protein expression but does not alter COX-1 protein expression. NS-398 does not affect the expression of COX-1, COX-2 or 15-PGDH. Cells treated as in (A). (**C**) PGE_2 promotes increased viability of RG/C2 cells during glucose deprivation. RG/C2 cells were subjected to glucose deprivation ± 1 μM PGE_2 for 72 h. Cell viability assessed by MTT assay. Data presented as percentage of vehicle control. Columns indicate the mean of three independent experiments, each performed in sextuplicate. Bars indicate standard error of the mean. Statistical analysis performed using Student's *t*-test; $^{**}P < 0.01$ and $^{***}P < 0.001$.

phosphorylation. Glucose deprivation resulted in a significant and sustained increase in Akt phosphorylation, whereas there was little change in ERK1/2 phosphorylation (Figure 3A).

Given reports that PI3K/Akt signalling can regulate COX-2 (29,30) and 15-PGDH (19) expression, we investigated whether this increase in PI3K/Akt activity during glucose deprivation was responsible for the changes in COX-2 and 15-PGDH protein expression when HT29

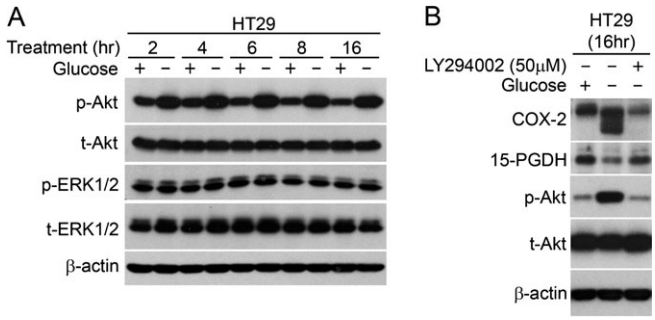

Fig. 3. PI3K/Akt signalling contributes to the regulation of COX-2 and 15-PGDH during glucose deprivation. (**A**) Glucose deprivation causes a large and sustained phosphorylation of Akt, but not ERK1/2, in HT29 cells. Cells were treated with Dulbecco's modified Eagle's medium containing or deficient in glucose and examined for phosphorylated (p-) and total (t-) Akt and ERK1/2. (**B**) The PI3K inhibitor LY294002 inhibited the increase in COX-2 and the decrease in 15-PGDH expression. HT29 cells were preincubated with LY294002 or vehicle for 2 h prior to treatment of cells for 16 h with glucose-containing Dulbecco's modified Eagle's medium or glucose deprivation ± LY294002.

cells were deprived of glucose. Incubation of HT29 cells with LY294002 (a PI3K inhibitor) inhibited the increase in COX-2 expression during glucose deprivation and partly, but not fully, inhibited the repression in 15-PGDH expression by glucose deprivation (Figure 3B). These findings suggest that increased activation of the PI3K/Akt pathway during glucose deprivation promotes not only an increase in COX-2 expression but also a decrease in 15-PGDH expression.

Increased expression of CHOP, an effector of the UPR, can suppress 15-PGDH expression

Inhibiting the activity of the PI3K/Akt pathway during glucose deprivation did not fully rescue 15-PGDH expression. This suggests that other mechanism(s) are also important in the repression of 15-PGDH expression during glucose deprivation. Since glucose deprivation can result in ER stress and induction of the UPR through a reduction in N-linked glycosylation (34), and earlier, it was shown that glucose deprivation inhibited the N-linked glycosylation of COX-2 (Figure 1B), we examined whether the UPR had a role in down-regulating 15-PGDH expression during glucose deprivation. Glucose deprivation, as expected, caused an increase in expression of CHOP (Figure 4A), a transcription factor which is commonly up-regulated during the UPR (34) and, as observed previously (Figure 1), glucose deprivation also decreased 15-PGDH expression. Interestingly, the increase in CHOP expression preceded the reduction in 15-PGDH expression, suggesting that the UPR might have a role in the repression of 15-PGDH expression by glucose deprivation.

To examine further if activation of the UPR was involved in the down-regulation of 15-PGDH expression by glucose deprivation, two other well-known inducers of ER stress, tunicamycin and thapsigargin, were also examined for their ability to regulate 15-PGDH expression. Both compounds activated the UPR, as demonstrated by an increase in CHOP expression. Significantly, these reagents also decreased 15-PGDH expression (Figure 4B), thus providing further evidence that activation of the UPR may reduce 15-PGDH expression.

Since the increase in CHOP expression preceded the reduction of 15-PGDH expression during glucose deprivation, it was hypothesized that this increase in CHOP could have an important role in the repression of 15-PGDH expression by glucose deprivation. To test this hypothesis, two independent siRNA sequences against CHOP were transfected into cells to inhibit CHOP induction by glucose deprivation. Inhibiting CHOP expression in HT29 cells during glucose deprivation partially rescued 15-PGDH expression (Figure 4C) (also observed in SW480 cells, data not shown), suggesting that elevated CHOP expression contributes to the repression of 15-PGDH expression

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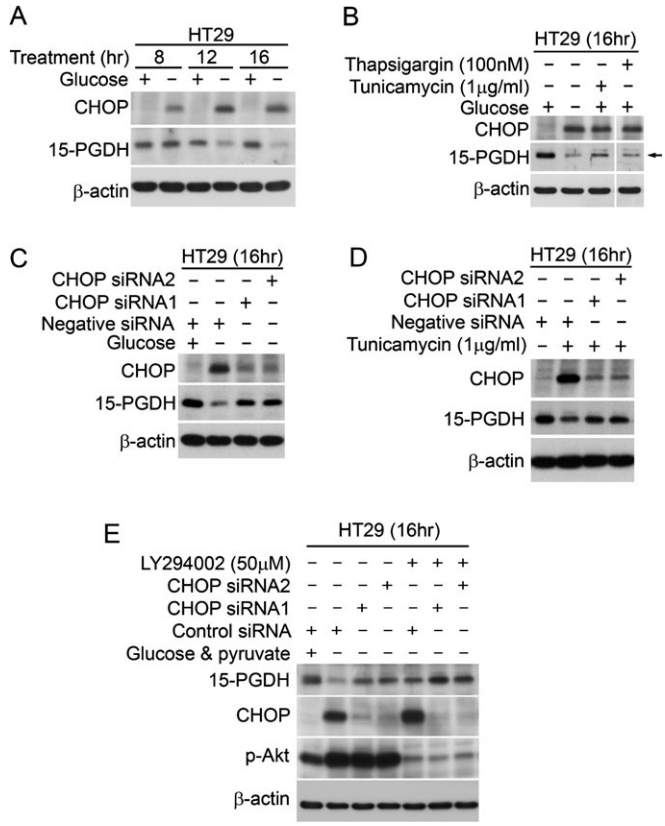

Fig. 4. The increase in CHOP expression represses 15-PGDH expression during glucose deprivation independently of PI3K activity. (**A**) Glucose deprivation causes an increase in CHOP expression, which precedes the reduction in 15-PGDH expression. HT29 cells were treated with Dulbecco's modified Eagle's medium containing or deficient in glucose and examined for CHOP and 15-PGDH expression. (**B**) Tunicamycin and thapsigargin treatment of HT29 cells also cause an increase in CHOP and a decrease in 15-PGDH expression. Cells were treated with tunicamycin or thapsigargin or deprived of glucose and examined 16 h after treatment. (**C** and **D**) Inhibiting the expression of CHOP during glucose deprivation or treatment with tunicamycin partially rescues 15-PGDH expression. CHOP expression in HT29 cells was inhibited using two independent siRNA sequences and cells subjected to glucose deprivation or treated with tunicamycin for 16 h. (**E**) Inhibiting CHOP expression by siRNA and inhibiting PI3K/Akt activity using LY294002 causes an additive recovery of 15-PGDH expression during glucose deprivation.

during glucose deprivation. Inhibiting CHOP expression by siRNA in HT29 cells treated with tunicamycin also partially rescued 15-PGDH expression during tunicamycin treatment (Figure 4D), suggesting that increasing CHOP expression may be a common means of tumour cells to suppress 15-PGDH expression in ER stress-inducing conditions.

Having shown that both PI3K/Akt activation and increased expression of CHOP can reduce the expression of 15-PGDH, we examined the effects of inhibiting both pathways on the expression of 15-PGDH during glucose deprivation. Interestingly, LY294002 treatment combined with CHOP siRNA leads to the complete recovery of 15-PGDH expression during glucose deprivation (Figure 4E). Reducing CHOP expression by siRNA during glucose deprivation did not inhibit Akt phosphorylation and likewise, LY294002 did not inhibit CHOP expression during glucose deprivation. Thus, it is likely that the repression of 15-PGDH expression during glucose deprivation by CHOP and by PI3K/Akt activity is via independent mechanisms. Taken together, these data suggest an important role for both the PI3K/AKT pathway and the UPR in the regulation of 15-PGDH expression during glucose deprivation.

Discussion

Adaptation to the tumour microenvironment is critical for tumour cell survival. Therefore, understanding the mechanisms employed by tumour cells to adapt to their microenvironment may identify novel targets for chemopreventative and chemotherapeutic agents. In the current study, we report for the first time that glucose deprivation regulates both COX-2 and 15-PGDH expression and stimulates an increase in extracellular PGE_2 and that an increase in PGE_2 provides a novel means to promote tumour cell survival in conditions of limited glucose availability. We have previously demonstrated that hypoxia causes an increase in extracellular PGE_2 through a hypoxia inducible factor-1-mediated increase in COX-2 expression, which promotes survival in low oxygen conditions (18). Thus, increasing PGE_2 may be a common adaptive response to promote tumour cell survival in diverse microenvironments (Figure 5). Furthermore, as PGE_2 has been implicated in promoting angiogenesis (35,36), the increase in extracellular PGE_2 levels during glucose deprivation or hypoxia could be implicated in both short-term adaptive measures (i.e. survival) and long-term adaptive measures (i.e. angiogenesis) to adverse microenvironments (Figure 5).

Given the importance of COX-2 and PGE_2 in tumorigenesis, targeting this pathway is of great clinical interest (13). Although chemoprevention using both non-selective cyclooxygenase inhibitors such as aspirin (37) or COX-2-selective inhibitors such as celecoxib (12) have been proven to reduce the incidence of colorectal cancer, administration of these drugs can cause side effects. This has led to great interest in identifying other targets that regulate PGE_2 levels or signalling, such as 15-PGDH. A potentially effective approach would be to lower PGE_2 levels by increasing 15-PGDH expression. Indeed, the clinical importance of 15-PGDH expression was recently demonstrated when it was shown that lower colonic 15-PGDH expression can be linked to resistance to celecoxib administered for colonic adenoma prevention (38). This, taken together with evidence that 15-PGDH plays an important tumour-suppressive role in the colon (14), emphasizes the importance of increasing our understanding of how

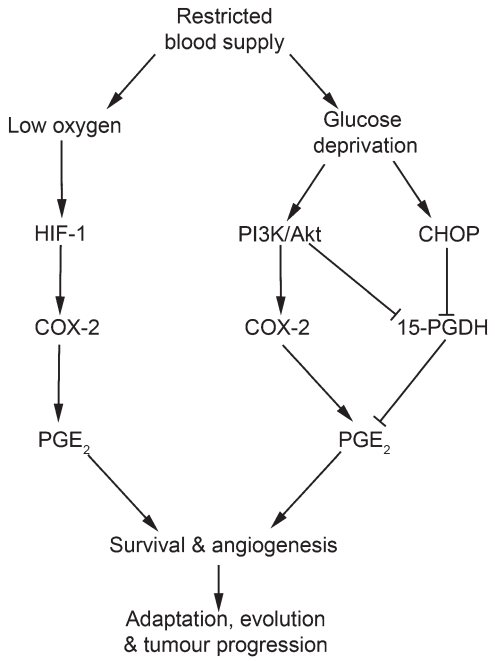

Fig. 5. Model for the regulation of PGE_2. Glucose deprivation increases PGE_2 by up-regulating COX-2 and down-regulating 15-PGDH expression via PI3K/Akt- and CHOP-dependent mechanisms. Hypoxia increases PGE_2 levels by up-regulating COX-2 expression via hypoxia inducible factor-1. Elevated PGE_2 increases survival of colon tumour cells exposed to both glucose deprivation and hypoxic conditions.

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15-PGDH—as well as COX-2—can be regulated to enable novel chemopreventative and therapeutic approaches that reduce colonic PGE_2 levels to be developed. While it has been established that 15-PGDH expression is repressed in colorectal tumours (39,40), the mechanisms by which 15-PGDH expression is regulated during tumorigenesis are relatively poorly understood and, surprisingly, there have been no previous studies investigating the regulation of 15-PGDH by microenvironmental stresses experienced by colorectal tumour cells such as hypoxia or glucose deprivation. Therefore, findings presented here add significantly to our understanding of how a critical PGE_2-regulating enzyme can be regulated during tumorigenesis.

The PI3K/Akt pathway is frequently deregulated in colorectal cancer and has been associated with increasing COX-2 expression (29,30) and repressing 15-PGDH expression (19). Our findings reported here that increased PI3K/Akt signalling in glucose-deprived conditions can regulate the expression of these two key proteins which control PGE_2 levels highlight the importance of the PI3/AKT pathway in colorectal tumorigenesis and in the adaptation of tumour cells to the tumour microenvironment. Furthermore, it supports the potential for exploiting the PI3K/AKT pathway for chemoprevention and cancer drug discovery (41).

Glucose is important not only as an energy and biosynthetic source for tumour cells but also in maintaining ER homeostasis via its role in glycosylation of proteins. Glucose deprivation, which we showed inhibited the N-linked glycosylation of COX-2 (Figure 1B), is known to activate the UPR (34). This raised the interesting hypothesis that increased ER stress and activation of the UPR during glucose deprivation could have contributed to the reduction in 15-PGDH expression. Significantly, we have shown for the first time that two other well-known inducers of ER stress, tunicamycin and thapsigargin, also repressed 15-PGDH expression, supporting the hypothesis that a cellular response to ER stress could be to repress 15-PGDH expression. We subsequently showed using an siRNA approach that the transcription factor CHOP, the expression of which can increase as a consequence of activation of the UPR, contributed to the repression of 15-PGDH during both glucose deprivation and tunicamycin treatment.

Taken together, our data suggest that 15-PGDH can be regulated by stress-activated signalling. This finding is of particular interest as it demonstrates a previously unreported means for stressed cells to increase PGE_2. Activation of the UPR has been reported to occur in a range of tumour types and has been linked with both cytoprotection and induction of cell death (42). The ability of tumour cells to elevate PGE_2 levels through CHOP-mediated repression of 15-PGDH expression may thus form part of the cytoprotective defence of the UPR in tumour cells. Consequently, this may have important implications for promoting tumour cell survival not only in the adaptation to a tumour microenvironment with low glucose availability but also following the exposure of tumour cells to cancer therapies reported to induce ER stress, such as radiation (43) or certain chemotherapeutic agents (44–46). Subsequently, our novel findings suggest that lowering stress-associated PGE_2 levels may enhance the effectiveness of ER stress-inducing anticancer therapies.

It is of interest to note that glucose deprivation leads to reduced 15-PGDH in both adenoma and carcinoma cells suggesting that glucose deprivation may contribute to 15-PGDH down-regulation at early stages of tumorigenesis. Furthermore, glucose deprivation reduces 15-PGDH in tumours with a wide range in COX-2 expression including cells in which COX-2 is very low–undetectable.

In the colon, COX-2 expression is very low–undetectable in normal epithelial cells (9). However, low levels of PGE_2 can be detected in normal colonic tissue (47), which may be derived from COX-1 activity in epithelial cells or COX-1- and COX-2-expressing cells within the stroma. Our finding that glucose deprivation can suppress 15-PGDH expression in adenoma-derived and carcinoma-derived cell lines, even when COX-2 is low–undetectable, may have important implications for tumour cell survival. Even during the early benign stages of carcinogenesis (as well as later, malignant stages), tumour cells exposed to deficiencies in glucose availability may be primed, perhaps through COX-1-derived prostaglandins, to increase survival-promoting PGE_2 through a reduction in 15-PGDH expression, even before COX-2 is up-regulated. Therefore, our findings may provide a fresh insight into the mechanisms regulating PGE_2 levels in relatively early stage carcinogenesis and which may also give rise to a potential PGE_2-mediated survival mechanism in COX-2-negative as well as COX-2-positive tumours through down-regulation of 15-PGDH expresssion. Although not investigated in this study, PGE_2 has been reported to protect colorectal tumours from apoptosis through a number of mechanisms including increasing Bcl-2 (48) and suppressing Bim (27) expression.

In summary, we have shown that a novel response to depriving colon tumour cells of glucose is an up-regulation of PGE_2 with both an increase in COX-2 expression and a decrease in 15-PGDH expression, which is mediated via enhanced PI3K/Akt signalling. We also report the novel finding that glucose deprivation leads to activation of the UPR which, through increased levels of CHOP, can lead to the suppression of 15-PGDH, a key tumour suppressor gene. These findings may have important implications for the ability of tumour cells to not only adapt to their microenvironment but also to resist a range of currently used therapeutic agents which induce ER stress. Our data suggest that diverse microenvironmental stresses converge to regulate PGE_2 as a common and crucial mediator of cell survival during adaptation to the fluctuating tumour microenvironment and have implications for the development of chemopreventive and therapeutic strategies not only for colorectal cancer but also for other major cancers where PGE_2 is known to have an important role.

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