Oncogene (2014), 1–7

www.nature.com/onc

SHORT COMMUNICATION

UAP1 is overexpressed in prostate cancer and is protective against inhibitors of *N*-linked glycosylation

HM Itkonen[1], N Engedal[1], E Babaie[2], M Luhr[1], IJ Guldvik[1], S Minner[3], J Hohloch[3], MC Tsourlakis[3], T Schlomm[4,5] and IG Mills[1,6,7]

Prostate cancer is the second most common cause of cancer-associated deaths in men, and signaling via a transcription factor called androgen receptor (AR) is an important driver of the disease. Consequently, AR target genes are prominent candidates to be specific for prostate cancer and also important for the survival of the cancer cells. Here we assess the levels of all hexosamine biosynthetic pathway (HBP) enzymes in 15 separate clinical gene expression data sets and identify the last enzyme in the pathway, UDP-*N*-acetylglucosamine pyrophosphorylase 1 (UAP1), to be highly overexpressed in prostate cancer. We analyzed 3261 prostate cancers on a tissue microarray and found that UAP1 staining correlates negatively with Gleason score ($P = 0.0039$) and positively with high AR expression ($P < 0.0001$). Cells with high UAP1 expression have 10-fold increased levels of the HBP end-product, UDP-*N*-acetylglucosamine (UDP-GlcNAc). UDP-GlcNAc is essential for *N*-linked glycosylation occurring in the endoplasmic reticulum (ER) and high UAP1 expression associates with resistance against inhibitors of *N*-linked glycosylation (tunicamycin and 2-deoxyglucose) but not with a general ER stress-inducing agent, the calcium ionophore A23187. Knockdown of UAP1 expression re-sensitized cells towards inhibitors of *N*-linked glycosylation, as measured by proliferation and activation of ER stress markers. Taken together, we have identified an enzyme, UAP1, which is highly overexpressed in prostate cancer and protects cancer cells from ER stress conferring a growth advantage.

Oncogene advance online publication, 22 September 2014; doi:10.1038/onc.2014.307

INTRODUCTION

Prostate cancer is the second most common cause of cancer-associated deaths in men. The majority of prostate cancers express androgen receptor (AR), which has been identified as a key mediator of the disease.[1] The vast majority of patients respond initially to AR-targeted therapies, while a subset of patients goes on to develop castration-resistant disease over time. In cell line models, AR activates anabolic and catabolic metabolism and glycolysis.[2,3] These metabolic networks have provided both highly specific prostate cancer biomarkers, such as alpha-methylacyl-CoA racemase,[4,5] and promising drug targets, such as fatty acid synthase.[6] However, AR-driven metabolic networks are highly complex, and inhibition of a single enzyme has not proven successful in clinical setting, with the exception of a steroid biosynthesis enzyme CYP17A1 targeted by abiraterone acetate.[7]

We recently reported that AR activates the expression of both the rate-limiting and final enzymes in the hexosamine biosynthetic pathway (HBP).[8] HBP functions as an integration point of multiple metabolic pathways, as it requires glucose, glutamine, acetyl-coenzyme A and nucleotides to produce UDP-*N*-acetylglucosamine (UDP-GlcNAc).[9,10] UDP-GlcNAc can be utilized by two principal processes, (1) *O*-GlcNAcylation, which is a single sugar conjugation, catalyzed by *O*-GlcNAc transferase (OGT) in the cytoplasm, nucleus and mitochondria[11] and (2) *O*- and *N*-linked glycosylation occurring in the endoplasmic reticulum (ER) and the Golgi apparatus leading to complex sugar conjugates on target proteins.[12] We recently showed that OGT is overexpressed in prostate cancer and can be targeted with a small-molecule inhibitor, which decreases cell viability and results in the loss of c-Myc.[8]

N-linked glycosylation is required for plasma-membrane localization of growth factor receptors, and increased receptor glycosylation prolongs surface retention, thereby enabling cells to drive growth-promoting signals according to the availability of nutrients.[13,14] Inhibition of *N*-linked glycosylation has been shown to inhibit plasma-membrane localization of growth factor receptors and processing of secretory proteins.[15–17] Core 2 1,6-*N*-acetylglucosaminyltransferase is an example of Golgi enzymes, which is overexpressed in prostate cancer, and activation of its expression results in significantly higher tumor burden in nude mice.[18]

The flux through HBP has emerged as an important metabolic integration point, and in this paper we evaluated the expression of different HBP enzymes in prostate cancer. Having identified the most strongly overexpressed enzyme at the mRNA level (UDP-*N*-acetylglucosamine pyrophosphorylase 1 (UAP1)), we analyzed 3261 prostate cancers on a tissue microarray by immunohistochemistry and elucidated its biological function in prostate cancer cell lines.

RESULTS AND DISCUSSION

HBP is upregulated in early stages of prostate cancer

The HBP has been implicated in tumorigenesis in a number of cancers,[19–21] while no study has systematically assessed the

[1]Prostate Cancer Research Group, Centre for Molecular Medicine (Norway), University of Oslo and Oslo University Hospitals, Oslo, Norway; [2]The Biotechnology Center of Oslo, University of Oslo, Oslo, Norway; [3]Institute for Pathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [4]Department of Urology, Section for translational Prostate Cancer Research University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [5]Martini Clinic, Prostate Cancer Center, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [6]Department of Cancer Prevention, Oslo University Hospitals, Oslo, Norway and [7]Department of Urology, Oslo University Hospitals, Oslo, Norway. Correspondence: Dr IG Mills or Dr HM Itkonen, Centre for Molecular Medicine Norway (NCMM), University of Oslo, Gaustadalleen 21, P.O. Box 1137, Oslo 0318, Norway. E-mail: ian.mills@ncmm.uio.no or harri.itkonen@ncmm.uio.no
Received 29 July 2013; revised 13 August 2014; accepted 16 August 2014

potential overexpression of each HBP enzyme in prostate cancer. We therefore utilized the Oncomine database[22] and found that both the first and the last enzymes of the HBP are significantly overexpressed in localized prostate cancer (Figures 1a and b). In 15 separate prostate cancer expression array data sets, UAP1 was the most overexpressed gene in the pathway. In order to determine the expression pattern of UAP1 in normal tissues, we carried out an *in silico* analysis using the Genesapiens database (http://ist.genesapiens.org/).[23] High levels of UAP1 are found in testis and adult stem cells, reflecting the identification of the enzyme and its alternative name, sperm-associated antigen 2[24] (Supplementary Figure 1A). On this basis, we evaluated the protein level expression of UAP1 by means of immunohistochemistry. In a large cohort of 3261 patients, a total of 1828 tissue samples could be evaluated for UAP1 staining (see Supplementary Material). Representative images of the staining intensity are shown in Figure 1c. Interestingly, UAP1 staining was negatively associated with the Gleason score across all staining intensities ($P = 0.0039$; Table 1). Our patient cohort has previously been evaluated for the expression of AR, which enabled us to evaluate the relationship between the two. Tumors with moderate and strong UAP1 immunostaining revealed strong AR staining in 57.6% while tumors with no UAP1 staining revealed strong AR immunostaining in only 11.8% of tumors ($P < 0.0001$; Table 1 and Supplementary Figure 1b).

This is in good agreement with induction of UAP1 at the mRNA level upon androgen stimulation *in vitro*, as reported previously.[8] Notably, the expression of most of the HBP enzymes is androgen responsive *in vitro* (Supplementary Figure 2A). Given the prominent overexpression of UAP1 in prostate cancer, we went on to evaluate its role *in vitro*.

UAP1 regulates the flux through the HBP

A null mutation in UAP1 is lethal in *Saccharomyces cerevisiae*, and this can be rescued by overexpression of the human enzyme indicating evolutionary conservation.[25] However, no study has explored the importance of UAP1 in human cells. The function of UAP1 has been elucidated *in vitro*, and the enzyme was found to utilize *N*-acetylglucosamine-1-phosphate and *N*-acetylgalactosamine-1-phosphate to produce UDP-GlcNAc and UDP-*N*-acetylgalactosamine (UDP-GalNAc).[25,26] In order to elucidate the importance of UAP1 expression for the production of UDP-GlcNAc and UDP-GalNAc, we used three prostate cancer cell lines (LNCaP, VCaP and PC3) and two non-tumorigenic prostate-derived cell lines (RWPE-1[27] and PNT2[28]). LNCaP and VCaP cell lines have an approximately threefold higher protein expression of UAP1 in comparison to PC3, PNT2 and RWPE-1 cells (Figure 2a), as previously reported,[17] and we next assessed the levels of sugar nucleotides using a recently published method.[29] Interestingly, cell lines with high UAP1 expression have 10-fold higher UDP-GlcNAc and UDP-GalNAc content (Figures 2a–c). This is in good agreement with Sreekumar *et al.*[30] who detected high levels of *N*-acetylglucosamine and *N*-acetylgalactosamine in localized

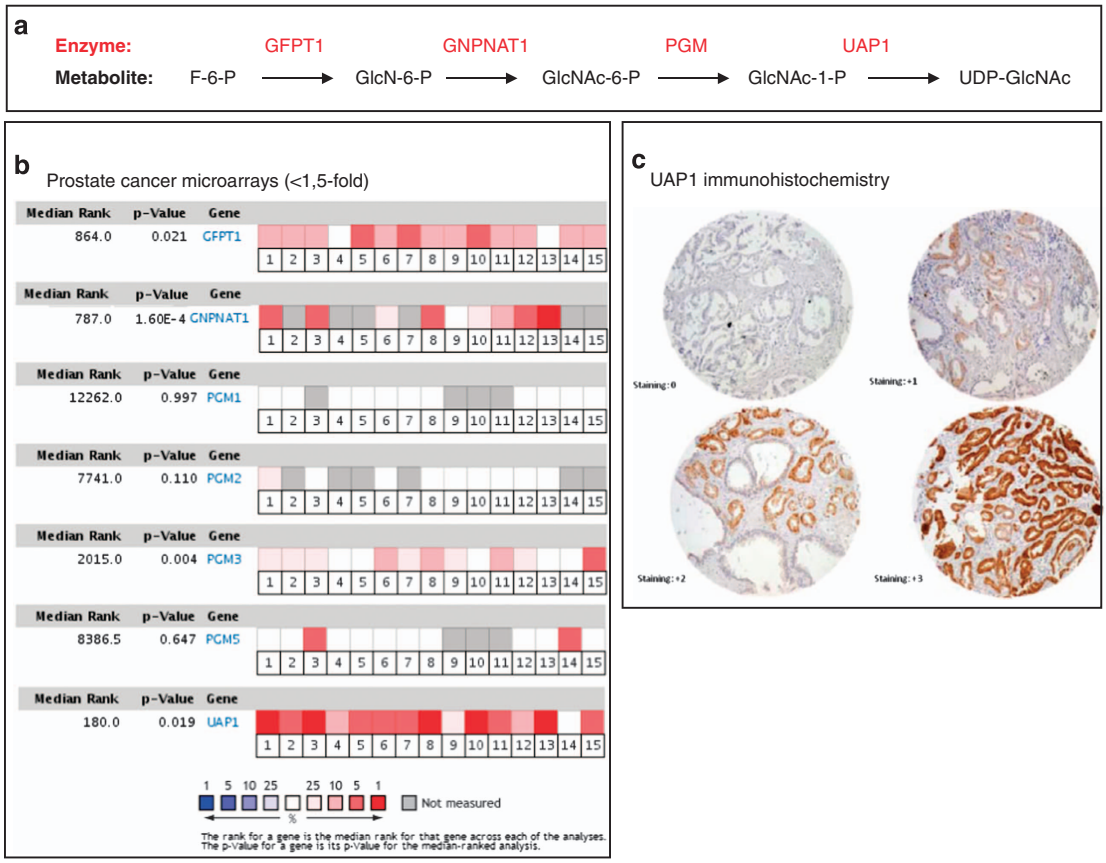

Figure 1. UAP1 is upregulated in the early stages of prostate cancer. (**a**) The hexosamine biosynthetic pathway. Each enzyme is depicted in red fonts and metabolites are highlighted below. Appreviations: GFTP1, glutamine-fructose-6-phosphate transaminase 1; GNPNAT, glucosamine-phosphate *N*-acetyltransferase; PGM, phosphoglucomutase; UAP1, UDP-*N*-acetylglucosamine pyrophosphorylase 1; F-6-P, fructose-6-phosphate; GlcN-6-P; glucosamine-6-phosphate; GlcNAc-6-P, *N*-acetylglucosamine-6-phosphate. (**b**) Evaluation of UAP1 mRNA expression in prostate cancer. Oncomine database[22] was used to assess the expression of UAP1 (>1.5-fold increased expression and $P < 0.05$). (**c**) Immunohistochemistry of UAP1 in prostatectomy samples. UAP1 staining was classified into four groups (staining intensity 0 to +3), which were used in the analysis (Table 1).

Table 1. Clinical parameters of UAP1 staining

Parameter	n	*Int0*	*Int1*	*Int2*	*Int3*	P-*value*
Staining	1828	322 (17.6)	580 (31.7)	866 (47.4)	60 (3.3)	
PSA						
<4	273	59 (21.6)	89 (32.6)	117 (42.9)	8 (2.9)	0.3989
4–10	985	160 (16.2)	307 (31.2)	480 (48.7)	38 (3.9)	
10–20	386	74 (19.2)	120 (31.1)	181 (46.9)	11 (2.8)	
>20	140	23 (16.4)	49 (35)	66 (47.1)	2 (1.4)	
pT stage						
pT2	1163	211 (18.1)	361 (31)	555 (47.7)	36 (3.1)	0.1135
pT3a	401	67 (16.7)	123 (30.7)	197 (49.1)	14 (3.5)	
pT3b	224	35 (15.6)	86 (38.4)	93 (41.5)	10 (4.5)	
pT4	20	5 (25)	2 (10)	13 (65)	0 (0)	
pN stage						
Nx	883	164 (18.6)	290 (32.8)	402 (45.5)	27 (3.1)	0.5834
N0	863	143 (16.6)	260 (30.1)	428 (49.6)	32 (3.7)	
N+	56	10 (17.9)	19 (33.9)	26 (46.4)	1 (1.8)	
Gleason Score						
$\leqslant 3+3$	764	151 (19.8)	231 (30.2)	360 (47.1)	22 (2.9)	0.0039
3+4	821	120 (14.6)	269 (32.8)	402 (49)	30 (3.7)	
4+3	191	35 (18.3)	66 (34.6)	86 (45)	4 (2.1)	
$\geqslant 4+4$	32	12 (37.5)	6 (18.8)	10 (31.3)	4 (12.5)	
Margin						
R0	1401	258 (18.4)	437 (31.2)	661 (47.2)	45 (3.2)	0.3835
R1	406	60 (14.8)	135 (33.3)	197 (48.5)	14 (3.4)	
AR						
Negative	67	35 (52.2)	20 (29.9)	11 (16.4)	1 (1.5)	<0.0001
Weak	135	42 (31.1)	44 (32.6)	47 (34.8)	2 (1.5)	
Moderate	266	59 (22.2)	98 (36.8)	106 (39.8)	3 (1.1)	
Strong	1144	135 (11.8)	350 (30.6)	608 (53.1)	51 (4.5)	

Abbreviations: AR, androgen receptor; PSA, prostate-specific antigen; UAP1, UDP-*N*-acetylglucosamine pyrophosphorylase 1. Samples were analyzed based on the staining intensities (Int) shown in Figure 1c for UAP1, and examples of AR staining at each scored intensity (Int0—negative; Int1—weak; Int2—moderate; Int3—strong) are shown is Supplementary Figure 1b. The number of patients in each group is reported, and the percentage is shown in brackets.

prostate cancer. In order to confirm the importance of UAP1 expression for the production of UDP-GlcNAc and UDP-GalNAc, we inhibited its expression with siRNA. This led to a consistent ~60% decrease in the levels of these hexosamines with two different siRNAs (Figure 2e).

HBP activity has been shown to be important for the regulation of glucose metabolism and cell growth.[31,32] However, inhibition of UAP1 expression did not have any effect on either glucose uptake or growth rate (Supplementary Figure 2B and Figure 3a). Perturbation of the HBP causes diabetes in murine models[33] and in 3T3-L1 adipocytes; insulin-stimulated glucose uptake results in 30% increase in the production of UDP-GlcNAc.[34] Of special interest, development of insulin resistance is associated with a fourfold increase in the levels of UDP-GlcNAc in the skeletal muscles,[35] whereas we observed that inhibition of UAP1 expression results in a significant decrease in the levels of UDP-GlcNAc (Figure 2e). High-glucose-induced development of insulin resistance can be prevented by an inhibitor of GFPT1,[33] and it remains to be determined whether UAP1 could be pharmacologically regulated to affect insulin responsiveness.

High levels of UAP1 expression contributes to resistance to inhibitors of *N*-linked glycosylation

Inhibition of UAP1 expression with two siRNAs did not have prominent effects on the growth rate. However, inhibition of UAP1 expression led to a 60% decrease in the levels of UDP-GlcNAc. UDP-GlcNAc is required for both (1) *O*-GlcNAcylation, which is a single sugar conjugation, catalyzed by OGT in the cytoplasm, nucleus and mitochondria[11] and (2) *O*- and *N*-linked glycosylation of proteins occurring in the ER and the Golgi apparatus.[12] In order to distinguish between the impact of UAP1 knockdown on *O*-GlcNAcylation and on *N*-linked glycosylation, we used various drugs. These were (1) 2-deoxyglucose (2DG), a non-hydrolysable sugar-analogue, which acts in two principal ways, by inhibiting glycolysis and by inhibiting *N*-linked glycosylation thereby activating ER-stress response,[36,37] (2) tunicamycin targeting *N*-linked glycosylation,[16] (3) a calcium ionophore (A23187) as a general ER-stress inducer and (4) an OGT inhibitor (ST045849).[38] ER-stress markers CHOP, GRP78 and GRP94 were used to assess the induction of ER stress,[36] and knockdown of UAP1 expression enhanced the induction of all three ER-stress markers by treatment with 2DG and tunicamycin but not by treatment with calcium ionophore (Supplementary Figure 2C). OGT inhibitor decreased the levels of total *O*-GlcNAcylation, but UAP1 knockdown did not further enhance this effect, suggesting that the major effects of UAP1 silencing are predominantly on *N*-linked glycosylation.

We next assessed the steady-state responses of the different prostate cell lines to inhibitors of *N*-linked glycosylation, testing the growth inhibitory effects of increasing doses of tunicamycin and 2DG. Interestingly, while the growth of the PNT2 and RWPE-1 cell lines was blocked by tunicamycin treatment at doses as low as 0.1 μg/ml, the growth of the PC3 cell line was abolished with 0.4 μg/ml, whereas the LNCaP and VCaP cells continued to grow in the presence of 0.5 μg/ml tunicamycin (Supplementary Figure 3A).

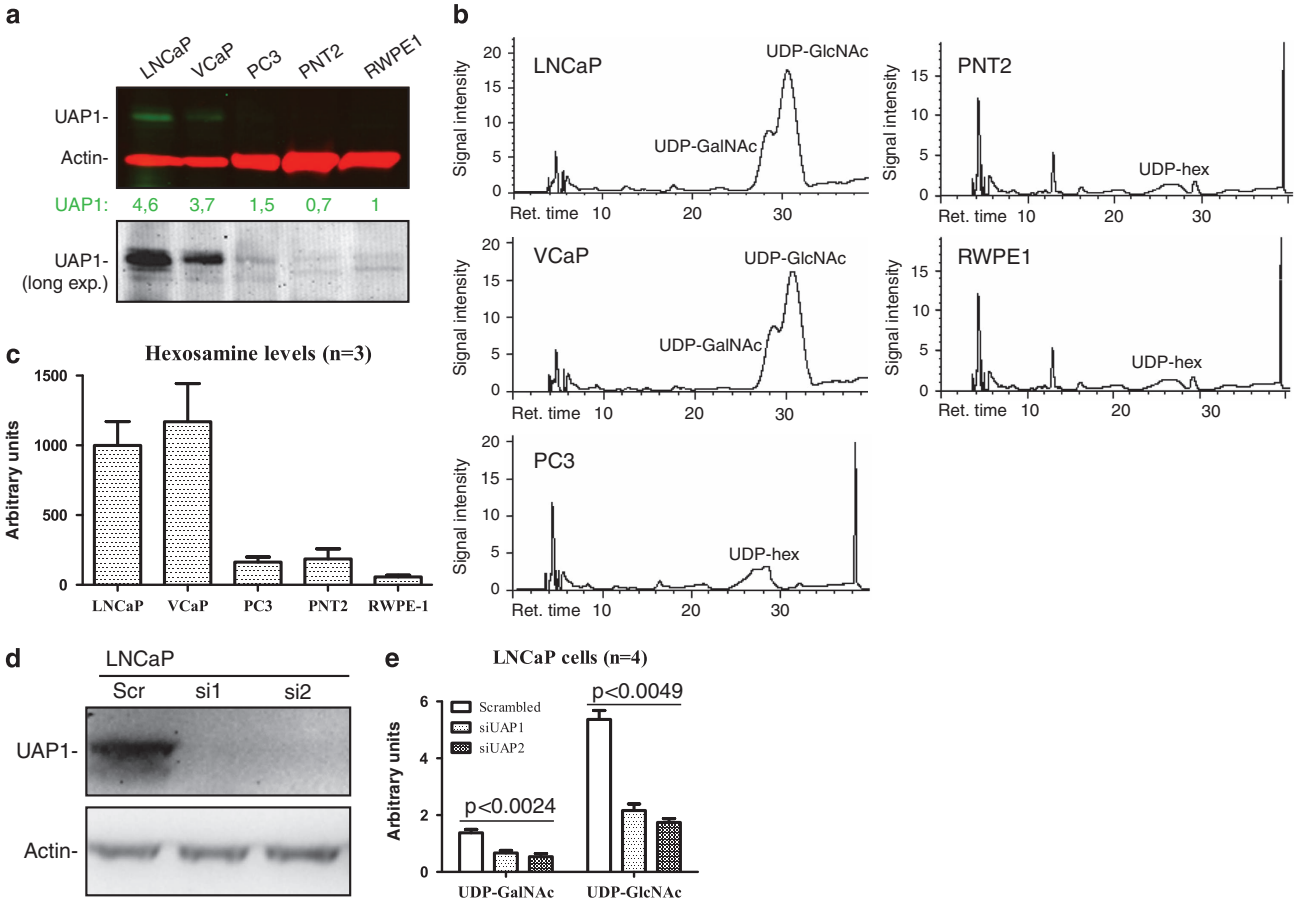

Figure 2. The activity of UAP1 is required for the flux through the hexosamine biosynthetic pathway. (**a**) Cell lysates were collected from LNCaP, VCaP, PC3, PNT2 and RWPE-1 cells and blotted for UAP1, and actin was used as a loading control. The intensity of each band was measured, normalized against actin and the value in RWPE-1 cells was set to one. (**b**) High-pressure liquid chromatograph (HPLC)-based evaluation of the levels of sugar nucleotides in cells. Examples of typical running profiles for each cell line are shown, and the metabolites of interest are depicted. Identification of each peak was based on the analysis of purified compounds. We were unable to separate UDP-GalNAc and UDP-GlcNAc in PC3, PNT2 and RWPE-1 cells, and both peaks were therefore combined to represent hexosamines (UDP-hex). (**c**) Quantitation of the HPLC data. The obtained values were first normalized to cell count. The data shown was obtained from three biological replicates, and the average values with s.e.m. are shown. LNCaP cells were reverse-transfected, and cell lysates were collected after 72 h either for western blotting (**d**) or for HPLC (**e**). The data shown are average of four biological replicates, and values were first normalized to internal control GDP-glucose and then to protein concentration. The significance of the data was evaluated with the Student's *t*-test, and s.e.m. is depicted.

The direct target of tunicamycin is DPAGT1 (dolichyl-phosphate *N*-acetylglucosaminephosphotransferase 1), which transfers UDP-GlcNAc to the dolichyl-diphosphate to be used for *N*-linked glycosylation.[39] Of special interest, high concentrations of UDP-GlcNAc relative to tunicamycin protect DPAGT1 from inhibition,[40] offering a potential explanation for the lack of growth inhibition in LNCaP and VCaP cells.

Similar data were obtained for 2DG, where treatment with 5 mM 2DG completely blocked the growth of PC3, PNT2 and RWPE-1 cells (low UAP1 expressors), whereas treating LNCaP and VCaP cell lines (high UAP1 expressors) with 10 mM 2DG did not abolish growth (Supplementary Figure 3B). Overall, this raised the possibility that high UAP1 expression contributes to resistance to inhibitors of *N*-linked glycosylation in LNCaP and VCaP cell lines.

UAP1 protects cells against inhibitors of *N*-linked glycosylation

We next went on to more directly assess whether UAP1 knockdown could be used to sensitize cells to inhibitors of *N*-linked glycosylation working with the LNCaP cell line. We performed the knockdown for 72 h and added a low dose of tunicamycin (0.1 μg/ml), which is not growth inhibitory in this cell line (Supplementary Figure 3A). Knockdown of UAP1 sensitized LNCaP cells to this low dose of tunicamycin and decreased the growth (Figure 3a). Similar results were obtained for VCaP cells (Supplementary Figure 4A). Additionally, inhibition of UAP1 expression also sensitized LNCaP cells to 2DG (Supplementary Figure 4B). 2DG inhibits both glycolysis and *N*-linked glycosylation, and the inhibitory effect on *N*-linked glycosylation can be alleviated by the addition of exogenous mannose.[36] Importantly, the combinatorial effects of UAP1 knockdown and 2DG-induced growth inhibition were reversed with mannose (Supplementary Figure 4C).

An important feature of transformed cells is the ability to form colonies in soft agar. In order to determine whether UAP1 is important for this process, we performed colony-forming assays using the LNCaP cell-line. UAP1 knockdown decreased colony-forming ability of the cells, while there was only modest additional effect of combining this knockdown with either tunicamycin or 2DG (Supplementary Figure 4D). Cell detachment is known to induce metabolic stress, which leads to decreased glucose uptake, thereby limiting glucose availability for the HBP.[41] Inhibition of UAP1 expression appears to affect anchorage-independent

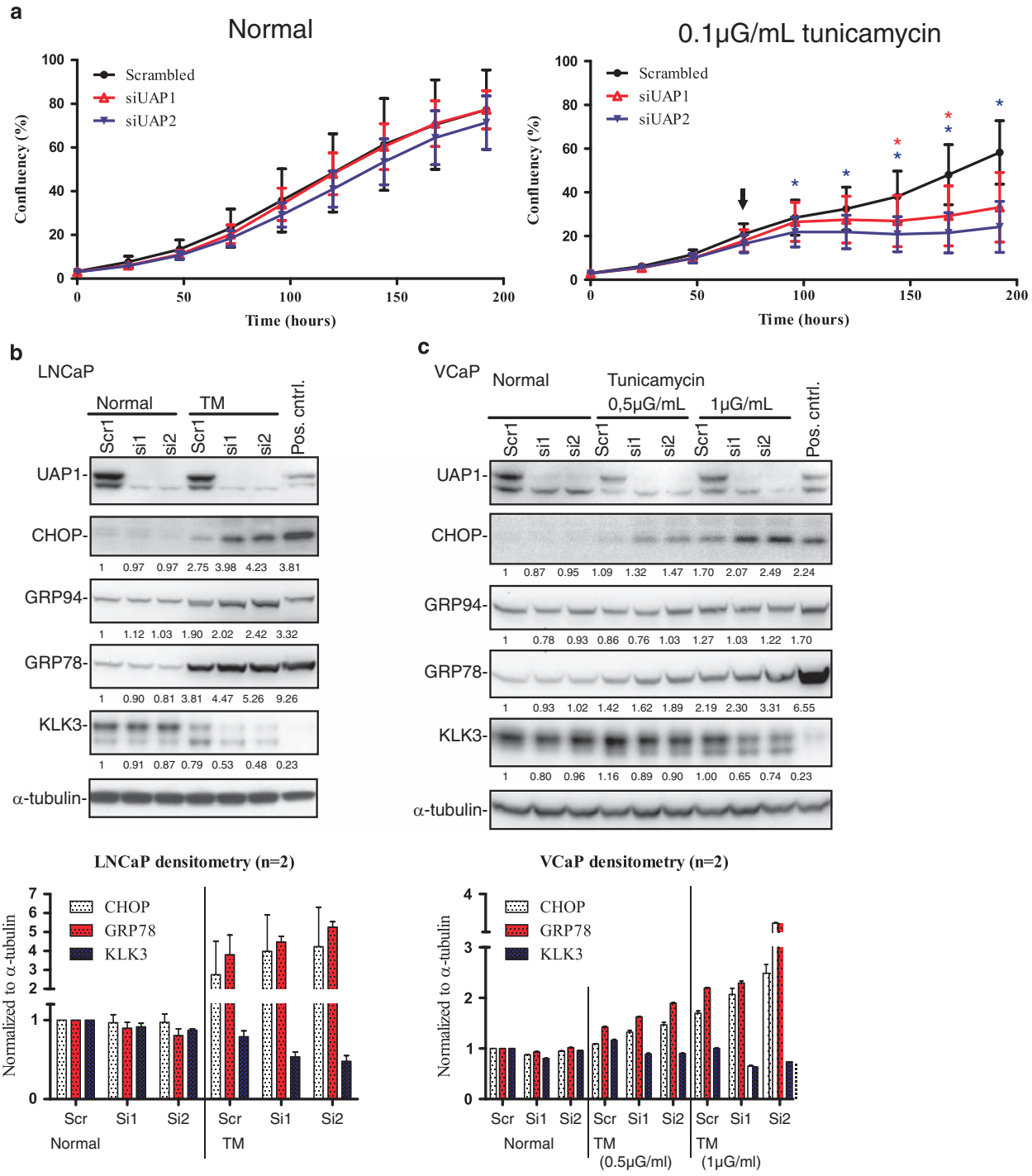

Figure 3. Inhibition of UAP1 expression sensitizes cells to tunicamycin. (**a**) LNCaP cells were plated into 384-plates on transfection reagent, placed into IncuCyte and imaged every 24 h. At 72 h, cells were treated with tunicamycin (indicated with an arrow). The data shown represent average of four independent experiments, s.e.m. is shown, and the significance was assessed with the paired samples Student's *t*-test (red stars are a comparison between siUAP1 and scrambled, while blue stars are a comparison between siUAP2 and scrambled, $*P < 0.05$). (**b**) LNCaP and (**c**) VCaP cells were reverse transfected for 72 h, treated with tunicamycin (TM; 0.6 μg/ml for LNCaP cells, 0.5 and 1 μg/ml for VCaP cells), and cell lysates were collected after 24 h. The intensity of each band was determined with densitometry, normalized to alpha-tubulin and normal scrambled sample was set to the value of 1. Data shown are representative of at least two biological replicates. Positive control is 5 μM thapsigargin.

growth of cells, further positioning it as an important factor for the survival of prostate cancer cells.

We next used two different siRNAs against UAP1 with tunicamycin treatment and evaluated the effects on the expression of ER-stress markers and a secretory glycoprotein KLK3 in LNCaP and VCaP cells. We first confirmed the increased induction of ER-stress markers GRP78, GRP94 and CHOP (Figures 3b and c). KLK3 is a secretory glycoprotein, and its levels are

measured in blood samples in order to assess the presence/recurrence of prostate cancer. The total level of KLK3 decreased upon treatment with tunicamycin, and this effect was further enhanced by UAP1 knockdown. Given that tunicamycin has also been reported to induce autophagy,[42] we assessed the effects on an autophagy marker LC3,[43] the induction of which was further enhanced by the knockdown of UAP1 in LNCaP cells but not further affected in VCaP cells (Supplementary Figures 5A and B). Inhibition of the growth rate suggested that UAP1 knockdown combined with tunicamycin treatment might also induce apoptosis, but we could not detect any increase in the levels of cleaved poly ADP-ribose polymerase. We next checked the possible effects on a marker of cycling cells, cyclin D1,[44] and this decreased in LNCaP cells and was unaltered in VCaP cells upon UAP1 knockdown. In addition, we observed an increase in the cyclin-dependent kinase inhibitor protein p27 and a decrease in the phosphorylation of S6 kinase (positive regulator of protein synthesis) in LNCaP cells, in agreement with inhibition of cell cycle progression. VCaP cells have a longer doubling time than LNCaP cells, and it remains possible that the effect on autophagy and especially cell cycle arrest becomes more apparent at later time points. Knockdown of UAP1 did not affect the levels of total *O*-GlcNAcylation either on its own or in the presence of tunicamycin.

GFPT1 is the rate-limiting enzyme in the HBP, and increased GFPT1 expression protects heart cells from cell death activation during ischemia/reperfusion.[45] Furthermore, we had observed that it is also overexpressed in prostate cancer (Figure 1b). Consequently, it was important to determine whether GFPT1 knockdown also impacted on responses to inhibitors of *N*-linked glycosylation in prostate cells. GFPT1 knockdown led to decreased KLK3 already in the absence ER stress-inducers (Supplementary Figures 5C and D) in agreement with previous studies reporting growth-inhibitory effects upon targeting GFPT1.[46] However, unlike UAP1, knockdown of GFPT1 did not enhance the expression of ER-stress markers when cells were treated with inhibitors of *N*-linked glycosylation (Supplementary Figures 5C and D). This suggests that inhibition of UAP1 activity might offer selectivity to sensitize prostate cancer cells to ER-stress-inducing agents.

In order to assess whether UAP1 knockdown specifically sensitizes prostate cancer cells to ER-stress-inducing agents, we knocked down UAP1 in RWPE-1 cells, representing normal prostate tissue, and treated cells with 2DG, tunicamycin and A23187. All three compounds led to the accumulation of ER stress and autophagy markers, but, importantly, knockdown of UAP1 had no effect on the levels of any of these markers (Supplementary Figure 6). In addition, inhibitors of *N*-linked glycosylation did not induce apoptosis in this cell line.

In conclusion, inhibition of UAP1 expression sensitizes cancer cells with high UAP1 expression, accompanied by high hexosamine levels, to inhibitors of *N*-linked glycosylation. High levels of UAP1 expression and/or HBP activity appear to confer a growth advantage to cancer cells upon induction of ER stress.

GFPT1 and UAP1 are co-expressed in prostate cancer patients

In order to assess the extent of the contribution of UAP1 to resistance to inhibitors of *N*-linked glycosylation, we overexpressed it in RWPE-1 cells. We were able to obtain over threefold increased expression of UAP1, but this did not increase resistance against 2DG, tunicamycin or A23187, as measured by the accumulation of ER-stress markers and growth rate (Supplementary Figures 7A and B). We therefore speculate that the expression of both the rate-limiting enzyme in the HBP, GFPT1, and UAP1 are needed for the resistance against inhibitors of *N*-linked glycosylation. Interestingly, GFPT1 and UAP1 are tightly co-expressed in prostate cancer patients (Supplementary Figure 7C).

In conclusion, we have identified a pathway that is overexpressed in prostate cancer and shown for the first time that UAP1 can be targeted to inhibit the flux through this pathway. Levels of UAP1 expression affect responses to inhibitors of *N*-linked glycosylation, and we therefore propose that the HBP can function as a 'buffer' against this type of ER stress. UAP1 is highly prostate cancer specific, and we showed that inhibition of UAP1 can specifically sensitize prostate cancer cells to the inhibitors of *N*-linked glycosylation. On the other hand, inhibition of GFPT1 might lead to serious complications as it is the rate-limiting enzyme in the HBP implicated in insulin resistance[33] and has important functions in normal tissue, such as the heart.[45] The structure of UAP1 has been solved,[26] which will significantly aid in the design and development of small-molecule inhibitors to target this enzyme. The main treatment for prostate cancer, androgen-deprivation therapy, induces hyperinsulinemia,[47] which is associated with rapid treatment failure[48] and new-onset of diabetes mellitus.[49] In this context, the role of UAP1 in maintaining insulin responsiveness warrants further investigation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

HMI is funded by an Early Stage Researcher fellowship as part of the EU FP7 Marie Curie Integrated Training Network, PRO-NEST (Prostate Research Organizations – Network Early Stage Training) Norwegian Cancer Society and Finnish Cultural Foundation. IGM is supported by funding from the Norwegian Research Council, Helse and the University of Oslo through the Centre for Molecular Medicine (Norway), which is a part of the Nordic EMBL (European Molecular Biology Laboratory) partnership. IGM is also supported by the Norwegian Cancer Society and by EU FP7 funding. IGM holds a visiting scientist position with Cancer Research UK through the Cambridge Research Institute and a Senior Visiting Research Fellowship with Cambridge University through the Department of Oncology. We thank Professor Olli Kallioniemi's group (Institute for Molecular Medicine Finland, Helsinki, Finland) for technical support.

REFERENCES

1 Harris WP, Mostaghel EA, Nelson PS, Montgomery B. Androgen deprivation therapy: progress in understanding mechanisms of resistance and optimizing androgen depletion. *Nat Clin Pract Urol* 2009; **6**: 76–85.
2 Massie CE, Lynch A, Ramos-Montoya A, Boren J, Stark R, Fazli L *et al*. The androgen receptor fuels prostate cancer by regulating central metabolism and biosynthesis. *EMBO J* 2011; **30**: 2719–2733.
3 Moon JS, Jin WJ, Kwak JH, Kim HJ, Yun MJ, Kim JW *et al*. Androgen stimulates glycolysis for de novo lipid synthesis by increasing the activities of hexokinase 2 and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2 in prostate cancer cells. *Biochem J* 2011; **433**: 225–233.
4 Humphrey PA. Diagnosis of adenocarcinoma in prostate needle biopsy tissue. *J Clin Pathol* 2007; **60**: 35–42.
5 Luo J, Zha S, Gage WR, Dunn TA, Hicks JL, Bennett CJ *et al*. Alpha-methylacyl-CoA racemase: a new molecular marker for prostate cancer. *Cancer Res* 2002; **62**: 2220–2226.
6 Kridel SJ, Axelrod F, Rozenkrantz N, Smith JW. Orlistat is a novel inhibitor of fatty acid synthase with antitumor activity. *Cancer Res* 2004; **64**: 2070–2075.
7 Sartor O, Pal SK. Abiraterone and its place in the treatment of metastatic CRPC. *Nat Rev Clin Oncol* 2013; **10**: 6–8.
8 Itkonen HM, Minner S, Guldvik IJ, Sandmann MJ, Tsourlakis MC, Berge V *et al*. O-GlcNAc transferase integrates metabolic pathways to regulate the stability of c-MYC in human prostate cancer. *Cancer Res* 2013; **73**: 5277–5287.
9 Slawson C, Copeland RJ, Hart GW. O-GlcNAc signaling: a metabolic link between diabetes and cancer? *Trends Biochem Sci* 2010; **35**: 547–555.
10 Wellen KE, Thompson CB. A two-way street: reciprocal regulation of metabolism and signalling. *Nat Rev Mol Cell Biol* 2012; **13**: 270–276.
11 Butkinaree C, Park K, Hart GW. O-linked beta-N-acetylglucosamine (O-GlcNAc): extensive crosstalk with phosphorylation to regulate signaling and transcription in response to nutrients and stress. *Biochim Biophys Acta* 2010; **1800**: 96–106.
12 Schwarz F, Aebi M. Mechanisms and principles of N-linked protein glycosylation. *Curr Opin Struct Biol* 2011; **21**: 576–582.

13 Dennis JW, Lau KS, Demetriou M, Nabi IR. Adaptive regulation at the cell surface by N-glycosylation. *Traffic* 2009; **10**: 1569–1578.
14 Lau KS, Partridge EA, Grigorian A, Silvescu CI, Reinhold VN, Demetriou M *et al.* Complex N-glycan number and degree of branching cooperate to regulate cell proliferation and differentiation. *Cell* 2007; **129**: 123–134.
15 Chen R, Li J, Feng CH, Chen SK, Liu YP, Duan CY *et al.* c-Met function requires N-linked glycosylation modification of pro-Met. *J Cell Biochem* 2013; **114**: 816–822.
16 Dricu A, Carlberg M, Wang M, Larsson O. Inhibition of N-linked glycosylation using tunicamycin causes cell death in malignant cells: role of down-regulation of the insulin-like growth factor 1 receptor in induction of apoptosis. *Cancer Res* 1997; **57**: 543–548.
17 Itkonen HM, Mills IG. N-linked glycosylation supports cross-talk between receptor tyrosine kinases and androgen receptor. *PLoS ONE* 2013; **8**: e65016.
18 Hagisawa S, Ohyama C, Takahashi T, Endoh M, Moriya T, Nakayama J *et al.* Expression of core 2 beta1,6-N-acetylglucosaminyltransferase facilitates prostate cancer progression. *Glycobiology* 2005; **15**: 1016–1024.
19 Krzeslak A, Forma E, Bernaciak M, Romanowicz H, Brys M. Gene expression of O-GlcNAc cycling enzymes in human breast cancers. *Clin Exp Med* 2012; **12**: 61–65.
20 Lynch TP, Ferrer CM, Jackson SR, Shahriari KS, Vosseller K, Reginato MJ. Critical role of O-Linked beta-N-acetylglucosamine transferase in prostate cancer invasion, angiogenesis, and metastasis. *J Biol Chem* 2012; **287**: 11070–11081.
21 Mi W, Gu Y, Han C, Liu H, Fan Q, Zhang X *et al.* O-GlcNAcylation is a novel regulator of lung and colon cancer malignancy. *Biochim Biophys Acta* 2011; **1812**: 514–519.
22 Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D *et al.* ONCOMINE: a cancer microarray database and integrated data-mining platform. *Neoplasia* 2004; **6**: 1–6.
23 Kilpinen S, Autio R, Ojala K, Iljin K, Bucher E, Sara H *et al.* Systematic bioinformatic analysis of expression levels of 17,330 human genes across 9,783 samples from 175 types of healthy and pathological tissues. *Genome Biol* 2008; **9**: R139.
24 Diekman AB, Olson G, Goldberg E. Expression of the human antigen SPAG2 in the testis and localization to the outer dense fibers in spermatozoa. *Mol Reprod Dev* 1998; **50**: 284–293.
25 Mio T, Yabe T, Arisawa M, Yamada-Okabe H. The eukaryotic UDP-N-acetylglucosamine pyrophosphorylases. Gene cloning, protein expression, and catalytic mechanism. *J Biol Chem* 1998; **273**: 14392–14397.
26 Peneff C, Ferrari P, Charrier V, Taburet Y, Monnier C, Zamboni V *et al.* Crystal structures of two human pyrophosphorylase isoforms in complexes with UDPGlc(Gal)NAc: role of the alternatively spliced insert in the enzyme oligomeric assembly and active site architecture. *EMBO J* 2001; **20**: 6191–6202.
27 Bello D, Webber MM, Kleinman HK, Wartinger DD, Rhim JS. Androgen responsive adult human prostatic epithelial cell lines immortalized by human papillomavirus 18. *Carcinogenesis* 1997; **18**: 1215–1223.
28 Berthon P, Cussenot O, Hopwood L, Leduc A, Maitland N. Functional expression of sv40 in normal human prostatic epithelial and fibroblastic cells—differentiation pattern of nontumorigenic cell-lines. *Int J Oncol* 1995; **6**: 333–343.
29 Nakajima K, Kitazume S, Angata T, Fujinawa R, Ohtsubo K, Miyoshi E *et al.* Simultaneous determination of nucleotide sugars with ion-pair reversed-phase HPLC. *Glycobiology* 2010; **20**: 865–871.
30 Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J *et al.* Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. *Nature* 2009; **457**: 910–914.
31 Monauni T, Zenti MG, Cretti A, Daniels MC, Targher G, Caruso B *et al.* Effects of glucosamine infusion on insulin secretion and insulin action in humans. *Diabetes* 2000; **49**: 926–935.
32 Wellen KE, Lu C, Mancuso A, Lemons JM, Ryczko M, Dennis JW *et al.* The hexosamine biosynthetic pathway couples growth factor-induced glutamine uptake to glucose metabolism. *Genes Dev* 2010; **24**: 2784–2799.
33 Buse MG. Hexosamines, insulin resistance, and the complications of diabetes: current status. *Am J Physiol Endocrinol Metab* 2006; **290**: E1–E8.
34 Bosch RR, Pouwels MJ, Span PN, Olthaar AJ, Tack CJ, Hermus AR *et al.* Hexosamines are unlikely to function as a nutrient-sensor in 3T3-L1 adipocytes: a comparison of UDP-hexosamine levels after increased glucose flux and glucosamine treatment. *Endocrine* 2004; **23**: 17–24.
35 Patti ME, Virkamaki A, Landaker EJ, Kahn CR, Yki-Jarvinen H. Activation of the hexosamine pathway by glucosamine in vivo induces insulin resistance of early postreceptor insulin signaling events in skeletal muscle. *Diabetes* 1999; **48**: 1562–1571.
36 Kurtoglu M, Gao N, Shang J, Maher JC, Lehrman MA, Wangpaichitr M *et al.* Under normoxia, 2-deoxy-D-glucose elicits cell death in select tumor types not by inhibition of glycolysis but by interfering with N-linked glycosylation. *Mol Cancer Ther* 2007; **6**: 3049–3058.
37 Xi H, Kurtoglu M, Liu H, Wangpaichitr M, You M, Liu X *et al.* 2-Deoxy-D-glucose activates autophagy via endoplasmic reticulum stress rather than ATP depletion. *Cancer Chemother Pharmacol* 2011; **67**: 899–910.
38 Gross BJ, Kraybill BC, Walker S. Discovery of O-GlcNAc transferase inhibitors. *J Am Chem Soc* 2005; **127**: 14588–14589.
39 Bassik MC, Kampmann M. Knocking out the door to tunicamycin entry. *Proc Natl Acad Sci USA* 2011; **108**: 11731–11732.
40 Keller RK, Boon DY, Crum FC. N-Acetylglucosamine- 1 -phosphate transferase from hen oviduct: solubilization, characterization, and inhibition by tunicamycin. *Biochemistry* 1979; **18**: 3946–3952.
41 Grassian AR, Coloff JL, Brugge JS. Extracellular matrix regulation of metabolism and implications for tumorigenesis. *Cold Spring Harb Symp Quant Biol* 2011; **76**: 313–324.
42 Ding WX, Ni HM, Gao W, Hou YF, Melan MA, Chen X *et al.* Differential effects of endoplasmic reticulum stress-induced autophagy on cell survival. *J Biol Chem* 2007; **282**: 4702–4710.
43 Johansen T, Lamark T. Selective autophagy mediated by autophagic adapter proteins. *Autophagy* 2011; **7**: 279–296.
44 Musgrove EA, Caldon CE, Barraclough J, Stone A, Sutherland RL. Cyclin D as a therapeutic target in cancer. *Nat Rev Cancer* 2011; **11**: 558–572.
45 Wang ZV, Deng Y, Gao N, Pedrozo Z, Li DL, Morales CR *et al.* Spliced x-box binding protein 1 couples the unfolded protein response to hexosamine biosynthetic pathway. *Cell* 2014; **156**: 1179–1192.
46 Ying H, Kimmelman AC, Lyssiotis CA, Hua S, Chu GC, Fletcher-Sananikone E *et al.* Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. *Cell* 2012; **149**: 656–670.
47 Braga-Basaria M, Dobs AS, Muller DC, Carducci MA, John M, Egan J *et al.* Metabolic syndrome in men with prostate cancer undergoing long-term androgen-deprivation therapy. *J Clin Oncol* 2006; **24**: 3979–3983.
48 Gunter JH, Sarkar PL, Lubik AA, Nelson CC. New players for advanced prostate cancer and the rationalisation of insulin-sensitising medication. *Int J Cell Biol* 2013; **2013**: 834684.
49 Derweesh IH, Diblasio CJ, Kincade MC, Malcolm JB, Lamar KD, Patterson AL *et al.* Risk of new-onset diabetes mellitus and worsening glycaemic variables for established diabetes in men undergoing androgen-deprivation therapy for prostate cancer. *BJU Int* 2007; **100**: 1060–1065.

Supplementary Information accompanies this paper on the Oncogene website (http://www.nature.com/onc)