Human Cancer Biology

Clinical Cancer Research

Nupr1-Aurora Kinase A Pathway Provides Protection against Metabolic Stress-Mediated Autophagic-Associated Cell Death

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Abstract

Purpose: The limited supply of oxygen and nutrients is thought to result in rigorous selection of cells that will eventually form the tumor.

Experimental Design: Nupr1 expression pattern was analyzed in human tissue microarray (TMA) and correlated with survival time of the patient. Microarray analysis was conducted on MiaPaCa2 cells subjected to metabolic stress in Nupr1-silenced conditions. DNA repair and cell cycle–associated gene expression was confirmed by real-time quantitative PCR (qRT-PCR). Nupr1 and AURKA protective role were analyzed using RNA interference (RNAi) silencing or overexpression. DNA damage and autophagy were analyzed by Western blot analysis and immunofluorescence.

Results: We showed that both Nupr1 and HIF1α are coexpressed in human pancreatic ductal adenocarcinoma (PDAC) samples and negatively correlate with survival time. PDAC-derived cells submitted to hypoxia and/or glucose starvation induce DNA damage–dependent cell death concomitantly to the overexpression of stress protein Nupr1. Affymetrix-based transcriptoma analysis reveals that Nupr1 knockdown enhances DNA damage and alters the expression of several genes involved in DNA repair and cell-cycle progression. Expression of some of these genes is common to hypoxia and glucose starvation, such as *Aurka* gene, suggesting that Nupr1 overexpression counteracts the transcriptional changes occurring under metabolic stress. The molecular mechanism by which hypoxia and glucose starvation induce cell death involves autophagy-associated, but not caspase-dependent, cell death. Finally, we have found that *AURKA* expression is partially regulated by Nupr1 and plays a major role in this response.

Conclusions: Our data reveal that Nupr1 is involved in a defense mechanism that promotes pancreatic cancer cell survival when exposed to metabolic stress. *Clin Cancer Res; 18(19); 5234–46. ©2012 AACR.*

Introduction

The incidence of pancreatic ductal adenocarcinoma (PDAC) is increasing with more than 38,000 predicted new cases in the United States and 65,000 in Europe every year. After years of research, we are now beginning to unveil the genetic determinants of premalignant lesions of PDAC. PDAC arises from precursor lesions known as pancreatic intraepithelial neoplasia (PanIN) and, less frequently, from 2 types of cystic tumors, the mucinous cystic neoplasms (MCN) and the intraductal papillary mucinous neoplasms (IPMN). Recurrent genetic alterations associated with these lesions have been identified, including the mutational activation of the *KRAS* proto-oncogene, present in virtually all cases (1), and loss-of-function mutations of the G_1 cyclin–dependent kinase inhibitor INK4A/ADP ribosylation factor (ARF; 80% of cases), the SMAD4/DPC4 (50%), and the *TP53* (50%) tumor suppressor genes (2). In turn, this research has increased our knowledge of the genetic alterations required for pancreatic cancer development. Nevertheless, the irreversibility of these mutations and absence of drugs that would counteract their consequences has hindered the development of effective therapies.

Surgery, the most effective treatment of pancreatic cancer to date, results in a mean life expectancy of only 15 to 18

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

In this work, we showed that both Nupr1 and HIF1α are coexpressed in human pancreatic ductal adenocarcinoma (PDAC) samples and negatively correlate with survival time, and therefore could be used as prognosis markers. In addition, we also showed that PDAC cells become resistant to the metabolic stress by activating an efficient intracellular defense pathway involving a Nupr1-dependent AURKA activation pathway. These findings pointed at a strong protumoral function of AURKA and, in consequence, at the potential efficacy of inhibitors of AURKA to treat cancers. Such a rationale was based on the effect of AURKA on mitosis, and data from our study provide an additional argument for its targeting. We suggest that inhibition of AURKA will sensitize preferentially intrapancreatic tumor cells located near hypovascularized regions, which should correspond to the most resistant cells because they have been selected by exposure to an adverse microenvironment, and also to the areas of the tumor in which the anticancer drug results are less accessible.

months in the 15% to 20% of patients who harbor resectable tumors. Moreover, in the fraction of patients who qualify for surgery, chemotherapy/radiotherapy–resistant metastases often arise after surgical resection of the primary tumor. The resistance to chemo and radiotherapy is thought to be associated both with the intrinsic nature of pancreatic cancer cells (3) and with the abundant fibrotic stroma of the tumors, which favors rapid tumor progression and creates a physical barrier preventing drug delivery and immune cell infiltration (4, 5). In addition to the importance of fibrotic stroma, PDAC histolopathology is characterized by poor vascularization indicating that pancreatic cancer cells must cope with an adverse microenvironment with nutrient and oxygen shortage. However, the mechanism underlying the resistance to such metabolic stress remains obscure. The limited supply of oxygen and nutrients is thought to result in rigorous selection of cells that will eventually form the tumor. Nupr1 is a stress response protein systematically overexpressed in pancreatic cancer and associated with bad prognosis (6, 7). Nupr1 regulates TGF-β responsiveness, inhibits apoptosis, and promotes cancer cell resistance to antitumoral treatments (8–12). We hypothesized that, under chronic hypoxic conditions as well as nutrient starvation, pancreatic cancer cells activate intracellular pathways responsible for their resistance. Here, we show that Nupr1 mediates resistance to metabolic stress–induced autophagic cell death through the regulation of AURKA-dependent DNA damage.

Materials and Methods

Immunohistochemistry

Tissue sections were deparaffinized, rehydrated, antigen unmasked, and quenched in hydrogen peroxide to block endogenous peroxidase activity using standard conditions. Then, slides were incubated with primary antibody diluted in phosphate saline buffer. Antibodies were revealed using the avidin–biotin peroxidase complex method (Dako) following recommendation of the manufacturer and counterstained with hematoxylin. Finally, slides were dehydrated, cleared, and mounted. HIF1α antibody (Bethyl) was used at 1:100 dilution and the monoclonal anti-Nupr1 (homemade) at 1:200.

Cell culture

MiaPaCa2, Panc1, and BxPC-3 cells were obtained from the American Type Culture Collection and maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen) supplemented with 10% FBS at 37°C with 5% CO_2. To avoid any supplementary stress, all media were preheated at 37°C before rinsing or changing media. Glucose starvation was obtained by cultivating cells with DMEM (no glucose) (Invitrogen, Ref# 11966) supplemented with 10% FBS. Hypoxia experiments were carried out using C-Shuttle Glove Box coupled hypoxia chamber (BioSpherix). Nupr1-deficient and Nupr1-restored mouse embryonic fibroblasts (MEF) were previously reported (13). N-acetyl-L-cysteine (NAC; Sigma–Aldrich) was used at 15 mmol/L.

Plasmid transfection

pCS2-Myc and pCS2-Myc-Aurka expression vectors were kindly given by Dr. Paolo Sassone-Corsi (Department of Biological Chemistry, University of California, Irvine, Irvine, CA; ref.14). MiaPaCa2 cells were seeded a day before transfection using Lipofectamine2000 reagent (Invitrogen), according to the manufacturer's protocol.

siRNA transfection

Cells were plated at 70% confluence in 100 mm dishes. Nupr1, Atg5, Beclin 1, and AURKA were knocked down using 140 ng of specific siRNA. Scrambled siRNA targeting no known gene sequence was used as negative control. INTERFErin reagent (POLYPLUS transfection) was used to conduct siRNA transfection according to the manufacturer's protocol. The sequences of the siRNA used are shown in Supplementary Table S1A.

DNA microarray

Total RNA (15 μg) was isolated and reverse transcribed for hybridization to the human oligonuleotide array U133 Plus 2.0 (Genechip, Affymetrix) as described previously (15). Arrays were processed using the Affymetrix GeneChip Fluidic Station 450 (protocol EukGE-WS2v5_450) and scanned using a GeneChip Scanner 3000 G7 (Affymetrix). The GeneChip Operating Software (Affymetrix GCOS v1.4) was used to obtain chip images with quality control conducted using the AffyQCReport software.

Cell viability and caspase-3/7 activity assay

Cells were seeded at a density of 15,000 cells per well in 6-well plates. Cells were allowed to attach overnight, RNA interference (RNAi) treated, and finally subjected to stress. At the end of the experiment, cells were harvested, and cell

viability was determined using a cell counter (Countess, Invitrogen). To conduct caspase-3/7 activity assay, cells were seeded on 96-well plates at a density of 7,000 cells per well. At the end of the experiment, cell number and caspase-3/7 activity were monitored on the same sample using CellTiter-Blue (Promega G8081) and Apo-ONE Caspase-3/7 assay (Promega G7790). Caspase-3/7 activity was estimated as the ratio Apo-ONE/CellTiter-Blue signals.

RNA extracts/real-time quantitative PCR

Cells were washed once with PBS and cell lysis was conducted using total RNA extraction kit (Norgen Biotek) according to the manufacturer's protocol. cDNA was obtained using the ImProm-II Reverse Transcription System (Promega). The sequences of the primers used to amplify human genes are shown in Supplementary Table S1B.

Immunoblotting

Protein extraction was conducted on ice using total protein extraction buffer as previously reported (9). Protein samples (80 μg) were denaturated at 95°C and subsequently separated by 12.5% SDS-PAGE. After transfer to nitrocellulose membrane and blocking with BSA 1%, samples were probed with LC3 rabbit polyclonal antibodies (Cell Signaling), p62 mouse monoclonal antibody (BD Bioscience), γ-H2AX and HIF1α (Bethyl), Aurora Kinase A (Abcam), and β-tubulin (Sigma) using SNAP i.d. protein detection system (Millipore).

Immunofluorescence

Cells cultured on glass coverslips were treated as previously described, fixed, permeabilized, and incubated with rabbit anti-γ-H2AX (Bethyl, A300-081A) followed by mouse Alexa Fluor antirabbit IgG 568 (Invitrogen Life Technologies) secondary antibody. Nuclei were stained using Prolong DAPI (Invitrogen Life Technologies). For counts of γ-H2AX foci, 4 independent cell fields with about 50 cells each were examined at × 20.

Patients and tissue microarray

PDAC samples were formalin-fixed surgical specimens obtained from the Pathology Department of the Marseille University (Marseille, France). A 2-year follow-up data was recorded from 34 patients. The procedure for construction of tissue microarrays (TMA) was as previously described (16, 17). The immunoperoxidase procedures were conducted using an automated Ventana Benchmark XT autostainer. Measurements of immunoprecipitates densitometry in cores were assessed for each marker in individual core after digitizalition and "cropping" of microscopic images as previously reported (16, 17).

Statistics

Statistical analyses were conducted using the Student *t* test. The density of the bands corresponding to p62, γ-H2AX, LC3-I, and LC3-II were measured with the Image J software (NIH, Bethesda, MD) and normalized against the density of the bands for β-tubulin. All values were expressed as mean ± SEM, with significance set at $P \leq 0.05$.

Results

Nupr1 is upregulated in human PDAC hypovascularized areas

Given the low vascularization of pancreatic cancer tissue, tumor cells are subjected to high metabolic stress consisting in a low nutrient and oxygen concentrations. We evaluated whether Nupr1 protein responds to metabolic stress *in vivo* by comparing its expression with the hypoxia-sensitive factor HIF1α in human PDAC samples using immunohistochemistry. As shown in Fig. 1A and B, Nupr1 protein is expressed in tumor lesions in which HIF1α is also expressed. These data support the hypothesis that Nupr1 expression is induced in PDAC cells subjected to metabolic stress (6).

We investigated the relationship between the expression levels of Nupr1 and HIF1α and the prognosis of patients with PDAC. Levels of both Nupr1 and HIF1α were assessed by immunohistochemistry on a TMA containing PDAC samples from 34 patients with a follow-up of 24 months. We found a coexpression of the 2 proteins with a significant correlation of the intensities of their expressions. Moreover, an inverse correlation was found between Nupr1 and HIF1α expression and patient's time of survival (Fig. 1C). Importantly, HIF1α accumulation in hypoxic conditions *in vitro* seems to be partially dependent on Nupr1 expression (Fig. 1D and data not shown). These data support the hypothesis by which metabolic stress–induced proteins facilitate the progression of PDAC and may constitute markers of poor prognosis.

Upregulation of Nupr1 is required to maintain the expression of DNA damage and cell cycle–related genes upon glucose starvation and hypoxia

In an effort to identify factors regulating the response to metabolic stress in pancreatic cancer cells, we evaluated the changes in gene expression occurring in conditions of hypoxia and glucose starvation. The expression of Nupr1 was dramatically increased upon glucose starvation and hypoxia. Nupr1 mRNA level increased after glucose starvation (23.4 ± 2.1 folds after 24 hours) and, to a lower extent, after prolonged hypoxia treatment (5.1 ± 1.4 folds after 24 hours). Combination of glucose starvation with hypoxia resulted in an increased expression of the Nupr1 transcript and protein similar to that observed with glucose starvation alone (21.5 ± 1.8 folds after 24 hours; Fig. 2A–C). Therefore, expression of Nupr1 is elevated in pancreatic cancer cells in response to the metabolic stresses generated by hypoxia and glucose starvation.

Nupr1, which shares homology with the HMG I/Y family of cotranscription factors, has been previously involved in the regulation of gene expression (18). To elucidate the consequences of Nupr1 upregulation by metabolic stress, we carried out a microarray profiling (Affymetrix) on MiaPaCa2 cells transfected with siCtrl or siNupr1 siRNAs and subjected to either severe hypoxia (0.2% oxygen

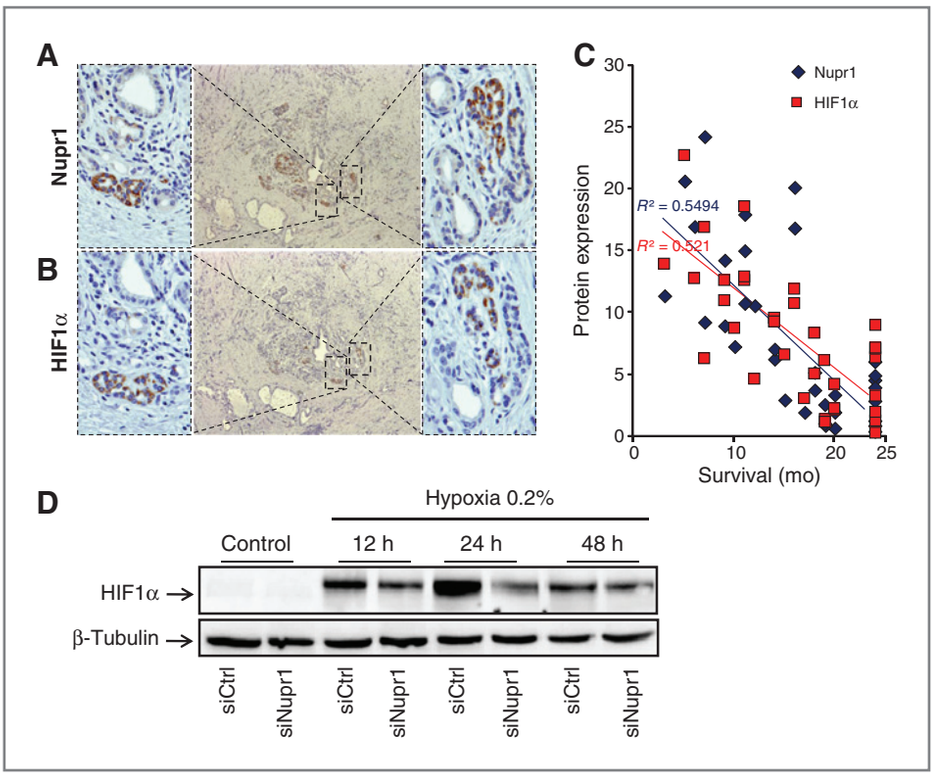

Figure 1. Nupr1 is expressed in PDAC cells subjected to metabolic stress. Human PDAC tissue sections were stained with anti-Nupr1 (A) and anti-HIF1α (B). Magnification is × 40. C, PDAC successive samples were stained with anti-Nupr1 and anti-HIF1α antibodies and their expression values plotted against survival time of 34 patients. D, HIF1α expression was assessed in MiaPaCa2 siNupr1- or siCtrl-transfected cells subjected to 12, 24, and 48 hours of hypoxia.

concentration) or glucose starvation, alone or in combination. In line with the known function of Nupr1 in the regulation of transcription, we found that under normal growth conditions, Nupr1-silencing affected the expression of several genes (see Table 1 and Supplementary Fig. S1), and that the expression of a subset of these genes was modified by hypoxia and by glucose starvation (Supplementary Fig. S1). We focused our attention on Nupr1-dependent genes, which are regulated by metabolic stress as candidates to mediate Nupr1 function in conditions of metabolic stress. Using the GO-ANOVA analysis from the Partek Genomics Suite (Partek GS; ref. 19), we found that these genes can be assigned to 2 functional families associated with DNA repair or cell cycle (Table 1, validation of the microarray data in Fig. 3A). Using the Ingenuity Systems Pathways Analysis (IPA) software (Ingenuity Systems), we represented in Supplementary Fig. S2 the DNA repair genes regulated by Nupr1. To functionally validate the role of Nupr1 in these processes, we assessed DNA repair by monitoring γ-H2AX activation levels and cell-cycle progression by flow cytometry analysis. Nupr1-silencing resulted in an increase of γ-H2AX protein (2.01 ± 0.6 fold), as estimated by Western blot analysis (Figs. 4A and 4B, line 1 and 2) or by the counting of γ-H2AX–positive nuclear dots (Figs. 4C and 4D, line 1 and 2). In addition, we also found that in Nupr1-depleted pancreatic cancer cells, reactive oxygen species were partially responsible for DNA damage, as treatment with the antioxidant NAC decreases the γ-H2AX protein as shown in Fig. 4E. Furthermore, γ-H2AX level was highly increased in $Nupr1^{KO}$-transformed MEFs subjected to glucose starvation or hypoxia. Restoration of Nupr1 expression in $Nupr1^{KO}$-transformed MEFs decreased γ-H2AX protein content under basal, glucose starvation, or hypoxia as shown by Western blot analysis and immunofluorescence (Supplementary Fig. S3). Nupr1-silencing also decreased percentage of cells in G_1 phase (24.93% ± 2.56% vs. 33.10% ± 3.67%) and a consistently lower percentage of cells in S-phase (26.86% ± 2.34% vs. 18.13% ± 1.78%; Fig. 4F). These data indicate that Nupr1 is involved in the transcriptional regulation of DNA repair and cell cycle in pancreatic cancer.

Nupr1 silencing sensitizes cells to death upon glucose starvation or hypoxia

Next, we tested whether Nupr1, through the regulation of the previously described cellular functions contributes to cell survival in conditions of metabolic stress. To this end, Nupr1 expression was silenced and cell survival was assessed 48 hours later in conditions of normoxia and hypoxia (0.2% oxygen concentration). As previously described, Nupr1 knockdown resulted in a slight but significant decrease of MiaPaCa2 cell survival (9.12%; ref. 11). Hypoxia and glucose starvation induced death in control siCtrl-transfected cells (14.45% and 20.10%, respectively; Fig. 5A). In contrast,

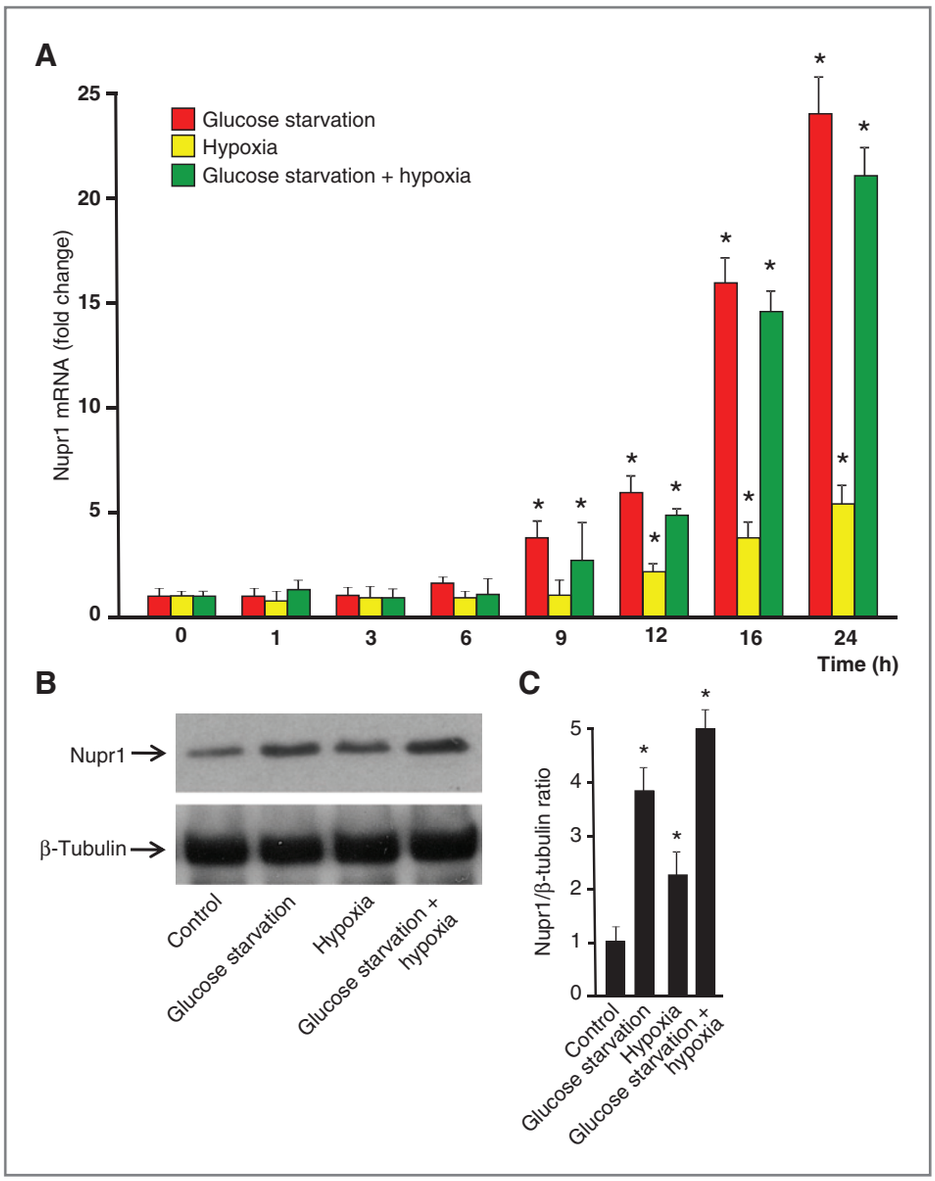

Figure 2. Nupr1 is upregulated upon hypoxia and glucose starvation. A, MiaPaCa2 cells were subjected to glucose starvation and hypoxia alone or in combination for different times. Total RNAs were extracted to monitor Nupr1 mRNA level using qRT-PCR. B, twenty-four hours after treatment, proteins were extracted and Nupr1 protein level was assessed by Western blot analysis, quantified, and expressed as Nupr1/β-tubulin ratio (C). Data are means of triplicates ± SEM. Statistically different from siCtrl untreated control (*, $P \leq 0.05$).

we found that 42.20% of the cells transfected with siNupr1 and exposed to hypoxia died, as shown in Fig. 5A. The cytoprotective effect of Nupr1 was also checked by analyzing the behavior of transformed MEFs from Nupr1-deficient mice in which Nupr1 deficiency had rescued by reintroducing human Nupr1 (13). Cells expressing Nupr1 were not sensitive to hypoxia, whereas 43% of Nupr1-deficient cells died, thus confirming that Nupr1 is involved in cell resistance to hypoxia-induced death, not only in pancreatic cancer cells but also probably in other cell types. Similarly, Nupr1 silencing increased sensitivity of MiaPaCa2 pancreatic cancer cells to death induced by glucose starvation (48.82% ± 3.40% vs. 78.99% ± 7.09% of living cells in siNupr1- and siCtrl-treated cells, respectively; Fig. 5A) and MEFs in which Nupr1 expression had been rescued were more resistant than control (83.10% ± 5.72% vs. 57.24% ± 3.80% of living cells; Fig. 5B). Finally, the same situation was observed in pancreatic cancer cell lines (75.88% ± 5.90% vs. 38.30% ± 3.48% of living cells; Fig. 5A) and in Nupr1^{KO} MEFs (66.44% ± 4.40% vs. 27.49% ± 3.80% of living cells; Fig. 5B) exposed to a combination of glucose deprivation and hypoxia. Similar data were obtained in 2 other pancreatic cancer cell lines named Panc-1 and BxPC-3 (Supplementary Figs. S4 and S5). Together, these results strongly indicate that Nupr1 protects cells from death induced by hypoxia or glucose starvation.

Nupr1 silencing increases cytotoxic autophagy elicited by hypoxia and glucose starvation

Autophagy is considered as a dual response leading either to cytoprotection or to cytotoxicity depending on the nature and the strength of the inducing stress. Nupr1 is involved in

Table 1. Nupr1-dependent genes in MiaPaCa2 cells under normal growth conditions

Gene symbol	Fold-change	Gene symbol	Fold-change	Gene symbol	Fold-change	Gene symbol	Fold-change	Gene symbol	Fold-change	Gene symbol	Fold-change	Gene symbol	Fold-change	Gene symbol	Fold-change	Gene symbol	Fold-change	Gene symbol	Fold-change	Gene symbol	Fold-change
FAM72D	6.11	*HIST1H2AB*	3.16	*TNS3*	2.63	*LRRC16A*	2.36	*VPS45*	2.164259744	*HFE*	2.00	*RCN2*	1.77	*HBEGF*	−8.51	*SLC16A6*	−2.97	*GMEB1*	−2.21	*AREG*	−1.89
FAM173B	5.96	*KIF23*	3.16	*BRCA1*	2.62	*BRCC3*	2.36	*GBP2*	2.161146252	*EXTL2*	2.00	*ADAM22*	1.77	*EPGN*	−7.41	*TSC22D2*	−2.93	*NT5DC3*	−2.20	*TMEM136*	−1.89
HIST1H3A	5.93	*PPWD1*	3.15	*GATSL1*	2.62	*SLC39A8*	2.35	*RAD51*	2.15	*MYD88*	1.99	*MYH10*	1.77	*ADM*	−7.31	*CXCL5*	−2.91	*SRF*	−2.19	*MAP1LC3B2*	−1.89
ZNF737	5.86	*ATR*	3.12	*GATSL1*	2.62	*LOC100133315*	2.35	*MCM10*	2.14	*API5*	1.99	*C14orf147*	1.76	*SIK1*	−6.59	*MMP10*	−2.86	*FHL2*	−2.19	*KIAA0284*	−1.86
GINS1	5.85	*SKA2*	3.09	*CCDC77*	2.61	*ZNF488*	2.34	*SIX4*	2.14	*RBMS1*	1.98	*HIST1H2AL*	1.76	*NDRG1*	−6.42	*C20orf111*	−2.84	*LHFPL2*	−2.19	*CHMP1B*	−1.85
ASPM	5.72	*LMAN2L*	3.09	*EME1*	2.60	*LRRC33*	2.33	*DAGLA*	2.13	*HIST1H2BB*	1.98	*PM20D2*	1.76	*UPP1*	−6.20	*TRIB3*	−2.82	*KLF6*	−2.18	*IRF2BP2*	−1.83
FAM72D	5.64	*SP110*	3.07	*MX2*	2.60	*LARS2*	2.32	*PLEKHM3*	2.13	*MMD*	1.98	*IL13RA1*	1.76	*HK2*	−6.06	*SNORD53*	−2.74	*PHLDA1*	−2.18	*ZFP36L1*	−1.83
TRERF1	5.62	*EPHA7*	3.06	*FBXO3*	2.60	*DENND5B*	2.32	*PHF15*	2.13	*NFIB*	1.97	*SPAG5*	1.75	*IL8*	−6.05	*CAMK2N1*	−2.68	*RAB11FIP5*	−2.17	*NCKIPSD*	−1.81
SLC2A12	5.36	*HLTF*	3.05	*KIF2C*	2.58	*PCYOX1*	2.32	*HNRNPA2B1*	2.13	*TMEM194A*	1.97	*CDCA3*	1.73	*ENO2*	−6.03	*SLC2A1*	−2.67	*TNIP1*	−2.17	*LOC100129112*	−1.78
AS3MT	5.27	*ALG8*	2.99	*RMI1*	2.58	*FAM54A*	2.32	*GTF3C2*	2.12	*SLC16A10*	1.96	*ACCN2*	1.73	*DDIT3*	−5.90	*TRIB1*	−2.66	*ZNF259*	−2.16	*LAMP3*	−1.78
HIST1H1B	5.08	*TCTN2*	2.99	*NAT11*	2.58	*PIK3R1*	2.31	*TMCO7*	2.12	*HIST1H2AI*	1.96	*NUP50*	1.73	*LIF*	−5.34	*C5orf26*	−2.66	*ELMOD1*	−2.16	*RNF19B*	−1.76
SAMHD1	4.85	*FAM120B*	2.95	*ALDH5A1*	2.56	*GTSE1*	2.31	*DKFZp434H1419*	2.12	*LRP8*	1.96	*NRSN2*	1.72	*PFKFB4*	−4.93	*UPRT*	−2.61	*ST6GALNAC6*	−2.15	*LOC100132426*	−1.74
HIST1H3J	4.81	*ESPL1*	2.93	*PMPCA*	2.56	*PTPRJ*	2.31	*HCN1*	2.12	*LPHN3*	1.95	*CCDC134*	1.71	*PIM1*	−4.89	*IRS2*	−2.60	*XBP1*	−2.13	*C3orf58*	−1.73
KIF18A	4.72	*FAM111A*	2.92	*ACAD10*	2.56	*CYB5B*	2.30	*ARMC7*	2.12	*SKP2*	1.95	*SIGMAR1*	1.71	*GADD45A*	−4.78	*LOC654433*	−2.59	*UCA1*	−2.13	*MAFG*	−1.73
FAM111B	4.57	*CEP97*	2.90	*UNQ3104*	2.55	*IFIT2*	2.29	*ETV6*	2.11	*BRI3BP*	1.94	*ACSS1*	1.71	*CD68*	−4.74	*TMEM158*	−2.58	*FAM115C*	−2.12	*CREB5*	−1.72
LBR	4.52	*KIFC1*	2.88	*KIFC1*	2.55	*SFRS1*	2.29	*COQ10A*	2.11	*CTPS2*	1.94	*CCNB2*	1.70	*C7orf68*	−4.73	*CCDC9*	−2.58	*RAB20*	−2.12	*RP5–1022P6.2*	−1.71
FAM72D	4.41	*ZC3HAV1*	2.88	*IGF1R*	2.54	*FAM114A1*	2.28	*CKAP2L*	2.11	*KANK2*	1.94	*MSH6*	1.70	*PPP1R15A*	−4.64	*PLK3*	−2.57	*AEN*	−2.10	*PRNP*	−1.71
CHD4	4.37	*RAB7L1*	2.87	*C3orf62*	2.54	*MKL2*	2.28	*PCTP*	2.10	*WDR76*	1.94	*CHUK*	1.69	*SAT1*	−4.56	*FUT11*	−2.57	*USP31*	−2.10	*UAP1*	−1.70
NAPEPLD	4.37	*NEK9*	2.87	*COL1A2*	2.54	*C20orf72*	2.28	*NSF*	2.10	*ST3GAL2*	1.91	*NUCKS1*	1.68	*SERPINE1*	−4.54	*CXCR4*	−2.56	*RAB32*	−2.09	*OSBPL6*	−1.67
E2F8	4.24	*C15orf42*	2.87	*MKI67*	2.53	*CDC25C*	2.28	*SHPK*	2.09	*C6orf167*	1.90	*CCNA2*	1.68	*FGF1*	−4.43	*RCL1*	−2.55	*NR1D2*	−2.08	*C9orf91*	−1.67
SHOX2	4.23	*CDCA2*	2.87	*ATAD5*	2.52	*HIST1H3C*	2.27	*PRND*	2.09	*TFAP2A*	1.90	*CACNA2D2*	1.68	*CYR61*	−4.37	*RNU11*	−2.55	*P4HA2*	−2.07	*MGLL*	−1.66
KIF20A	3.96	*GNE*	2.84	*POLE2*	2.52	*HIST1H3B*	2.27	*TNFAIP8L1*	2.07	*ZNF483*	1.90	*TARDBP*	1.67	*PDK1*	−4.30	*TP53BP2*	−2.54	*MYC*	−2.07	*TESK1*	−1.65
FAM72D	3.93	*PARP1*	2.84	*PHTF1*	2.51	*TP53BP1*	2.27	*HSPA2*	2.07	*BUB1*	1.89	*APOBEC3B*	1.67	*KLF4*	−4.28	*ABL2*	−2.49	*CITED2*	−2.07	*FAM162A*	−1.65
CDC23	3.85	*KIF11*	2.84	*C1orf83*	2.51	*SAFB*	2.26	*GCNT2*	2.07	*CASP2*	1.89	*ANK1*	1.67	*ALOXE3*	−4.22	*LOC642838*	−2.49	*ING2*	−2.06	*UBIAD1*	−1.60
RTN4IP1	3.85	*STIL*	2.83	*RBM14*	2.51	*MAT2A*	2.26	*ADCY9*	2.07	*SLC6A6*	1.89	*SYTL2*	1.66	*DUSP8*	−4.21	*LOC642838*	−2.49	*ATF3*	−2.06	*TRIM16*	−1.60
SLC25A12	3.81	*C2orf64*	2.81	*C2orf44*	2.50	*CNPY4*	2.26	*CERK*	2.06	*TUBGCP5*	1.89	*FCF1*	1.65	*SESN2*	−4.15	*C6orf145*	−2.45	*STX3*	−2.05	*NRBF2*	−1.56
PARP9	3.78	*ACTR3B*	2.80	*IP6K2*	2.50	*B3GAT3*	2.26	*KIAA0196*	2.05	*TFAP2A*	1.88	*FANCD2*	1.65	*SERTAD2*	−4.15	*C8orf58*	−2.44	*DNTTIP1*	−2.05	*SAMD4A*	−1.55
DEPDC6	3.59	*GCH1*	2.80	*LEPRE1*	2.49	*FUCA1*	2.26	*MTFMT*	2.05	*ATP8B1*	1.88	*HIST1H3F*	1.65	*MXD1*	−4.06	*USP36*	−2.42	*C19orf33*	−2.04	*ALDOC*	−1.54
C7orf69	3.57	*DSN1*	2.79	*IPP*	2.48	*C6orf203*	2.25	*TRAFD1*	2.05	*PPFIBP2*	1.88	*ASB9*	1.64	*RIMKLA*	−3.91	*MT1X*	−2.41	*RELB*	−2.03	*AKAP12*	−1.53
BUB1B	3.57	*RNFT2*	2.78	*FLVCR1*	2.47	*SPATS2L*	2.23	*AGRN*	2.04	*GRAMD3*	1.88	*WDR91*	1.64	*NGFR*	−3.84	*ITPR3*	−2.37	*PRAGMIN*	−2.03	*GABBR1*	−1.53
MUDENG	3.55	*BLM*	2.77	*TDRKH*	2.47	*NSL1*	2.23	*MEGF9*	2.04	*COL3A1*	1.88	*DDX46*	1.64	*KDM3A*	−3.82	*B3GNT5*	−2.37	*RUSC2*	−2.02		
ATPBD4	3.50	*CASC5*	2.75	*RFX5*	2.46	*UEVLD*	2.23	*TEP1*	2.03	*DNMT3B*	1.87	*HIST1H2AI*	1.64	*BTG1*	−3.71	*EGLN1*	−2.37	*TMEM167B*	−2.02		
FAM5C	3.45	*HIST1H2BM*	2.74	*ANKRD32*	2.46	*DHCR24*	2.23	*RILPL2*	2.03	*SPIN4*	1.87	*HNRNPM*	1.62	*HSN2*	−3.70	*PRELID2*	−2.37	*C5orf32*	−2.01		
VANGL1	3.44	*CSTF2T*	2.74	*ERMP1*	2.46	*RPA1*	2.21	*DPYSL5*	2.03	*NAAA*	1.87	*GK*	1.60	*GDF15*	−3.70	*MMP12*	−2.37	*UNC5B*	−2.01		
CROT	3.40	*TMPO*	2.71	*HIST1H3E*	2.46	*CARD8*	2.21	*ABCC5*	2.03	*HIST1H2AM*	1.87	*PLK1*	1.59	*SNORA8*	−3.53	*LOC388796*	−2.36	*RAB38*	−2.01		
ATP6V0A1	3.36	*ZNF512*	2.70	*HIST1H2AH*	2.44	*DHTKD1*	2.21	*PEX12*	2.03	*ANK3*	1.86	*C1orf112*	1.59	*NR1D1*	−3.52	*GAB2*	−2.36	*SH2D5*	−2.00		
ARHGAP11A	3.34	*SRGAP2*	2.70	*SGEF*	2.44	*CDCA8*	2.20	*SPG7*	2.03	*MYO18A*	1.86	*ANP32A*	1.59	*DUSP5*	−3.47	*RNF122*	−2.33	*PRDM4*	−1.99		
SPC25	3.34	*CCDC99*	2.70	*SPC24*	2.44	*MRS2*	2.20	*JKAMP*	2.03	*HIST2H3D*	1.86	*MAN2B2*	1.57	*FOXD1*	−3.42	*KAT5*	−2.32	*THG1L*	−1.98		
ZDHHC13	3.32	*C1orf124*	2.68	*OSGEPL1*	2.43	*NOX3*	2.20	*PRR12*	2.03	*GSTA4*	1.84	*PRKACA*	1.54	*ETS1*	−3.31	*LCE1F*	−2.31	*PEA15*	−1.97		
SUOX	3.27	*SYNE2*	2.66	*NEIL3*	2.42	*STK38*	2.20	*HIST2H3D*	2.03	*IQGAP2*	1.84	*PODNL1*	1.53	*SIPA1L2*	−3.30	*FOXO3*	−2.29	*TOLLIP*	−1.97		
HIST1H4L	3.26	*ZMYM3*	2.66	*KBTBD7*	2.41	*RIBC2*	2.20	*DIDO1*	2.02	*HIST1H2AJ*	1.84	*RFTN2*	1.53	*NFIL3*	−3.27	*CEBPB*	−2.28	*RAB39B*	−1.96		
HOXB5	3.26	*TMEM19*	2.65	*FCHSD2*	2.41	*MKKS*	2.20	*ABHD15*	2.02	*IGF2BP3*	1.83	*DPY19L2P2*	1.51	*PFKFB3*	−3.24	*FAM83G*	−2.27	*IL6R*	−1.95		
CCNF	3.26	*XRCC2*	2.65	*SHROOM3*	2.40	*CNIH2*	2.18	*HJURP*	2.02	*MAGI1*	1.81	*PER3*	1.51	*AVPI1*	−3.23	*GPR56*	−2.25	*ELL2*	−1.95		
CENPI	3.24	*SLC25A20*	2.64	*EXO1*	2.40	*HIST2H3A*	2.18	*ANP32E*	2.01	*ANGEL1*	1.81			*ZSWIM6*	−3.18	*C9orf30*	−2.25	*FZD8*	−1.94		
LOC100289612	3.23	*POLA2*	2.63	*DGCR8*	2.39	*C16orf53*	2.18	*GPSM2*	2.01	*LOC440243*	1.80			*JOSD1*	−3.18	*RASAL2*	−2.24	*ZBTB34*	−1.92		
ERCC6L	3.23	*POLH*	2.63	*CHTF8*	2.38	*PFTK2*	2.18	*BCL9*	2.01	*LOC440243*	1.80			*CTU1*	−3.17	*EREG*	−2.23	*GPR1*	−1.92		
DLG1	3.18	*POLQ*	2.63	*CENPC1*	2.38	*HDAC8*	2.17	*CENPL*	2.01	*CXADR*	1.79			*LIF*	−3.14	*CXCL3*	−2.22	*ZNF296*	−1.92		
AURKA	3.17	*LMNB1*	2.63	*SHCBP1*	2.38	*TRERF1*	2.17	*SERF1A*	2.00	*HIST1H4A*	1.79			*RYBP*	−3.07	*ISCA1*	−2.22	*ADRB2*	−1.91		
ETV1	3.17	*TOB1*	2.63	*HIVEP3*	2.37	*RANBP6*	2.16	*SERF1A*	2.00	*RAB17*	1.78			*ZFAND2A*	−3.02	*SNORA5A*	−2.21	*C19orf50*	−1.90		

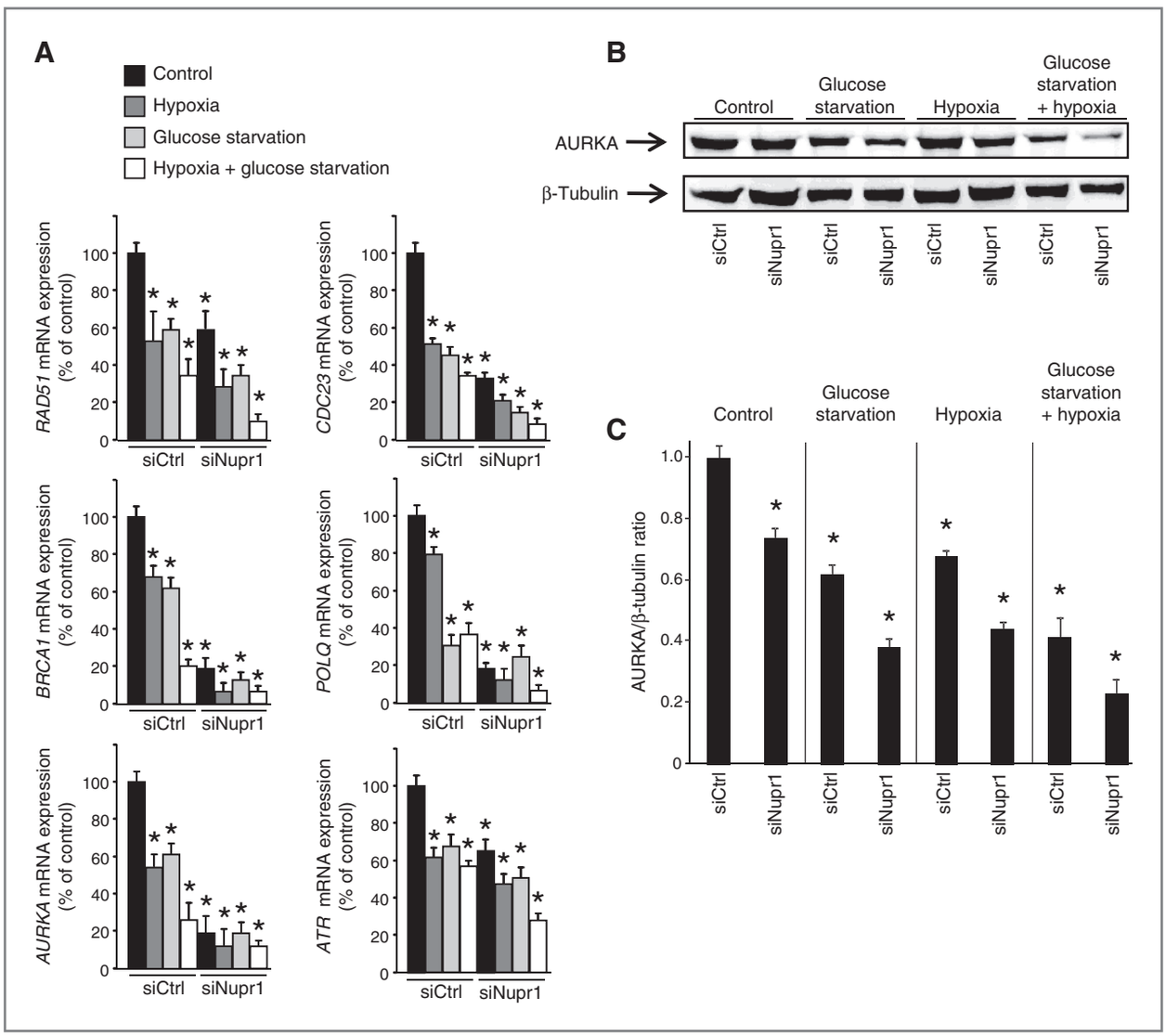

Figure 3. Expression of DNA repair- and cell cycle–associated transcripts upon hypoxia and glucose starvation. A, expression of *RAD51*, *BRCA1*, *CDC23*, *POLQ*, *ATR*, and *AURKA* was measured by qRT-PCR on MiaPaCa2 cells subjected to 24 hours of hypoxia or glucose starvation alone or together in siCtrl- or siNupr1-transfected conditions. B, MiaPaCa2 cells were subjected to 24 hours of hypoxia or glucose starvation alone or together in siCtrl- or siNupr1-transfected conditions; AURKA protein level was assessed by Western blot analysis on total protein extracts. C, AURKA protein level quantifications. Data are means of triplicate ± SEM (*, $P \leq 0.05$).

autophagy (20–22), and its expression is activated in response to metabolic stress. In addition, glucose starvation and hypoxia can lead to autophagy. The hypothesis that Nupr1 regulates autophagy in response to such metabolic stress was tested by using complementary approaches. We evaluated by Western blotting the LC3-II/LC3-I ratio in MiaPaCa2 cells, as this ratio is a specific indicator of autophagy level (23). As shown in Fig. 6A–C, lipidated LC3 (LC3-II) concentration increased much more than that of LC3-I upon metabolic stress, an effect dramatically enhanced when Nupr1 was silenced. We also monitored the amounts of the mammalian protein p62/SQSTM1, which is selectively degraded by autophagy (24). In agreement with LC3 lipidation data, Western blot analysis showed that p62 was clearly and significantly degraded in siCtrl-transfected cells in response to metabolic stress (Fig. 6), and that this phenomenon was enhanced upon Nupr1 silencing. Similar results were obtained in Panc-1 or BxPC3 cells and when using another Nupr1-specific siRNA (Supplementary Figs. S4–S6). Altogether, these results show that Nupr1 functions as a promoter of cell survival in conditions of metabolic stress through the inhibition of autophagy.

Nupr1 protects from cell death by inhibiting autophagy in a caspase-independent manner

To evaluate whether autophagy plays a cytoprotective or cytotoxic role in these settings, we genetically inhibited autophagosome formation, through siRNA targeting of key

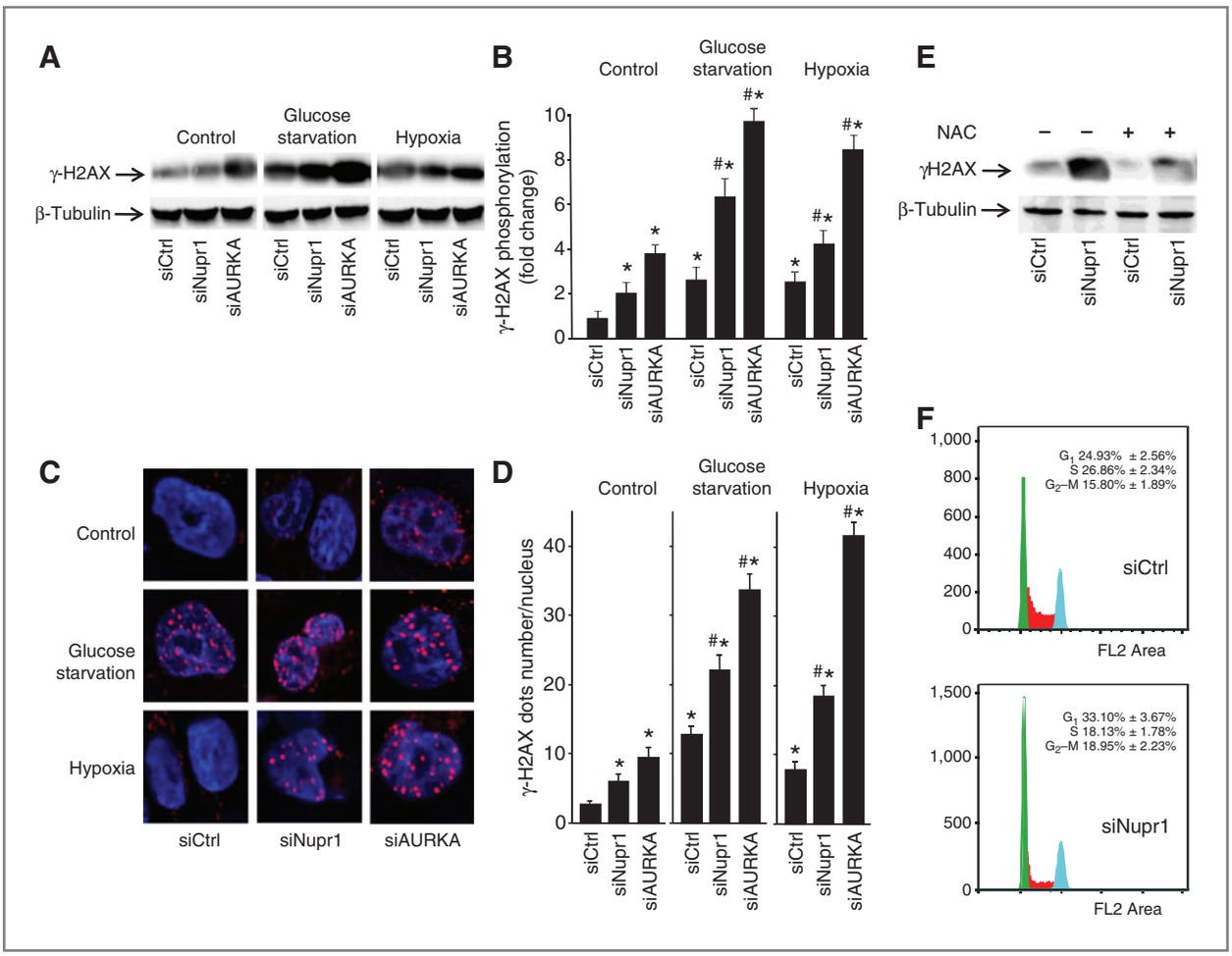

Figure 4. Nupr1 is required to maintain DNA repair activity in response to hypoxia and glucose starvation. A, γ-H2AX immunoblot after glucose starvation or hypoxia in MiaPaCa2 cells treated with siCtrl, siNupr1, or siAURKA. B, γ-H2AX level was quantified and expressed as γ-H2AX/β-tubulin ratio. C, γ-H2AX immunofluorescence. MiaPaCa2 cells were plated on coverslips, Nupr1 or *AURKA* expression was silenced using specific siRNAs and subjected to glucose starvation or hypoxia for 24 hours. The rabbit γ-H2AX was revealed using Alexa fluor antirabbit IgG568. D, γ-H2AX foci quantification. Four independent cell fields of approximately equal to 50 cells each were examined at × 20 and expressed as γ-H2AX foci dots per nucleus. E, γ-H2AX immunoblot in MiaPaCa2 cells pretreated or not with 15 mmol/L NAC for 24 hours and then transfected with siNupr1. Total proteins were extracted 24 hours later to conduct γ-H2AX immunoblot. F, MiaPaCa2 cells were plated in DMEM–10% FBS, Nupr1 expression was silenced using a specific siRNA. Forty-eight hours later, cells were harvested, fixed with ethanol, and stained with propidium iodure. Cell cycle was analyzed using FACSCalibur flow cytometer (BD Bioscience). Data are means of triplicates ± SEM. Statistically different from siCtrl-untreated control (*, $P \leq 0.05$) or siCtrl-treated cells (#, $P \leq 0.05$).

autophagic genes, such as *Atg5* and *Beclin1*, concomitantly with Nupr1 silencing. As shown in Fig. 5A, siBeclin1 and siAtg5 treatments rescued the viability defect of the Nupr1-silenced cells in response to both hypoxia and glucose starvation, alone or combined. Then, we evaluated the role of caspases in cell death upon severe hypoxia and glucose starvation by pharmacologic inhibition with the pan-caspase inhibitor Z-VAD. To our surprise, caspase inhibition did not significantly influence cell survival in response to either metabolic stress and was partially inhibitory of cell death induced by siNupr1 transfection (Fig. 5A). Similar results were obtained using Panc-1 or BxPC-3 cells and when using another Nupr1-specific siRNA (Supplementary Figs. S4–S6). Caspase-3/7 activity in MiaPaCa2-treated cells was presented in Fig. 7. Taken together, these results show that Nupr1, when induced in response to hypoxia and glucose starvation, protects pancreatic cancer cells from autophagic cell death independently of caspase activation.

Nupr1-regulated AURKA mediates the inhibition of the DNA damage and autophagic-cell death

Next, we aimed at elucidating the cytoprotective mechanism of Nupr1 in conditions of metabolic stress. We focused our attention on Aurora kinase A because this is one of the genes in which expression is regulated by metabolic stress, and it is particularly sensitive to Nurp1. *AURKA* links DNA damage with cell death by autophagy, in a caspase-insensitive manner (25–27; Table 1; and Fig. 3). Indeed, *AURKA* gene expression and protein level were downregulated in conditions of metabolic stress and

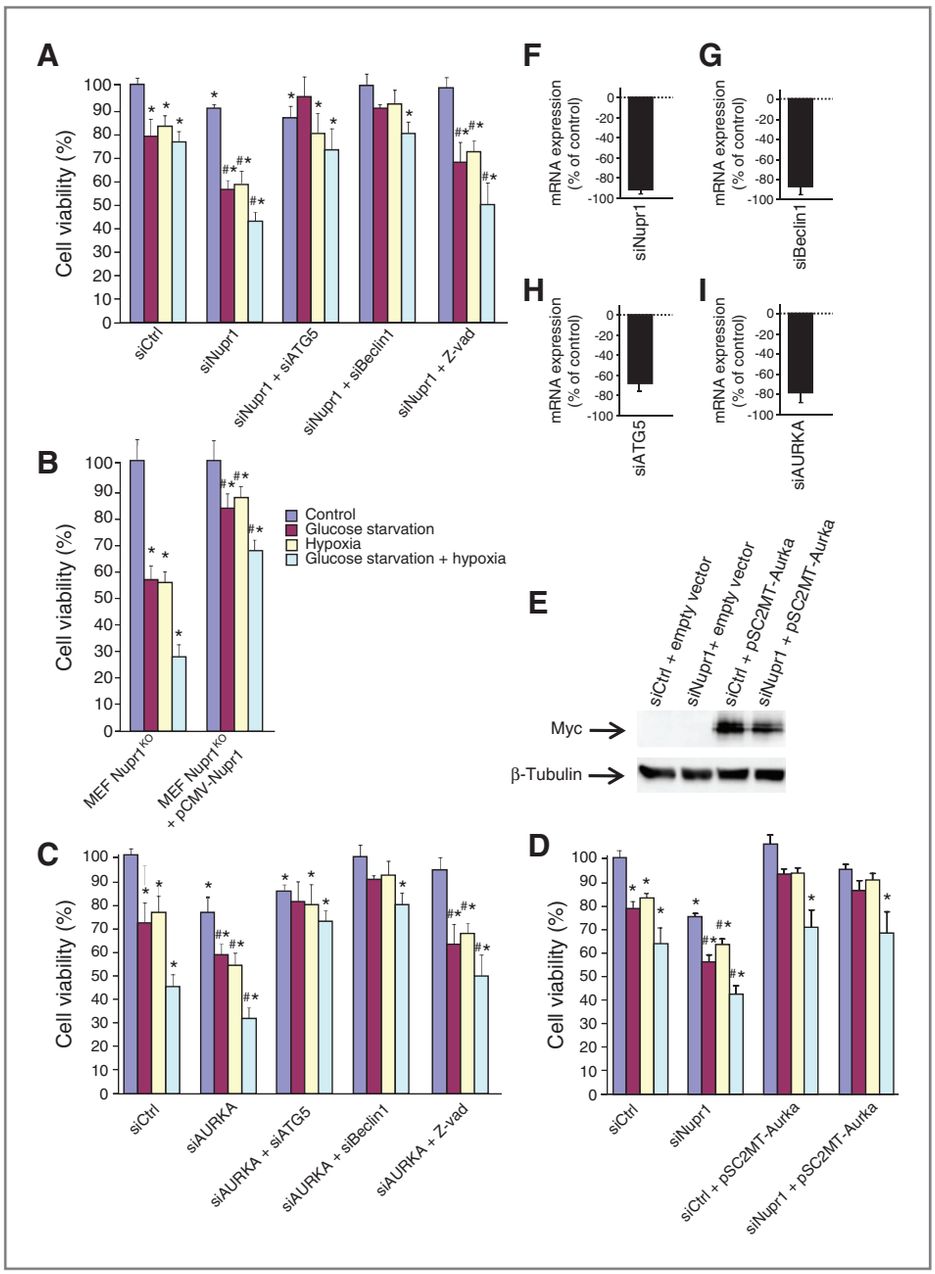

Figure 5. Nupr1 silencing sensitizes to cell death upon glucose starvation or hypoxia. A, MiaPaCa2 cells were transfected with siNupr1 alone or in combination with siATG5 or siBeclin1. Then, cells were subjected to glucose starvation or hypoxia alone or together for 48 hours. Z-VAD-FMK caspase inhibitor was added to the medium at 30 nmol/L. Cell survival analysis was conducted on 3 independent samples for each condition. B, $Ras^{V12/E1A}$-transformed $Nupr1^{KO}$ and $Nupr1^{KO}$+ pCMV-Nupr1 MEFs were subjected to glucose starvation and hypoxia alone or together for 48 hours. Cell survival was monitored as described earlier. C, MiaPaCa2 cells were transfected with siAURKA alone or together with siATG5 or siBeclin1. Cells were subjected to glucose starvation or hypoxia alone or together for 48 hours. Caspase inhibition was conducted as described before. D, MiaPaCa2 cells were transfected with siNupr1 or siCtrl alone or in combination with *AURKA* overexpression using pSC2–Myc–AURKA as vector. Cells were then subjected to glucose starvation, hypoxia, or both together. E, *AURKA* overexpression conducted in (D) was verified by Western blot analysis using anti-Myc Tag antibody. F–I, MiaPaCa2 cells were transfected with siNupr1, siBeclin1, siATG5, or siAURKA and expression of their targets was monitored using qRT-PCR. Data are means of triplicates ± SEM. Statistically different from siCtrl-untreated control (*, $P \leq 0.05$) or siCtrl-treated cells (#, $P \leq 0.05$).

dramatically dropped further when Nupr1 upregulation was prevented (Fig. 3B and C, Supplementary Fig. S1). These results indicate that, in pancreatic cancer cells, Nupr1 upregulation upon stress buffers the repression of *AURKA* in response to metabolic stress.

To evaluate the contribution of *AURKA* to the cytoprotective activity of Nupr1, we silenced *AURKA* in pancreatic cancer cells and subjected them to metabolic stress. siAURKA transfection sensitized MiaPaCa2 (Fig. 4), Panc1 cells (Supplementary Fig. S4C), and BxPC-3 cells (Supplementary Fig. S5C) to DNA damage (measured by γ-H2AX activation), in basal conditions (Fig. 4B, line 3) as well as under metabolic stress (Fig. 4B, lines 6 and 9). These data indicate that *AURKA* plays an important role of protection against DNA damage induced by hypoxia and glucose starvation in pancreatic cancer cells. On the other hand, *AURKA*-depletion using 2 siRNA sequences resulted in the amplification of the autophagic response to both hypoxia and glucose starvation, as measured by both LC3-II/LC3-I ratio and p62/SQSTM1 accumulation (Fig. 6D–F and Supplementary Fig. S6C). Furthermore, *AURKA* overexpression protected Nupr1-depleted MiaPaCa2 cells against metabolic stress (Fig. 5D and E). Finally, enhanced cytotoxicity upon *AURKA*-silencing in metabolically challenged cells was

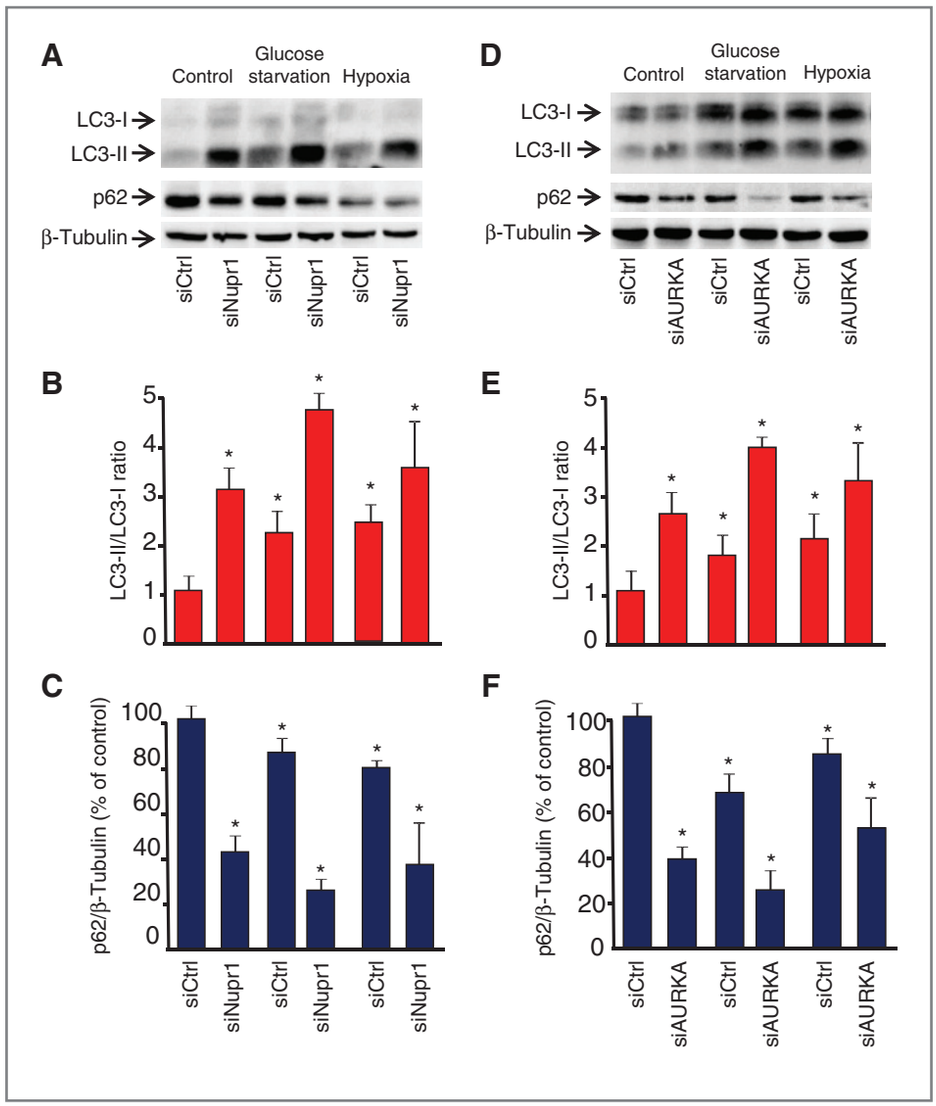

Figure 6. Nupr1 and AURKA inhibit autophagy induced by glucose starvation or hypoxia. A, MiaPaCa2 cells were transfected with siNupr1 and subjected to glucose starvation or hypoxia for 24 hours. LC3, p62, and β-tubulin amounts were measured by Western blot analysis and expressed as LC3 II/LC3 I (B) and p62/β-tubulin ratio (C). D, MiaPaCa2 cells were transfected with siAURKA and subjected to glucose starvation or hypoxia for 24 hours. LC3, p62, and β-tubulin amounts were measured and expressed as described earlier (E and F). Data are means of triplicates ± SEM. Statistically different from siCtrl-untreated control (*, $P \leq 0.05$).

Z-VAD–insensitive but Atg5- and Beclin1-dependent, similar to the observations in siNupr1-treated pancreatic cancer cells (Fig. 5C and Supplementary Figs. S4E, S5E, and S6D). Altogether, these results indicate that in response to hypoxia and glucose starvation, *AURKA* expression downstream from Nupr1 limits DNA damage and autophagic cell death.

Discussion

PDAC is characterized by hypovascularization (4), which induces in PDAC cells a permanent stress response with activation of Nupr1 expression. Nupr1 is a stress-induced transcription factor systematically overexpressed in cancer and associated with bad prognosis (refs. 7, 28–30 and Fig. 1C). Several functions, including cell transformation (6), inhibition of apoptosis (11), regulation of migration (9), and promotion of resistance to antitumoral treatments (10) have been attributed to Nupr1, all of them pointing at a protumoral activity. In this study, we report a novel role for Nupr1 in the cell response to metabolic stress, which is to favor cell survival by inhibiting autophagic cell death through *AURKA* expression.

Autophagy is a finely controlled process in which whole regions of the cytoplasm, including organelles, become sequestered into double membrane structures that eventually fuse with lysosomes, allowing digestion of the content by acid hydrolases. Many molecules that mediate autophagy have been identified and extensively characterized (31, 32). The prosurvival function of autophagy is an evolutionarily ancient process, conserved from yeast to mammals and well characterized in conditions of nutrient deficiency. Degradation of membrane lipids and proteins by the autolysosome generates free fatty acids and amino acids that can be reused to fuel mitochondrial ATP production and to sustain protein synthesis. Presumably, this recycling function of autophagy is linked mechanistically to its ability to sustain life during starvation. However, autophagy is also

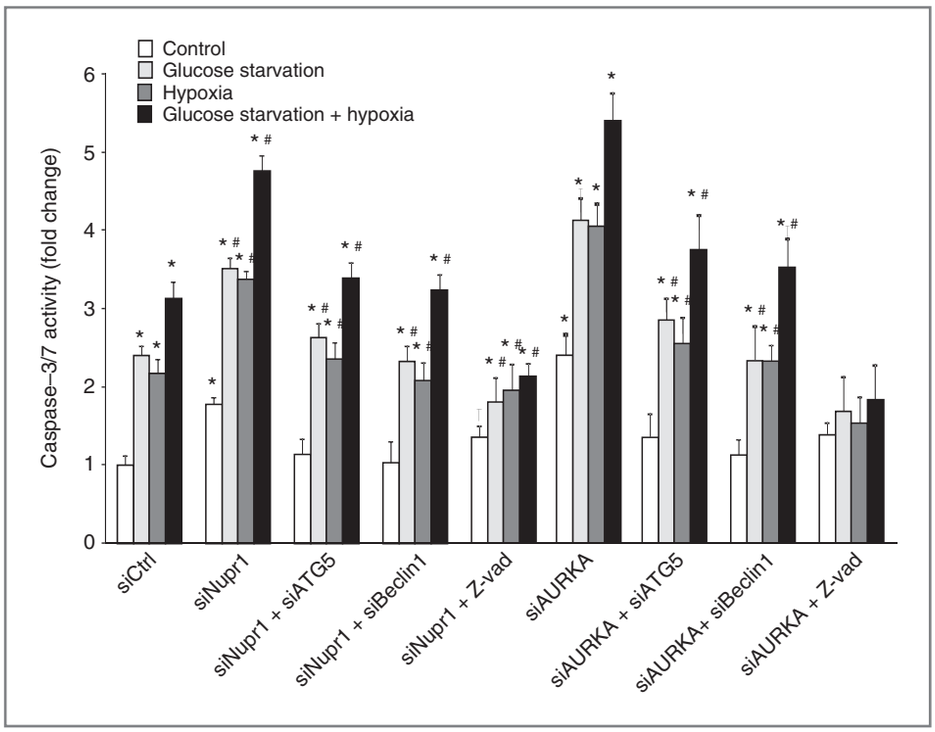

Figure 7. Nupr1 protects from cell death by inhibiting autophagy in a caspase-independent manner. MiaPaCa2 cells were transfected with siNupr1 and siAURKA alone or in combination with siATG5 or siBeclin1. Then, cells were subjected to glucose starvation or hypoxia alone or together for 48 hours. Z-VAD-FMK caspase inhibitor was added to the medium at 30 nmol/L. Caspase-3/7 activity was monitored using CellTiter-Blu/Apo-ONE as described in Materials and Methods. Data are means of triplicates ± SEM. Statistically different from siCtrl-untreated control (*, $P \leq 0.05$) or siCtrl-treated cells (#, $P \leq 0.05$).

observed in dying cells (33–36). In classical apoptosis, or type I programmed cell death, there is an early collapse of cytoskeletal elements but preservation of organelles until late in the process. In contrast, in autophagic, or type II, programmed cell death, there is early degradation of organelles but preservation of cytoskeletal elements until late stages. Recently, several studies have described autophagic cell death in compromised mammalian tissues and in tumor cell lines treated with chemotherapeutic agents. Moreover, some data suggest that pharmacologic or genetic inhibition of autophagy can prevent cell death. The pharmacologic inhibition of class III phosphoinositide 3-kinase (PI3K) activity by 3-methyladenine delays or partially inhibits cell death in several cell types. Furthermore, RNAi directed against ATG7 and Beclin1 prevent cell death in cells treated with the caspase inhibitor Z-VAD (37), and RNAis against ATG5 and Beclin1 block death in $Bak^{-/-}$ MEFs treated with staurosporine (38). Therefore, autophagy may mediate both cell survival and cell death (39).

In previous studies, we have shown that Nupr1 expression represses FoxO3 transcriptional activity by inhibiting its nuclear localization in cardiomyocytes. As a consequence, Nupr1 decreases FoxO3 binding to the BNIP3 promoter, a known proautophagic FoxO3 target, resulting in lower BNIP3 mRNA and protein levels (20) and decreasing autophagy. However, in the present work, we did not find alterations in BNIP3 levels after knocking down Nupr1, or after hypoxia and glucose starvation (data not shown), suggesting that a different mechanism is involved. Another important point is the fact that Nupr1 acute upregulation mediates the proautophagic effect of THC (δ(9)-tetrahydrocannabinol) in glioma and pancreatic cancer cells (21, 40, 41). Together, these observations suggest that Nupr1 may act as a pro- or antiautophagic factor depending of the context.

After silencing Nupr1 expression or exposing pancreatic cancer cells to hypoxia or glucose starvation, several genes involved in DNA repair and cell cycle were downregulated (Table 1 and Fig. 3). As a result, DNA is damaged and cells die (Fig. 5 and Supplementary Figs. S4 and S5). These effects were more intense when hypoxia or glucose starvation was combined with siNupr1 treatment indicating that Nupr1 expression is activated in pancreatic cancer cells, as part of the response against metabolic stress. Among genes, in which expression is both downregulated in response to metabolic stress and under the control of Nupr1, *AUR*KA was one of the most interesting, as its downregulation is involved in the response to DNA damage (42), and as its downregulation induces autophagy and cell death (43). Indeed, upon *AURKA* downregulation, spindle dysfunction and destruction of the G_2–M phase checkpoint results in DNA damage and cell death. Also, transcription of genes encoding mitotic regulators, including *AURKA*, is rapidly turned off following DNA damage, resulting in the blockade of mitosis (44). In fact, downregulation of *AURKA* and DNA damage generate a positive feedback in which downregulation of *AURKA* expression facilitates DNA damage and, in turn, DNA damage induces downregulation of *AURKA* expression. Moreover, analysis of *AURKA* function in HeLa cells revealed that depletion of *AURKA* by siRNA blocks almost complete entry into mitosis (45). Taken together, these findings pointed at a protumoral function of *AURKA*

when overexpressed and, in consequence, at a potential efficacy of inhibitors of *AURKA* to treat cancers. Such rationale was based on its effect on mitosis (46, 47), but results from this study provide an additional reason for its targeting. We suggest that inhibition of *AURKA* will sensitize preferentially intrapancreatic tumor cells located near hypovascularized regions, which should correspond to the most resistant cells because they have been selected after exposure to an adverse microenvironment.

Autophagy seems to be required for pancreatic cancer development (48), although in other conditions, it can also oppose cancer progression (49, 50). Therefore, autophagic process in cancer has different effects on cell fate depending on the cellular type and the nature of the induction. To clarify that situation, we analyzed the occurrence of autophagy in PDAC, taking advantage of the peculiarity of PDAC cells to be submitted to strong hypoxia and glucose starvation. Indeed, both stresses induce cell death, in part, through the induction of DNA damage (Fig. 4 and Supplementary Figs. S4 and S5) as previously reported (51, 52). In that context, we observed that knocking down Nupr1 expression increases cell death, induces DNA damage, and alters the expression of several genes involved in DNA repair, some of them being regulated by hypoxia and by glucose starvation. The molecular mechanism by which hypoxia and glucose starvation induces DNA damage, autophagy, and as a consequence cell death, is unknown. According to our results, Nupr1 and *AURKA* are major players in this response, although other factors might be involved (53, 54). In addition, knocking down Nupr1 results, to a lower extent, in a downregulation of genes involved in DNA repair and cell-cycle regulation. These data suggest that Nupr1 participates in a feedback mechanism, its activation by hypoxia and glucose starvation counteracting other transcriptional changes occurring in response to hypoxia and glucose starvation. Hence, Nupr1 belongs to a defensive pathway that promotes pancreatic cancer survival in conditions of metabolic stress.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: T. Hamidi, J.-C. Dagorn, R. Urrutia, J.L. Iovanna
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Hamidi, C.E. Cano, M.J. Sandi, E.L. Calvo
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.E. Cano, D. Grasso, M.N. Garcia, M.J. Sandi, E.L. Calvo, J.-C. Dagorn, R. Urrutia, J.L. Iovanna
Writing, review, and/or revision of the manuscript: T. Hamidi, C.E. Cano, D. Grasso, M.N. Garcia, M.J. Sandi, E.L. Calvo, J.-C. Dagorn, G. Lomberk, R. Urrutia, S. Goruppi, A. Carracedo, G. Velasco, J.L. Iovanna
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Hamidi, A. Carracedo, J.L. Iovanna
Study supervision: J.L. Iovanna

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