Expression of a Truncated Active Form of VDAC1 in Lung Cancer Associates with Hypoxic Cell Survival and Correlates with Progression to Chemotherapy Resistance

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Abstract

Resistance to chemotherapy-induced apoptosis of tumor cells represents a major hurdle to efficient cancer therapy. Although resistance is a characteristic of tumor cells that evolve in a low oxygen environment (hypoxia), the mechanisms involved remain elusive. We observed that mitochondria of certain hypoxic cells take on an enlarged appearance with reorganized cristae. In these cells, we found that a major mitochondrial protein regulating metabolism and apoptosis, the voltage-dependent anion channel 1 (VDAC1), was linked to chemoresistance when in a truncated (VDAC1-ΔC) but active form. The formation of truncated VDAC1, which had a similar channel activity and voltage dependency as full-length, was hypoxia-inducible factor-1 (HIF-1)-dependent and could be inhibited in the presence of the tetracycline antibiotics doxycycline and minocycline, known inhibitors of metalloproteases. Its formation was also reversible upon cell reoxygenation and associated with cell survival through binding to the antiapoptotic protein hexokinase. Hypoxic cells containing VDAC1-ΔC were less sensitive to staurosporine- and etoposide-induced cell death, and silencing of VDAC1-ΔC or treatment with the tetracycline antibiotics restored sensitivity. Clinically, VDAC1-ΔC was detected in tumor tissues of patients with lung adenocarcinomas and was found more frequently in large and late-stage tumors. Together, our findings show that via induction of VDAC1-ΔC, HIF-1 confers selective protection from apoptosis that allows maintenance of ATP and cell survival in hypoxia. VDAC1-ΔC may also hold promise as a biomarker for tumor progression in chemotherapy-resistant patients. Cancer Res; 72(8); 2140–50. ©2012 AACR.

Introduction

It is well established that cells exposed to the limiting oxygen microenvironment (hypoxia) of tumors acquire resistance to chemotherapy-induced apoptosis (1). However, the mechanisms involved and the implication of the key factor of the hypoxic response, the hypoxia-inducible factor (HIF), have not been extensively investigated (2). We recently reported that several types of cancer cells exposed to a hypoxic microenvironment showed enlarged mitochondria with reorganized cristae; a result of modifications to fusion/fission (3). In addition, we showed that these cells were resistant to chemotherapy-induced apoptosis.

Because mitochondria regulate both metabolism and apoptosis (4–6) and that fusion/fission participates in apoptosis (7), we investigated whether certain mitochondrial proteins implicated in these processes play a role in resistance to apoptosis in a HIF-dependent manner.

The voltage-dependent anion channel (VDAC) regulates mitochondrial import and export of Ca2+ and metabolites including ATP and NADH and interacts with antiapoptotic proteins such as Bcl-2 and hexokinase in controlling the release of cytochrome c (8–11). Of note, screening by RNA interference identified VDAC1 as a protein implicated in resistance to cisplatin-induced cell death (12). In mammals VDAC is present in 3 highly homologous isoforms: VDAC1, VDAC2, and VDAC3, and VDAC1 is composed of 19 amphipathic β strands that form a β barrel and of a mobile N-terminal α helix, located inside the pore (13). Through binding to VDAC1 hexokinase, the enzyme that catalyzes the first step of glycolysis is optimally positioned for ATP capture (14) and hexokinase expression is increase by HIF (15). Thus, these interactions influence the function of...
both hexokinase and VDAC in cell death and metabolism. However, the role of VDAC in metabolism and apoptosis in hypoxia is not known.

Materials and Methods

Cell culture
LS174, PC3, HeLa, 786-O, SKMel, and A549 cells were grown in Dulbecco’s Modified Eagle’s Medium (Gibco-BRL) supplemented with 5% or 10% inactivated FBS as appropriate in penicillin G (50 U/mL) and streptomycin sulfate (50 µg/mL). Dr. van de Wetering provided LS174 cells expressing the tetracycline repressor. A Bug-Box anaerobic workstation (Ruskind Technology Biotrace International Plc.) set at 1% oxygen, 94% nitrogen, and 5% carbon dioxide was used for hypoxia.

Transfection of short interfering RNA
The 21-nucleotide RNAs were synthesized (Eurogentec). siRNA sequences targeting SIMA (siCIL), and HIF-1α were described previously (16). The short interfering RNA (siRNA) sequences targeting human VDAC1, VDAC2, VDAC3, and hexokinase II are given in the Supplementary Materials and Methods. HeLa cells were transfected with 40 nmol/L of siRNA 24 hours before normoxia or hypoxia, as described (3).

Reconstitution of purified VDAC1 and VDAC1−ΔC into a planar lipid bilayer, single-channel current recording and data analysis

VDAC1 and VDAC1−ΔC were purified from hypoxic HeLa cells after solubilizing in dodecylmethylamine-octide (DDAO) and chromatography on hydroxypatite, as described (17). Elution with increasing Pi concentrations separated VDAC1 and VDAC1−ΔC. The fractions containing either VDAC1 or VDAC1−ΔC were used for channel reconstitution into a planar lipid bilayer (PLB). A PLB was prepared from soybean asolectin dissolved in n-decane (50 mg/mL) and purified VDAC1 or VDAC1−ΔC was added to the cis chamber containing 1 mol/L NaCl and 10 m mol/L HEPES, pH 7.4, unless otherwise indicated. After one or a few channels were inserted into the PLB, currents were recorded by voltage clamping with a Bilayer Clamp BC-525B Amplifier (Warner Instruments), the current trace duration was 4 or 10 seconds. Current was measured with respect to the trans side of the membrane (ground). The current was digitized on-line with a Digidata 1200 interface board and PCLAMP 6 software (Axon Instruments, Inc.).

Patients and tissue sample preparation

Forty-four patients who underwent surgery for lung adenocarcinoma between May 2007 and May 2010 at the Pasteur Hospital (Department of Thoracic Surgery, CHU de Nice, Nice, France) were selected. The patients received the necessary information concerning the study and consent was obtained. They obtained approval of the ethics committee (CHU de Nice). The main clinical and histopathologic data are summarized in Supplementary Table S1. Morphologic classification of the tumors was assigned according to the World Health Organization (WHO) criteria (18). The tumors were staged according to the international tumor-node-metastasis system (19). Follow-up data for all the patients were collected regularly. The median follow-up was 21 months (3.8–38.2 months). Among these patients, 13 relapsed (29.5%) and 6 (13.6%) died. Protein and miRNA were extracted from the same tissue sample using the protocol AllPrep DNA/RNA/Protein from QIAGEN.

Statistics

All values are the means ± SD of the indicated number of determinations (n), and significant differences are based on the Student t test and P values indicated. All categorical data used numbers and percentages. Quantitative data were presented using the median and range or mean. Differences between groups were evaluated using the χ2 test for categorical variables and the Student t test for continuous variables. SPSS 16.0 statistical software (SPSS Inc.) was used. All statistical tests were 2-sided, and P values less than 0.05 indicated statistical significance whereas P values between 0.05 and 0.10 indicated a statistical tendency.

Results

Hypoxic cells with enlarged mitochondria are resistant to chemotherapy and resistance implicates mitochondrial proteins

We reported that certain tumor-derived cell lines exposed to hypoxia showed a tubular mitochondrial network (PC3, SKMel) whereas others showed enlarged mitochondria (LS174, HeLa, A549; ref. 3). All cells showed a mitochondrial transmembrane potential (ΔΨm) that was unchanged compared with normoxic cells but the latter group was resistant to staurosporine (STS)-induced apoptosis. We now show that when hypoxic LS174 cells with enlarged mitochondria were treated with STS, the ΔΨm decreased in normoxia but remained unaffected in hypoxia (Fig. 1A). In normoxia, LS174 cells released mitochondrial cytochrome c when incubated with STS whereas hypoxic LS174 cells with enlarged mitochondria did not (Fig. 1B). To address the implication of antiapoptotic proteins of the Bcl-2 family in hypoxic resistance to STS-induced apoptosis, we tested the effect of the BH3 domain mimetic ABT-737, an inhibitor of Bcl-2 and Bcl-XL (20) on the apoptosis resistance of hypoxic cells. ABT-737 restored apoptosis as induced by STS in hypoxic LS174 cells (Fig. 1C), suggesting that association with a BH3 domain protein is implicated in resistance.

To better understand the molecular mechanisms behind resistance we compared the normoxic and hypoxic levels of anti- and proapoptotic proteins of the Bcl-2 family (Fig. 1D). LS174 and PC3 cells incubated in hypoxia (72 hours) were resistant or sensitive to STS-induced apoptosis, respectively. Bax and BID were not or only slightly detected in LS174 cells (Fig. 1D) while the expression of Bak and Bcl-XL were slightly enhanced in LS174 cells. Because Bcl-XL has been described to interact with VDAC1 (21), we examined the level of VDAC. We observed hypoxic induction of a faster migrating SDS-PAGE form of VDAC in LS174 but not in PC3 cells (Fig. 1D). Immunoblots of mitochondrial fractions confirmed mitochondrial origin (Supplementary Fig. S1).
The hypoxic induction of the formation of a smaller relative molecular mass form of VDAC is dependent on HIF-1 activation

Because an additional VDAC form was observed in hypoxic-resistant cells with enlarged mitochondria, and not in sensitive cells, we focused on the hypoxic induction of this form. As HIF-1 is essential in adaptation to hypoxia, we checked whether HIF-1 was involved in the formation of this form. When HIF-1α was silenced hypoxic cells did not contain the faster migrating form (Fig. 2A), but a normal mitochondrial morphology was restored (data not shown). Similar results were obtained for LS174 and A549 cells (data not shown). Thus HIF-1 initiates hypoxia-induced VDAC.

Expression of VDAC isoforms is not induced at the mRNA level by hypoxia and the hypoxia-mediated form of VDAC is a C-terminal–truncated VDAC1

We quantified the mRNA expression of VDAC1, VDAC2, and VDAC3 in normoxia and hypoxia but did not observe hypoxic induction of these isoforms (Fig. 2B). siRNA directed to the mRNA of the 3 isoforms gave knockdown of the corresponding VDAC isoform (Fig. 2B). Knockdown was confirmed at the protein level and identified the different isoforms (Fig. 2C). The top band corresponded to VDAC1, the intermediate band to VDAC3, and the bottom band to a faster migrating form of VDAC1. The identity of VDAC1 was confirmed with another VDAC1-specific antibody, but
directed to the N-terminus, and both forms were silenced with siRNA (Fig. 2D).

We considered the possibility that the fast migrating VDAC1 resulted from alternative splicing or hypoxia-mediated translation by internal ribosome entry but did not find any evidence to support either possibility (Supplementary Fig. S2).

Finally, the faster migrating VDAC1 was not detected with a VDAC1 antibody directed to the C-terminus (Fig. 2D), suggesting that the C-terminus of the protein was truncated (VDAC1-D). Doxycycline, a second-generation tetracycline that has cytoprotective and metal chelator effects, was found to diminish the formation of VDAC1-D (Fig. 2E). Minocycline, another tetracycline antibiotic, which exerts uncoupling and inhibiting effects on mitochondrial respiration (22), also inhibited formation (Fig. 2F), and partially restored normal mitochondrial morphology (Fig. 2G). Because tetracycline is an inhibitor of matrix metalloproteases we tested a number of protease inhibitors, but they did not inhibit formation of VDAC1-D (data not shown). The possibility of posttranslational cleavage of VDAC1, as described previously (23), is the most likely explanation for the appearance of VDAC1-D.

VDAC1-D Mediates Hypoxic Chemoresistance

We then hypothesized that VDAC1-D could be involved in hypoxic resistance to apoptosis. Silencing of VDAC1 and VDAC1-D decreased the number of enlarged mitochondria.

Figure 2. Hypoxia induced a HIF-1α-dependent novel form of VDAC1. A, induction in hypoxia of a faster migrating SDS-PAGE form of VDAC is dependent on HIF-1α. Immunoblot of HIF-1α and VDACs in HIF-1α silenced HeLa cells in normoxia or hypoxia in the absence (−) or presence (+) of HIF-1α siRNA. B, expression of the mRNA of VDAC1-3 in normoxia and hypoxia in HeLa cells. Expression of VDAC1, 2, and 3 after transfection with control (siCtl) or VDAC1 (siVDAC1), VDAC2 (siVDAC2), or VDAC3 (siVDAC3.1 or siVDAC3.2) siRNA, results are representative of 2 different siRNA for each isoform. C, immunoblot to HIF-1α and VDAC1 (ab15895) in control (siCtl) or VDAC1 (siVDAC1), VDAC2 (siVDAC2), or VDAC3 (siVDAC3.1 or siVDAC3.2) silenced HeLa cells in hypoxia. Hypoxia-induced fast migrating VDAC1. D, immunoblot using antibodies against the N- or C-terminus of VDAC1 in HeLa cells incubated in normoxia or hypoxia transfected or not with siRNA. E, immunoblot of HIF-1α and VDAC1 (ab15895) of LS174 cells incubated in hypoxia. Doxycycline was added for the first 24 hours of hypoxia. F, immunoblot of HIF-1α and VDAC1 (ab15895) of HeLa cells in hypoxia for 48 hours. Minocycline was added for the 48 hours of hypoxia. G, immunofluorescence to cytochrome c of HeLa cells in hypoxia without (+ DMSO) or with minocycline. DMSO, dimethyl sulfoxide.
and restored the tubular mitochondrial morphology (Fig. 3A). To evaluate the sensitivity to an apoptotic stimulus of normoxic and hypoxic LS174 cells, we determined the caspase-3 and -7 activity in cells exposed to STS, an inducer of mitochondrial-dependent apoptosis, and to etoposide, a topoisomerase II inhibitor used in cancer therapy (Fig. 3B). The caspase activity was the same in cells in normoxia or hypoxia, indicating that there was no induction of cell death in hypoxia. Silencing of VDAC1 in hypoxia partially reestablished the sensitivity of hypoxic LS174 cells to apoptosis (Fig. 3B).

Two additional cell lines were examined for enlarged mitochondria, VDAC1-ΔC, and resistance to STS-induced apoptosis. SKMel cells did not show any of these features whereas A549 cells showed all of them (Supplementary Fig. S3), as for HeLa cells (Figs. 2 and 3 and Supplementary Fig. S4). We then questioned which form of VDAC1 (full-length or truncated) was responsible for triggering resistance. In hypoxia, the level of VDAC1 decreased by around 50% (Fig. 3C) whereas VDAC1-ΔC increased by around 50%, which supported posttranslational cleavage of VDAC1. The silencing of vdac1 in normoxic...
cells was associated with a change in mitochondrial morphol-
ogy, as visualized with anticytochrome c (Fig. 3D) and a
decrease in VDAC1 in hypoxia (Fig. 3E). However, these cells
showed an increase in apoptosis with STS (Fig. 3B), suggesting
that the decrease in VDAC1 in normoxia was not responsible
for the protection against apoptosis. Moreover, silencing of
HIF-1α (+ Tet) in hypoxia with STS (Fig. 2A) restored sensi-
tivity to apoptosis (data not shown). We thus concluded that
resistance to apoptosis was associated with VDAC1–ΔC. To
evaluate this further, cells were placed in hypoxia and then
reoxygenated. The level of VDAC1–ΔC was maintained for 4
hours after reoxygenation, then progressively decreased after 8
hours and disappeared at 48 hours (Fig. 3F). As expected, the
level of VDAC1 was inversely proportionate to that of VDAC1-
ΔC. We showed previously that during the first 24 hours of
reoxygenation, cells were protected from apoptosis (3). Tran-
sient exogenous overexpression of a small form of VDAC1
truncated by 5 kDa in the C-terminus, pVDAC1-5kDaΔC (Fig.
3G), in cells exposed for 4 hours to STS showed a slight
resistance to apoptosis (Fig. 3H). Finally, in the presence of
doxycycline (Fig. 2E) or minocyclin (Fig. 2F), hypoxic cells
were no longer protected from STS (Fig. 3I). Taken together,
these results show that both the enlarged morphology of mitochon-
dria and VDAC1–ΔC participate in protection against apopto-
sis in hypoxic LS174, A549, and HeLa cells exposed to STS or
etoposide.

VDAC1–ΔC has the same channel activity and voltage
dependency as VDAC1 and binds Bcl-XL.

VDAC1 and VDAC1–ΔC proteins were purified from hypoxic
HeLa cells (Fig. 4A) and their channel activity was examined
following reconstitution into a PLB. The current through lipid
bilayer–reconstituted VDAC1 (fraction 12) or VDAC1–ΔC (frac-
tion 22) in response to a voltage step from 0 to −10 or to −40
mV (Fig. 4B and C) was the same for the 2 proteins. At −10 mV,
the channel conductance of both proteins was the same (30
pA). At a higher voltage of −40 mV, the full-length channel
showed 2 major conducting states with higher occupancy at
the closed substrate (S1) whereas VDAC1–ΔC showed so-
menly open-state occupancy (O) in comparison to the occupancy
of low-conducting substrates (S1, S2: Fig. 4C). Both channels
showed similar but not identical voltage-dependent conduc-
tance. At the high voltages, VDAC1–ΔC showed slightly higher
conductance than VDAC1 (Fig. 4D), in agreement with the
single-channel experiments [Fig. 4B(ii) and C(ii)]. The volt-
age sensitivity of VDAC1–ΔC suggests the presence of the
N-terminus, conferring voltage gating of the channel (8).
VDAC1–ΔC showed similar Ca2+ conductance to VDAC1,
but at higher voltages spent a longer time in its open state, as
reflected in the decreased voltage sensitivity (Fig. 4E). For
example, at +40 mV the Ca2+ conductance VDAC1–ΔC was
about 1.4-fold higher than that of VDAC1 (Fig. 4E).
VDAC1–ΔC, like VDAC1, interacted with purified Bcl-XL(ΔC)
and decreased its channel conductance (Fig. 4F). Similar
results were obtained with hexokinase I from rat brain (data
not shown). These results suggest that the C-terminal
domain is not required for the interaction of these anti-
apototic proteins with VDAC1.

VDAC1–ΔC forms a complex with hexokinase II and is
associated with cell survival in hypoxia.

As hexokinase II is a major player in maintaining the highly
malignant state of cancer cells (24), we focused on interaction
between hexokinase II and VDACs in hypoxia. Immunopreci-
pitates with antihexokinase II contained VDAC1, VDAC3, and
VDAC1–ΔC (Fig. 5A). In addition, the hexokinase II expression
level was substantially increased in hypoxia (Fig. 5B). Silencing
of more than 90% of the hypoxia-inducible expression of
hexokinase II decreased considerably the level of VDAC1–ΔC
(Fig. 5B). Silencing of hexokinase II in normoxia did not affect
the level of VDAC1. Conversely, silencing of VDAC1 in nor-
moxia and hypoxia decreased slightly the level of hexokinase II
(Fig. 5B). These results were confirmed by immunofluores-
cence; no or little labeling was observed with anti-VDAC in
cells silenced for either hexokinase II or VDACs (Fig. 5C).
In addition, a more intense and punctate immunofluorescence
was observed with anti-VDAC in VDAC1–ΔC–containing cells
incubated in hypoxia than in normoxia (Supplementary Fig.
S5). Clotrimazole (CTM) and bifonazole (BFN) induce apopto-
sis by detaching hexokinase from mitochondria (17, 25). Both
agents increased mortality to a similar extent to that for VDAC
silencing (Fig. 5D) and the mortality was enhanced in cells in
hypoxia in their presence. This suggested that VDAC1–ΔC
interacted with hexokinase II, as did purified hexokinase I,
which decreased VDAC1–ΔC channel conductance. To better
understand the role of VDAC1 and hexokinase II in cell survival
in hypoxia, we silenced VDAC1 or hexokinase II and tested cell
proliferation/death and ATP and lactate production in nor-
moxia and hypoxia. Hypoxia does not kill cells (26), but it slows
proliferation, as shown by a 3-fold decrease in the area of
colonies of cells after 10 days in hypoxia (Fig. 5E). Transient
silencing of VDAC1 (siVDAC1) in hypoxia had no impact on cell
survival but affected proliferation (P < 0.01), whereas silencing
of hexokinase II (siHKII) strongly inhibited survival (Fig. 5E).
As hexokinase II and VDAC1 form a complex and silencing of
hexokinase II decreased the level of VDAC1 in cells (Fig. 5B),
we hypothesized that hexokinase II interfered with ATP transport
and thereby its production via its interaction with VDAC1–ΔC.
Cells produced almost 2 times more ATP in hypoxia (Fig. 5F).
Silencing of VDAC1 decreased hypoxic but not normoxic
production of ATP, suggesting that VDAC1–ΔC influenced ATP
production (Supplementary Fig. S6). This could reflect the
impact of VDAC1 silencing on hexokinase II expression. As
expected the silencing of hexokinase II in hypoxia decreased
ATP. Lactate production, which reflects ATP synthesis via
glycolysis, was increased in hypoxia and diminished with
VDAC1 or hexokinase II silencing (Fig. 5G). Taken together,
these results confirm that VDAC1 is involved in energy homeo-
stasis and points to VDAC1–ΔC as an essential actor in both
glycolysis and mitochondrial energy production in hypoxia
probably through interaction with hexokinase.

VDAC1–ΔC is present in tissues of patients with lung
adenocarcinoma and is more frequently detected in
late-stage rather than in early-stage tumors.

Because we detected both VDAC1 and VDAC1–ΔC in
hypoxic A549 lung carcinoma cells (Supplementary Fig. S3),
we tested for VDAC1 and VDAC1-ΔC in lung adenocarcinomas tumor tissue from 44 patients. Tumor tissues were divided into 2 groups: stage IA and IB (n = 25) and stage IIIA and IIIB (n = 19). The clinical characteristics of the patients are listed in supplementary Table S1. The level of VDAC1-ΔC was determined in corresponding control matched healthy (C) and tumor (T) tissue of patients with lung cancer (Fig. 6A). The tumor tissue, but not healthy tissue, contained VDAC1-ΔC and the level of VDAC1-ΔC in stage III was several fold higher than in stage I (see also Figure 4.

Figure 4. VDAC1-ΔC channel activity and binding of Bcl-XL-ΔC are identical to that of VDAC1. A, VDAC1 and VDAC1-ΔC purification from HeLa cells identified by immunoblotting (anti-VDAC1; Calbiochem); fractions 21 or 22 were used. B and C, channel activity of bilayer-reconstituted purified VDAC1 or VDAC1-ΔC. Currents through bilayer-reconstituted VDAC1 or VDAC1-ΔC in response to a voltage step from 0 to –10 mV [B(i)] and C(i)] or to –40 mV [B(ii)] and C(ii)] were recorded. The dashed lines indicate the zero current level. The total current amplitude histogram traces (in the same recording), showing the relative occupancy of the open state (O) and closed substate (S) or, for VDAC1-ΔC, of 2 or more substates (S1 and S2) during a 4-second recording are shown (B and C). D, currents through the VDAC1 (●) or VDAC1-ΔC (○) channels were recorded in the presence of 1 mol/L NaCl and in response to a voltage step from 0 mV to voltages between –60 to +60 mV. Relative conductance was determined as the ratio of conductance at a given voltage (G) to the maximal conductance (G0). The results are representative of 9 similar experiments in which the value of each voltage represents the average of 3 to 6 swipes. E, currents through VDAC1 (●) or VDAC1-ΔC (○) as recorded in the presence of 0.2 mol/L CaCl2 and in response to a voltage step from 0 mV to voltages between –60 to +60 mV. The results are the average of 2 similar experiments with 3 swipes for each voltage. F, VDAC channel conductance was recorded before and 10 minutes after the addition of purified Bcl-XL-ΔC to the cis chamber. A representative experiment of 3 similar experiments is shown.
In addition, electron micrographs of mitochondria of tumor and matched normal patients' tissues showed enlarged mitochondria in only tumor samples (Supplementary Fig. S7).

The expression of carbonic anhydrase IX (CAIX; refs. 27, 28) and of miR-210 (ref. 29; Fig. 6B), 2 HIF-induced gene products, was analyzed to confirm the hypoxic status of the tissues (Fig. 6A and B). Positivity for CAIX was about 76% and 71% for tumor
tissues from stage I and stage III patients, respectively (Fig. 6A). Only rare control tissues showed minimal CAIX expression. The quality control of the miRNA in the extracts was confirmed by the level of miR-21. Significant relative expression of miR-210 was observed in tumors (Fig. 6B). The band intensity for VDAC1 and VDAC1-ΔC was determined with GeneTools software from Syngene, and the ratio of the intensity of the immunoblot signal of VDAC1-ΔC to VDAC1 determined with GeneTools software (Syngene) for healthy (C) and tumor (T) tissue from patients with lung cancer with either stage I or III tumors. The level of miR-21 indicated the quality of the miRNA in extracts whereas miR-210 is a hypoxia-induced miRNA. C, ratio of the intensity of the immunoblot signal of VDAC1-ΔC to VDAC1 determined with GeneTools software (Syngene) for healthy (C) and tumor (T) tissue. Patients with lung cancer with stage I tumors (left graph) and stage III tumors (right graph). E, evaluation of the number of patients with lung cancer either negative or positive for VDAC1-ΔC, respectively (VDAC1-ΔC neg.) and (VDAC1-ΔC pos.), with small- (T1 and T2) or large- (T3 and T4) sized tumors. F, evaluation of the number of patients with lung cancer either negative or positive for VDAC1-ΔC, respectively (VDAC1-ΔC neg.) and (VDAC1-ΔC pos.), with stages IA–IB–IIIA or stage IIIB tumors.

Figure 6. The ratio of VDAC1-ΔC to VDAC1 was higher in tumor tissue than in control tissue and proportionately more stage III tumors had a high ratio. A, representative immunoblots of VDAC in tissue extracts of healthy control (C) and tumor (T) tissue from 6 individual patients with lung cancer with either early-stage I or late-stage III tumors. CAIX was an indicator of hypoxia in tumors. B, the fold induction of miR-21 and miR-210 was determined for control and tumor tissues from patients with stage I and III tumors. The level of miR-21 indicated the quality of the miRNA in extracts whereas miR-210 is a hypoxia-induced miRNA. C, ratio of the intensity of the immunoblot signal of VDAC1-ΔC to VDAC1 determined with GeneTools software (Syngene) for healthy (C) and tumor (T) tissue from patients with lung cancer with either stage I or III tumors. D, ratio of the immunoblot signal to VDAC1-ΔC to VDAC1 for each individual patient for healthy (C) and tumor (T) tissue. Patients with lung cancer with stage I tumors (left graph) and stage III tumors (right graph). E, evaluation of the number of patients with lung cancer either negative or positive for VDAC1-ΔC, respectively (VDAC1-ΔC neg.) and (VDAC1-ΔC pos.), with small- (T1 and T2) or large- (T3 and T4) sized tumors. F, evaluation of the number of patients with lung cancer either negative or positive for VDAC1-ΔC, respectively (VDAC1-ΔC neg.) and (VDAC1-ΔC pos.), with stages IA–IB–IIIA or stage IIIB tumors.

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the patients’ tissues was neither high nor substantially different between stages I and III, 6% and 12%, respectively.

Discussion

Herein, we showed that hypoxia induces the appearance of a C-terminal truncated form of VDAC1C. The mechanism regulating formation was HIF dependent and the truncated form possessed channel activity, interacted with Bcl-XL and hexokinase I, both of which protect against apoptosis.

Interaction of VDACs with Bcl-2 family members is implicated in translocation of metabolites across the mitochondrial outer membrane (21). Nonetheless, it has been reported that the 3 isoforms are dispensable for mitochondrial-dependent cell death, but this was showed in a cellular and environmental context that was neither malignant nor hypoxic (30). In addition, VDAC2 but not VDAC1 has been shown to inhibit Bak-mediated mitochondrial apoptosis (31). It is possible to hypothesize that changes in the expression of Bcl-2 proteins are implicated directly in resistance. Cytoprotection of lung cancer cells to cisplatin correlated with suppression of activation of Bax but not Bak by cisplatin (12). However, in our study the Bax protein was not detected in LS174 cells. In addition, there exists an intricate crosstalk between the machineries of mitochondrial dynamics (fusion and fission), thus morphology, and apoptosis (32, 33). Both antiapoptotic (Bcl-2) and proapoptotic (Bak and Bax) proteins interact with proteins involved in mitochondrial fusion (mitofusins) and fission (dynamin-related GTPases). Thus, we may speculate that modifications in the expression of Bak and Bcl-XL correlate with the morphologic alterations observed. In addition, the increases in Bak and Bcl-XL in hypoxic LS174 cells correlated with the morphologic alteration in hypoxia.

It may also be hypothesized that the increase in the expression of Bcl-XL and the modification of the open configuration of the VDAC1C channel by Bcl-XL inhibits mitochondrial ATP/ADP exchange, which favors ATP production through glycolysis. A shift toward glycolysis is a characteristic of hypoxic cancer cells and may explain survival and thus resistance when confronted with a potentially lethal agent. The observed change in organization of the cristae of the mitochondria may also rupture the interaction between VDAC and the adenine nucleotide translocase thereby leading to a change in VDAC-mediated ATP transport. In addition, if VDAC oligomerization is responsible for cytochrome c release in apoptosis (11), a change in its conformation may block cytochrome c in the mitochondrial intermembrane space and thus diminish apoptosis.

The notion that resistance of hypoxic regions of tumors to chemotherapy (34) is associated with hypoxic VDAC1C and Bcl-XL is supported by reports showing that protection of HepG2 cells against etoposide-induced apoptosis was HIF-1α dependent (35) and that Bcl-XL is induced by HIF (36).

Because we detected VDAC1C in tumor tissue of patients (50%) and that the frequency of positivity for VDAC1C was higher in late-stage tumors than in early-stage tumors, we believe that VDAC1C represents a product of tumor progression. Gene expression of VDAC1 has been reported to predict poor outcome in early-stage non–small cell lung cancer (37). In addition, VDAC1 was shown to be upregulated in prednisolone-sensitive acute lymphoblastic leukemia cells but not in resistant cells (38).

In conclusion, our results point to modifications in mitochondrial dynamics and production of VDAC1C as a survival response in hypoxic cancer cells that resist apoptosis. Because agents that promote apoptosis may hold therapeutic benefit, these results may have important repercussions for combating cancer cell resistance to chemotherapy.

A synthetic lethality approach targeting RAS tumor cells identified a small-molecule inhibitor of VDAC2 that induced changes in mitochondrial morphology and cell death (39). We propose that VDAC1C may be a potential biomarker to stratify patients with respect to tumor progression and that the VDAC1C/hexokinase complex may be a cancer specific target for therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Table 1. Comparison of the characteristics of the patients and their tumors with VDAC1-ΔC detection

<table>
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<tr>
<th>Characteristic</th>
<th>VDAC1-ΔC detection</th>
<th>χ² (P value)</th>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>5 (50%)</td>
<td>P = 0.87</td>
</tr>
<tr>
<td>Male</td>
<td>16 (47.1%)</td>
<td></td>
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<td>Age, y</td>
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<tr>
<td>&lt;60</td>
<td>13 (52%)</td>
<td>P = 0.51</td>
</tr>
<tr>
<td>≥60</td>
<td>8 (42.1%)</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
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</tr>
<tr>
<td>T1 and T2</td>
<td>15 (41.7%)</td>
<td>P = 0.08</td>
</tr>
<tr>
<td>T3 and T4</td>
<td>6 (75%)</td>
<td></td>
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<tr>
<td>Node stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>14 (50%)</td>
<td>P = 0.69</td>
</tr>
<tr>
<td>N1 and N2</td>
<td>7 (43.8%)</td>
<td></td>
</tr>
<tr>
<td>Initial stage</td>
<td></td>
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<tr>
<td>IA, IB, IIIA</td>
<td>16 (42.1%)</td>
<td>P = 0.06</td>
</tr>
<tr>
<td>IIIB</td>
<td>5 (83.3%)</td>
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 published OnlineFirst March 2, 2012; DOI: 10.1158/0008-5472.CAN-11-3940
References


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M. Christiane Brahimi-Horn, Danya Ben-Hail, Marius Ilie, et al.


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