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Mitochondrial voltage-dependent anion channels (VDACs) as novel pharmacological targets for anti-cancer agents

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Abstract Recently, it was demonstrated that some anti-cancer agents used mitochondrial voltage-dependent anion channels (VDAC1–3 isoforms) as their pharmacological target. VDACs are expressed more highly in cancer cells than normal cells; thus the VDAC-dependent cytotoxic agents can have cancer-selectivity. Furanonaphthoquinones (FNQs) induced caspase-dependent apoptosis via the production of NADH-dependent reactive oxygen species (ROS) by VDAC1. The ROS production and the anti-cancer activity of FNQs were increased by VDAC1 overexpression. Meanwhile, erastin induced RAS-RAF-MEK-dependent non-apoptotic cell death via VDAC2. On the other hand, VDACs were needed for transporting ATP to hexokinase (HK), which was highly expressed in cancer cells. We hypothesized that the high glycolysis might induce up-regulation of VDAC. In this review, we propose that VDACs are novel candidates for effective pharmacological targets of anti-cancer drugs.

Keywords Mitochondria · Voltage-dependent anion channel (VDAC) · Pharmacological target · Anti-cancer agent · Apoptosis · Reactive oxygen species (ROS) · Adenine nucleotide translocator · Hexokinase

Introduction

In cancer therapy, it is important to determine whether anti-cancer drugs can discriminate between cancer cells and normal cells. Hence, a wide variety of oncogenes have been studied, and anti-cancer drugs with oncogenes-selective lethality have been developed. Voltage-dependent anion channels (VDAC1–3 isoforms), which are located in the mitochondrial outer membrane, have been studied mainly as one of the requisite actors for mitochondrial-dependent apoptosis. VDACs have been studied as one of the mediators of the mitochondrial permeability transition induced by Ca^{2+} overload (Shimizu et al. 2001) and oxidative damage (Madesh and Hajnoczky 2001). Recently, some groups reported focusing on VDACs as the pharmacologic target for novel molecules inducing cancer cell death.

In this review we first overview the functions of the VDAC family proteins, including the difference in the expression of VDACs between cancer and normal cells. Then, we categorize VDAC-dependent cytotoxic agents into three groups: (1) VDAC1-dependent chemicals, which induced caspase-dependent apoptosis by the production of reactive oxygen species (ROS) (Hirai et al. 1999; Pan et al. 2000; Simamura et al. 2003, 2006); (2) VDAC2 and/or VDAC3-dependent chemicals, which induced RAS-RAF-MEK-dependent oxidative cell death (Yagoda et al. 2007); and (3) phosphorothioate antisense oligonucleotide, which induced caspase-dependent apoptosis (Lai et al. 2006; Tan et al. 2007a, b). Finally, we discuss the cancer-selective VDAC activity in conjunction with other mitochondrial channels and hexokinase II (HK II) in cancer cells.

VDAC family proteins

Mitochondrial VDAC was found to be located in the mitochondrial outer membrane and to form the pores of the outer membrane (Colombini 1980). At least 2 human VDAC isoforms were found to be present (Blachly-Dyson

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et al. 1993). The VDAC proteins were thought to form the channel for adenine nucleotides through the outer membrane and were the binding site for HK and glycerol kinase. VDAC1 mRNA and protein were expressed ubiquitously, whereas VDAC2 mRNA was only expressed in the heart (Huizing et al. 1998).

During the induction of apoptosis, the permeability of the mitochondrial membrane was altered, cytochrome c leaked into the cytoplasm and the caspases were activated (Liu et al. 1996; Earnshaw 1999). The Bcl-2 family, whose proteins might be antiapoptotic or proapoptotic, regulated the permeability of the mitochondrial membrane during mitochondria-dependent apoptosis. The proapoptotic proteins Bax and Bak caused the opening of VDAC1 (Narita et al. 1998); in contrast, the antiapoptotic protein Bcl-xL prevented the release of cytochrome c by closing VDAC1 (Shimizu et al. 1999). On the other hand, VDAC2 complexed with Bak, which promotes cell death, and regulated the activity of Bak (Cheng et al. 2003). The overexpression of VDAC2 inhibited mitochondria-dependent apoptosis.

Shinohara et al. demonstrated that the transcript levels of the three VDAC1–3 isoforms in rat hepatoma AH130 cells were significantly higher than in the tissue of normal rat liver (Shinohara et al. 2000). In a comparison between human cancer cell lines and normal cell lines, the solid cancer cells were found to express higher VDAC1 contents than normal fibroblast cells (Simamura et al. 2006, 2008). The cancer-selectivity of VDAC-dependent agents could be caused by the higher expression of VDACs in cancer cells.

VDAC-dependent cytotoxic agents

Anti-cancer activity via VDAC1

FNQs, which were synthesized from 20 derivatives, were 10–14 times more toxic to human cancer cell lines than to normal epithelial cells (Hirai et al. 1999). FNQs caused damage to the structure of mitochondria via the production of ROS in human lung adenocarcinoma A549 cells (Fig. 1) (Simamura et al. 2003). Simamura et al. demonstrated that FNQ produced ROS on the outer membranes in an NADH-dependent manner (Simamura et al. 2006). The treatment of cancer cells with FNQs caused the collapse of the mitochondrial membrane potential and subsequently the leakage of cytochrome c from the mitochondria into the cytoplasm and the activation of procaspase-9, and finally led to apoptosis (51% of cells) (Simamura et al. 2003). In HeLa cells that overexpressed VDAC1, ROS production increased and cell survival decreased (Fig. 2a). On the other hand, HeLa cells transfected with *vdac1* siRNA caused decreases in the FNQ-induced ROS and in the frequency of cell death (Fig. 2b) (Simamura et al. 2006). Furthermore,

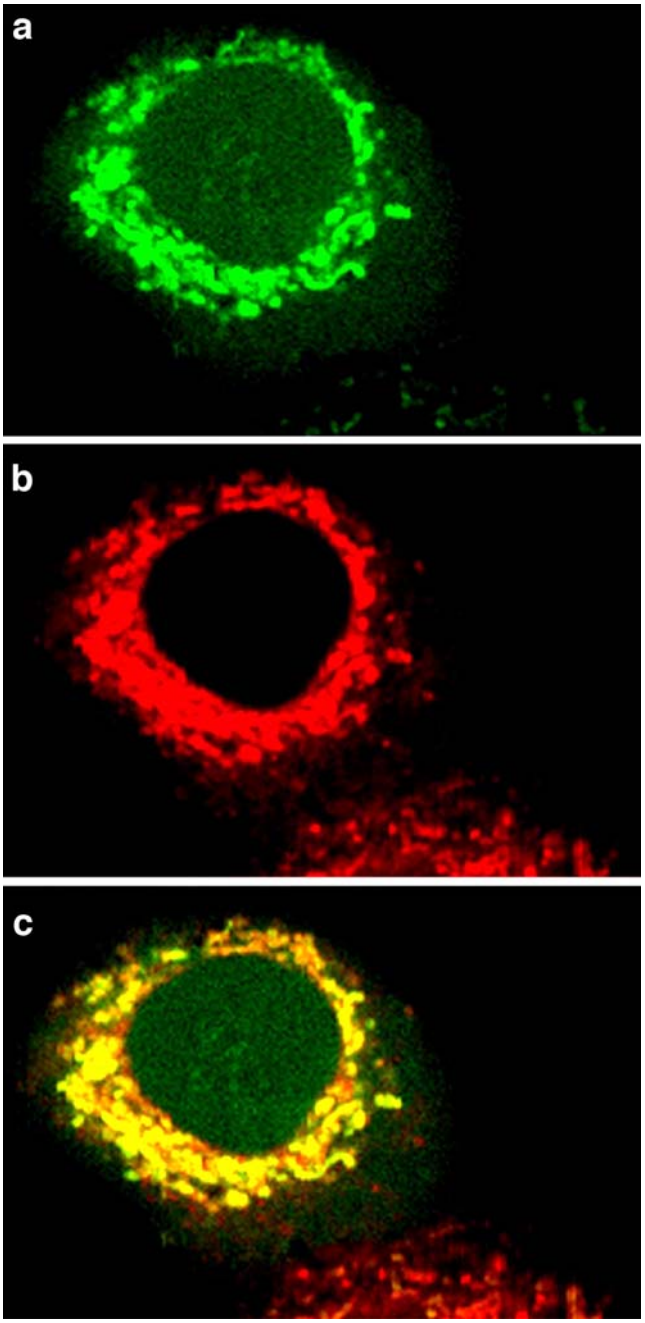

Fig. 1 Confocal microscopic observation of mitochondria. Fluorescence visualization of H_2O_2 production in A549 adenocarcinoma cells stimulated with FNQ3. **a** Intracellular production of H_2O_2 is shown by green fluorescence due to 2′,7′-dichlorofluorecein formation. **b** The localization of the mitochondria was granularly visualized by a tracker as red fluorescence. **c** These individual images were merged, and green and red fluorescence conspicuously formed yellow granular fluorescence. This figure incorporates modified data from (Simamura et al. 2003)

Simamura et al. showed that the VDAC1 protein had NADH-dependent quinone-reducing activity and activated menadione (vitamin K_3), but not mitomycin C or adriamycin (Simamura et al. 2008). Mitomycin C and adriamycin

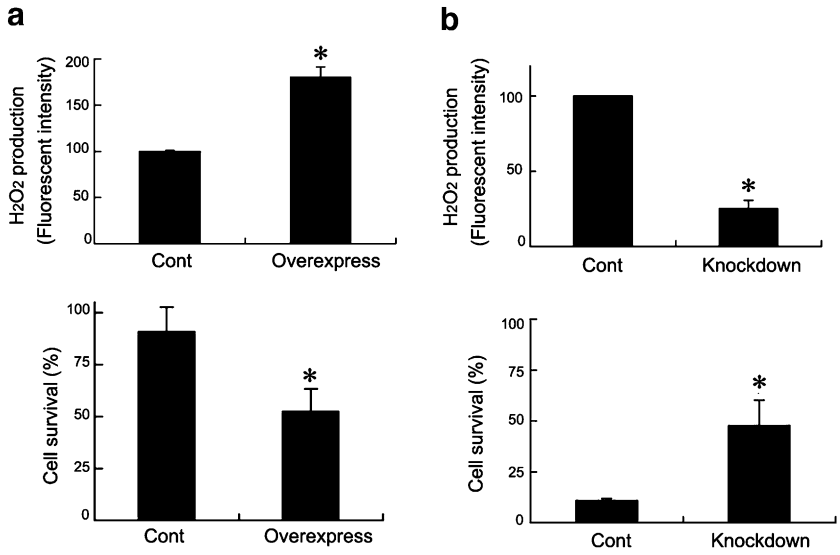

Fig. 2 ROS production and cell survival in HeLa cells that overexpressed VDAC1 or in which VDAC1 knockdown occurred. **a** VDAC1 overexpression. HeLa cells transfected with *vdac1* produced higher levels of H_2O_2 per cell by the addition of 4 μM FNQ. After a 48-h treatment with 0.6 μM FNQ, the survival of the cells decreased from that of control cells. Cont, cells transfected with a control vector; Overexpress, cells overexpressing pUC-CAGGS-human *vdac1*. **b** VDAC1 knockdown. HeLa cells transfected with *vdac1* siRNA produced lower levels of H_2O_2 per cell by the addition of 4 μM FNQ. After a 48-h treatment with FNQ, the survival of cells increased in comparison to that of control cells. Cont, cells transfected with a control siRNA. Knockdown, cells transfected with *vdac1* siRNA. Student's t test was performed on the different sets of data. *Differences were considered significant when the p value was $p<0.01$ vs control. These figures use modified data from (Simamura et al. 2006)

did not exhibit cancer-selective cytotoxicity (Hirai et al. 1999), and A549 cells treated with mitomycin C or adriamycin did not cause initial morphological alterations in the mitochondria (Simamura et al. 2001). As shown in Fig. 3, we propose a model schematic mechanism of apoptosis induction via VDAC1. VDAC1 would be preferentially targeted by FNQ and menadione, and ROS would induce mitochondria-dependent apoptosis (Madesh and Hajnoczky 2001; Simamura et al. 2006). The activity of NADH-dependent reductase is not unique to mitochondrial VDAC1; in fact, plasma membrane VDAC1 had the activity of NADH-ferricyanide reductase (Baker et al. 2004). These studies revealed that these anti-cancer agents produced ROS via the quinone-reducing activity of VDAC1 and induced caspase-dependent apoptosis in cancer cells.

The cancer cells with higher VDAC1 content in the mitochondria showed high sensitivity to FNQ (Fig. 4)

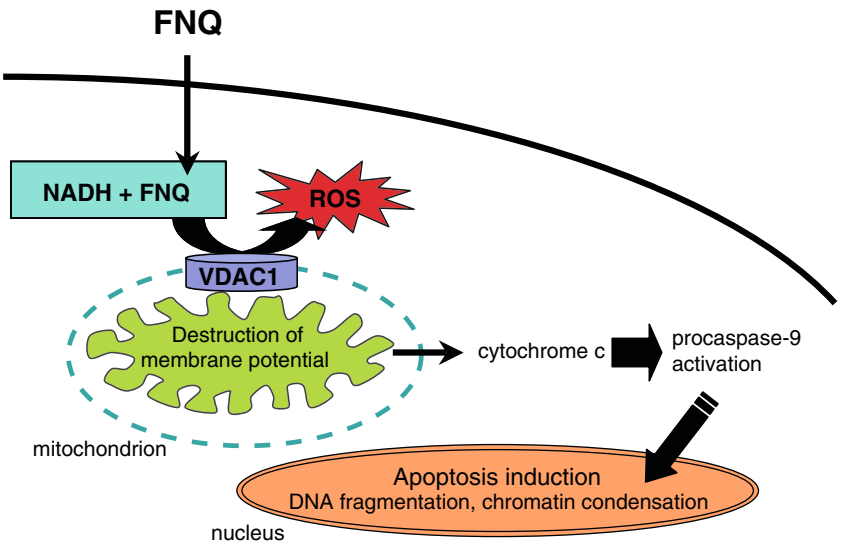

Fig. 3 The model schematic diagram of the mechanism of apoptosis induced via VDAC1. This figure shows a modified version of the scheme in (Simamura et al. 2006)

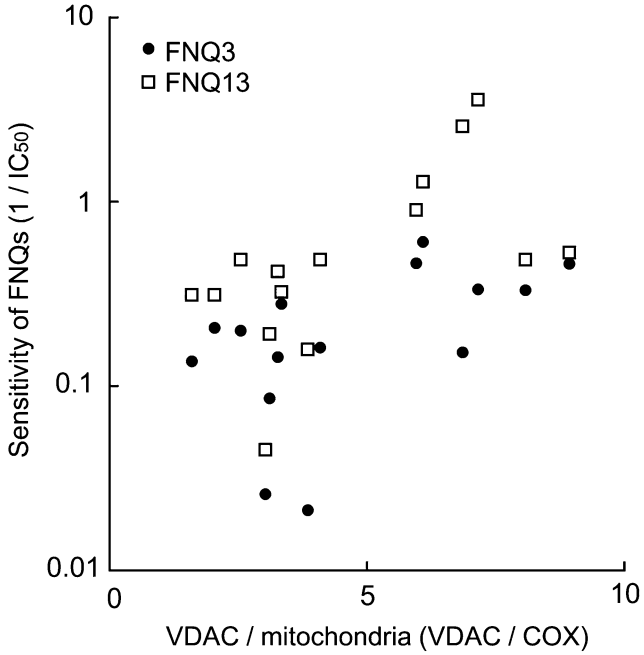

Fig. 4 The correlation between VDAC1 contents and the sensitivity of FNQs. The signal ratio of VDAC1 to COX (VDAC1/COX: cytochrome c oxidase) was used to compare the amount of VDAC1 among cell lines. The sensitivities are shown as a reciprocal plot of IC_{50} (μM). Solid cell lines: 5637 bladder carcinoma, T98G glioblastoma, T24 bladder carcinoma, A549 lung adenocarcinoma, HCT116 colon carcinoma, MKN1 (lymph node metastatic), MKN7 and MKN74 (liver metastatic) gastric cancer cells, RCC10RGB kidney cancer. Leukemia cell lines: HL60 human promyelocytic leukemia, Jurkat T cell leukemia, KU812 chronic myelogeneous leukemia and K562 chronic myelogeneous leukemia. Normal cell line: WI-38 fibroblast

(Simamura et al. 2008). In contrast, WI-38 cells, which are a wild-type fibroblast cell line and expressed a smaller amount of VDAC1 (VDAC/cytochrome c oxidase: 3.03), demonstrated lower sensitivity to FNQs than did cancer cells. The IC_{50} of FNQ derivatives was 4–20 times higher in WI-38 (IC_{50}; FNQ3: 36.6 μM, FNQ13: 22.2 μM) than in cancer cells. The sensitivity to FNQ of solid cancer cells and leukemia cells depends on the amount of VDAC1 in the mitochondria.

Anti-cancer activity via VDAC2 and VDAC3

Yagoda et al. (2007) reported that RAS-RAF-MEK-dependent oxidative cell death, not apoptosis, was induced via VDAC2 or VDAC3 by erastin. Erastin, a selective anti-cancer agent discovered in screening by Yagoda et al. (2007), exhibited greater lethality in human cancer cells having mutations in the oncogenes HRAS, KRAS or BRAF. It bound directly to VDAC2 and caused mitochondrial damage via ROS production in an NADH-dependent manner, which induces cell death, but not apoptosis. It was demonstrated that "ligands" of VDAC induced non-apoptotic cell death in some cancer cells having mutations in the RAS-RAF-MEK pathway.

Oligonucleotide interacting with VDACs

G3139 is an 18-mer phosphorothioate antisense oligonucleotide that targeted the initiation codon region of the Bcl-2 mRNA and down-regulated the expression of Bcl-2 (Klasa et al. 2002). Lai et al. (2005, 2006) demonstrated that G3139 caused VDAC closure, indicating the decay of channel conductance, by interacting directly with VDAC (isoform unspecified) and induced caspase-dependent apoptosis, VDAC is a pharmacological target for G3139, which represents quite a different strategy from FNQ or erastin.

Cancer selectivity of VDAC-dependent cytotoxicity

It remains unclear why VDACs were expressed more highly in cancer cells than in normal cells. We have hypothesized that the highly glycolytic phenotype in many cancers (Pedersen 1978; Warburg et al. 1930) may cause the up-regulation of VDACs. It was reported that VDACs were needed as one of the ATP-transporting proteins within the adenine nucleotide translocator (ANT)-VDAC-HK II complex in cancer cells (Mathupala et al. 2006). Chervollier et al. demonstrated that cells deprived of mitochondrial DNA expressed higher levels of ANT2 and VDAC than wild-type cells and concluded that ANT2 would be required for high glycolysis in cancer cells. HK II was highly expressed in malignant hepatomas (Pedersen 1978; Pedersen et al. 2002). Therefore, VDAC and ANT2 would be up-regulated in cancer mitochondria. Furthermore, because the amount of NADH increased in cells deprived of mitochondrial DNA in comparison to wild-type cells (Pelicano et al. 2006), an increase of NADH, which induces the activation of FNQ and erastin, may be caused in cancer cells. These studies support the hypothesis that VDACs are possible pharmacologic targets for anti-cancer drugs.

Concluding remarks

In this review, we have summarized the findings regarding VDACs acting as novel pharmacological targets for anti-cancer agents. FNQ and erastin were common to ROS production NADH-dependently and caused mitochondrial damage, but were different in terms of the isoform of VDAC required and the mechanisms of cell death. Because VDACs were expressed more highly in cancer cell lines than in normal cell lines, it would be appropriate to develop novel anti-cancer drugs that pharmacologically target VDACs. Because we hypothesize that high glycolysis

would regulate VDAC expression, additional research is needed to clarify the correlation of the expression of VDACs, ANT2 and HK II, i.e., the balance of glycolysis and mitochondrial effects in cancer cells.

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