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Review

Targeting a mitochondrial potassium channel to fight cancer

Luigi Leanza[a], Elisa Venturini[b], Stephanie Kadow[b], Alexander Carpinteiro[b], Erich Gulbins[b,**], Katrin Anne Becker[b,*]

[a] Department of Biology, University of Padova, Viale G. Colombo 3, 35131 Padova, Italy
[b] Department of Molecular Biology, University of Duisburg-Essen, Hufelandstrasse 55, 45122 Essen, Germany

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ABSTRACT

Although chemotherapy is able to cure many patients with malignancies, it still also often fails. Therefore, novel approaches and targets for chemotherapeutic treatment of malignancies are urgently required. Recent studies demonstrated the expression of several potassium channels in the inner mitochondrial membrane. Among them the voltage gated potassium channel Kv1.3 and the big-potassium (BK) channel were shown to directly function in cell death by serving as target for pro-apoptotic Bax and Bak proteins. Here, we discuss the role of mitochondrial potassium channel Kv1.3 (mitoKv1.3) in cell death and its potential function in treatment of solid tumors, leukemia and lymphoma. Bax and Bak inhibit mitoKv1.3 by directly binding into the pore of the channel, by a toxin-like mechanism. Inhibition of mitoKv1.3 results in an initial hyperpolarization of the inner mitochondrial membrane that triggers the production of reactive oxygen species (ROS). ROS in turn induce a release of cytochrome c from the cristae of the inner mitochondrial membrane and an activation of the permeability transition pore, resulting in opening of the intrinsic apoptotic cell death. Since mitoKv1.3 functions downstream of pro-apoptotic Bax and Bak, compounds that directly inhibit mitoKv1.3 may serve as a new class of drugs for treatment of tumors, even with an altered expression of either pro- or anti-apoptotic Bcl-2 protein family members. This was successfully proven by the *in vivo* treatment of mouse melanoma and *ex vivo* human chronic leukemia B cells with inhibitors of mitoKv1.3.

1. Introduction

Chemotherapeutic drugs target malignant tumors by a variety of mechanisms, including inhibition of proliferation, direct induction of tumor cell death by any form of apoptosis or necrosis, by activation of the immune system and by altering the niche of the tumor, in particular the tumor stem cell niche. Death of tumor cells is an important mechanism to contribute to a successful chemotherapy. Thus, it is of great clinical interest to define molecular mechanisms that mediate cell death. Since, unfortunately, chemotherapy still often fails and many patients die, it is also very important to identify novel targets for chemotherapy and, thereby, potential novel chemotherapeutic drugs. Here, we will focus on the role of potassium channel of the Shaker family, Kv1.3, in mitochondria (mitoKv1.3), its role in apoptosis and its potential function as targets in chemotherapy.

Mitochondria are in the center of many pathways that induce apoptosis or necrosis [1,2]. Death receptors, but also exogenous stress stimuli such as irradiation, converge to the activation of the pro-apoptotic Bcl-2 family proteins Bax and Bak. The critical role of Bax and Bak in apoptosis has been shown in numerous studies (for a recent review see Ref. [3]). Activation of Bax by pro-apoptotic stimuli results in its translocation and integration into the outer mitochondrial membrane. The molecular details of this process are still unknown.

Bak proteins seem to be loosely associated with the outer mitochondrial membrane [4]. Activation of Bak results in integration of the protein into the outer mitochondrial membrane [4]. Activated Bax assembles in oligomers that seem to form pores although these pores were detected at low pH and their *in vivo* relevance remains to be established [5–9]. Structural studies indicated that after incorporation of Bax into the outer mitochondrial membrane, only two amino acids stick out of the outer mitochondrial membrane and face the inner mitochondrial membrane, i.e. amino acids 127 and 128 located between the 5th and 6th helices of Bax [10]. The amino acid at position 128 is a positively charged lysine. However, it is well established that the integration of Bax mediates the release of cytochrome c and other pro-apoptotic molecules such as APAF1

* Corresponding author. Tel.: +49 201 723 1949; fax: +49 201 723 5974.
** Corresponding author. Tel.: +49 201 723 3118; fax: +49 201 723 5974.
E-mail addresses: erich.gulbins@uni-due.de (E. Gulbins), katrin.becker-flegler@uni-due.de (K.A. Becker).

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and caspase 9 from mitochondria into the cytoplasm (for a review see [11]). The function of Bak seems to be homologous to that of Bax and they are able to replace each other in genetic knock-out models [12].

Cytochrome c release from mitochondria is a key step in mitochondrial apoptosis and it was shown to occur in two independent steps [13,14]: first, cytochrome c, which physiologically binds to cardiolipin at the inner mitochondrial membrane, is released into the inter-membrane space [13,14]. The detachment of cytochrome c from cardiolipin has been shown to be mediated by reactive oxygen species (ROS) [13,14]. However, it is unknown if ROS intermediates directly change the oxidation of cardiolipin and whether that results in the release of cytochrome c, or whether oxygen radicals indirectly act to trigger the release of cytochrome c. Cytochrome c is released from the mitochondria into the cytoplasm by a Bax/Bak-dependent mechanism to execute cell death [15]. ROS also target the permeability transition pore (PTP) [16], which will be discussed below in the present review.

2. Kv1.3

Kv1.3 is a potassium channel belonging to the *Shaker* family of voltage-gated channels [17,18]. Voltage gated potassium channels (K_V) are a large family of potassium (K^+) channels expressed in both excitable and non-excitable cells. In humans, they represent the largest family of K^+ channels, comprehending at least 40 genes. K_V channels are divided in 12 subfamilies (K_V1-K_V12). As all K^+ channels, they have a homo- or hetero-tetrameric structure constituted by 4 subunits, named α subunits, and other additional subunits, i.e. β, γ and δ, which associate around the ion conducting central pore. The selectivity filter for K^+ is characterized by a distinctive P-loop structure formed by 5 conserved amino acidic residues (GYGD) located in the subunits [19]. These residues expose an electronegative carbonyl oxygen atom, which mimics the hydration sphere when the ions get access to the filter [19,20]. Kv channel α subunits possess 6 transmembrane helices (S1–S6) with both the N-terminus and C-terminus on the intracellular side of the membrane [21]. The first 4 helices form the voltage sensor, while the last two helices, together with the loop, form the pore region [22]. The S4 helix contains four positively charged arginine residues and acts as the voltage-sensor domain [23].

Plasma membrane Kv1.3 and the other members of the Kv family control resting and action potential in excitable cells, while in non-excitable tissues regulate cell volume and proliferation, but also cell death [24–28]. In particular, it has been demonstrated that plasma membrane Kv1.3 participates in controlling proliferation [18], apoptosis [29,30] and neurotransmitter release by excitable cells [31].

Kv1.3-deficient mice were generated [32] and show an increase in the platelet count, a finding that would be consistent with a role of Kv1.3 in apoptosis of platelets or precursors [33]. However, the lack of Kv1.3-expression seems to be compensated in most cells of these mice by up-regulation of Kv1.1 and of a chloride channel [34].

3. Kv1.3 inhibitors

Non-permeant Kv1.3 inhibitors can be peptide inhibitors or organic compounds [35]. Among peptide Kv1.3 inhibitors there are margatoxin, *Stichodactyla heliantus* toxin (ShK) and charybdotoxin [36]. Although the use of charybdotoxin demonstrated the crucial role of K^+ channels in lymphocytes activation, this compound, at low concentrations, also inhibits IK_{Ca} subfamily channels, therefore other inhibitors are preferred. *Stichodactyla heliantus* toxin is the most potent inhibitor of Kv1.3, blocking the channel at high affinity (Kd = 11 pM) and showing a 1000-fold higher selectivity with respect to other Kv and IK channels [36–40]. It contains 35 amino acid residues, bound together by three disulfide bounds [36–40]. This structure interacts with the negative charged residues of Kv1.3 (e.g. Asp^{386}) in the channel pore vestibule through its positive residues (e.g. His^{19}, Ser^{20}, Lys^{22}, Tyr^{23}) [36,37]. The substitution of a critical Lysine (ShK-Lys^{22}) with neutral residues substantially reduces the affinity of the toxin for the channel, indicating that other residues do not bind efficiently to the channel pore vestibule [38,40].

Permeant inhibitors are blockers that are able to cross the plasma membrane and have an effect on a mitochondrial K^+ channel. Among them there are: Psora-4, PAP-1 and clofazimine. The most potent Kv1.3 inhibitor is Psora-4 (EC 50 = 3 nM) [35]. It is a small molecule isolated from the plant *Ruta graveolens* and belongs to the group of psoralen-molecules. Psoralens are a class of photosensitive molecules used in the therapy of different skin diseases such as psoriasis [41,42]. Since Psora-4 also inhibits Kv1.5, a new derivative has been synthesized, which is more selective than the former, PAP-1 (EC 50 = 2 nM) [43]. Both Psora-4 and PAP-1, however, can act, at higher concentrations, also on other Kv family members.

The third membrane permeant inhibitor, clofazimine, is a highly lipophilic compound of riminophenazine family [44], and it is currently used in the treatment of different dermatological diseases such as leprosy [45]. Its use is under investigation in several infectious and non-infectious diseases, like antibiotic resistant tuberculosis, due to its apparent anti-mycobacterial and anti-inflammatory activity, although the precise mechanism is still unclear [46]. Clofazimine appears to inhibit bacterial proliferation by binding guanine bases of bacterial DNA, disrupting the cell cycle finally killing the bacterium [47].

4. Mitochondrial Kv1.3 and K^+ fluxes across the mitochondrial membrane

Besides the plasma membrane localization, Kv1.3, as well as other Kv channels (Kv1.1 and Kv1.5), was also found in the inner mitochondrial membrane [48–51]. Kv1.3 seems to be active in the inner mitochondrial membrane even at negative resting potential (−180 mV) as demonstrated by the hyperpolarization induced after incubation of isolated mitochondria with its specific inhibitors, such as margatoxin and *Stichodactyla heliantus* toxin [29]. In normal conditions, K^+ channels located to the inner mitochondrial membrane should mediate an inward K^+ flux from the cytosol to the mitochondrial matrix, following the electrochemical gradient of this ion [52] (Fig. 1). This positive flux is compensated by the efflux of protons (H^+) mediated by the respiratory chain and by the K^+/H^+ antiporter, to avoid volume changes and depolarization [52,53]. Patch clamp experiments on the inner mitochondrial membrane of lymphocyte mitochondria demonstrated that the inner mitochondrial membrane located Kv1.3 was active and showed the same electrophysiological properties as the correlated plasma membrane channel, i.e. slope conductance of 25 pS in 150 mM KCl, K^+ selectivity, slight rectification and inhibition by margatoxin and Psora-4, suggesting that both plasma membrane and mitochondrial channels are encoded by the same gene [48,49]. Since no targeting sequence is present in the Kv1.3 protein, the molecular mechanisms of mitochondrial targeting of Kv1.3 are still unknown.

As postulated by Mitchell in the 1960s, the mitochondrial ATPase activity is coupled to H^+ translocation across the inner mitochondrial membrane, characterized by a low permeability to H^+, cations and anions, and is mediated by the respiratory chain complexes [54]. This process is associated to a substrate-specific exchange-diffusion carrier system that leads to reversible transmembrane shuffle of anions and cations [54,55] (Fig. 1).