Ion channels and transporters in cancer. 5. Ion channels in control of cell cycle and cell apoptosis

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Themes

APOPTOSIS IS INTEGRAL to normal tissue homeostasis, and abnormalities in apoptotic functions underlie the pathogenesis of many diseases. In general, an excess of apoptosis can lead to tissue degeneration, whereas a deficiency can lead to cancer. The molecular machinery of apoptosis (from initiation to the final phagocytosis of cellular remnants) is complex, involving many molecular players and signaling pathways (30, 36, 66).

Over the past two or three decades, a clear and seemingly comprehensive picture of the biology of apoptosis has emerged (37). Originally identified through its characteristic cytological morphology (40), this mode of death is now known to result from activation of a common mechanism relevant in both physiological and pathological circumstances (15, 37). At the heart of this mechanism lie two families of proteins, the caspases and members of the Bcl2 extended family, as illustrated by Fig. 1. The caspases form a cascade in which initiator caspases are activated by lethal stimuli arising either at the cell membrane as a result of cytokine-receptor binding, or within the cell, in relation to internally determined signals, often generated in the microenvironment of particular organelles (87). The Bcl2 family is so called because of the relationship of its members to the B-cell lymphoma oncogene whose discovery led eventually to the identification of most of the other family members, but at the molecular level this family is remarkably diverse (84).

On the other hand, different inducers of apoptosis trigger plasma membrane potential depolarization (8), while the inhibition of apoptosis by Bcl-2 and Mcl-1 is associated with plasma membrane hyperpolarization (29, 82). Furthermore, irrespective of the inducer or whether apoptosis is a part of a physiological or pathological process, it always involves Ca2+-influx followed by the recruitment of three major Ca2+-dependent apoptotic mechanisms: mitochondrial, cytoplasmic, and endoplasmic reticulum (ER)-mediated (60, 65). Thus the ion fluxes mediated by ion channels are extremely important mechanisms of apoptosis regulation.

The first important role ascribed to plasma membrane ion channels, over 60 years ago, was their participation in cellular electrogenesis and electrical excitability. However, numerous subsequent studies have firmly established the contribution of ion channels to virtually all basic cellular behaviors, including such crucial ones for maintaining tissue homeostasis as proliferation, differentiation, and apoptosis (44, 69, 72). The major mechanisms via which ion channels contribute to these crucial processes include: providing the influx of essential signaling ions, regulating cell volume, and maintaining membrane potential. Malignant transformation of cells resulting from enhanced proliferation, aberrant differentiation, and impaired ability to die is the prime reason for abnormal tissue growth, which can eventually turn into uncontrolled expansion and invasion, characteristic of cancer (11, 34).

This review focuses on the aspects of programmed cell death influenced by various ion channels and how dysfunctions and misregulations of these channels may influence the development and progression of different cancers.

Role of Potassium Channels in Apoptosis

Potassium channels are involved in the maintenance of resting potential, thereby they represent an integral part of all cells. As K+ channels provide an efflux of K+, which is the dominant cation of the intracellular medium, they are also important regulators of cell volume. K+ channels represent one of the most diverse groups of channels, consisting of five major classes: 1) voltage-gated (Kv class), 2) Ca2+-activated (KCa class), 3) inwardly rectifying (Kir class), 4) ATP-sensitive (KATP class), and 5) background two-pore domain-containing (K2P class) (83). Some of them have been identified in various types of carcinomas where they are involved in the proliferation and apoptosis of tumor cells (83). This is consistent with the paradigm according to which the enhanced K+ efflux is associated with apoptosis promotion and, conversely, that apoptosis is attenuated if K+ efflux is decreased (70, 92). The mechanisms for proapoptotic effects of enhanced K+ efflux...
The pharmacological blockade of Kv1.5 channels in SGC7901 
significantly decreases AVD and activation of intracellular proapop- 
totic effectors (92). In particular, decreases in intracellular K 
and Ca2+ influx. For instance, this mechanism was implicated in the apoptosis of HepG2 human hepatoblastoma cells induced by the K+ channel blocker 4-aminopyridine (4-AP) (41). In addition, the involvement in the antiapoptotic effects of the expression of oncogenic TASK-3 channel (63) mentioned above cannot be excluded.

The importance of augmented K+ efflux in apoptosis was 
directly confirmed in experiments with KChAP, a K+-channel 
regulatory protein that increases K+-channel expression in a 
“chaperone-like” fashion in heterologous expression systems 
(4). Overexpression of KChAP in LNCaP cells decreased the 
average cell size due to enhanced AVD and promoted sponta- 
neous cells apoptosis (86). Moreover, repetitive overexpression 
of KChAP during 19 days in LNCaP and DU-145 tumor xenografts in nude mice significantly suppressed tumor growth 
due to the apoptosis of infected tumor cells. The mechanism of 
proapoptotic KChAP action consists of the direct interaction 
with K+ channels, thereby increasing their expression. Over- 
expression of KChAP in LNCaP cells also produced G0/G1 
cell-cycle arrest via the activation of p53 (the tumor suppressor 
protein) acting as a transcription factor. However, the involve- 
ment of p53 in proapoptotic KChAP activity was ruled out 
based on the fact that KChAP was able to induce similar 
apoptosis in DU-145 cells expressing mutated p53, rendering it 
nonfunctional as a transcription factor (86).

In conclusion, K+ channels seem to play an important role in 
the control of cancer cells apoptosis by regulating membrane 
potential and passive calcium influxes. However, further studies 
are needed to identify the precise role of each type of K+ 
channels in carcinogenesis and apoptosis resistance for their 
potential utilization as diagnostic markers or therapeutic tar-

Voltage-Gated Sodium Channels and Apoptosis

As compared with other channels very little is known about 
the involvement of voltage-dependent sodium channels. The notion that increased malignancy of cancer cells is associated 
with the shift to a “more excitable” phenotype of their plasma 
membrane is supported not only by the decrease in K+ 
conductances, as described above, but also by the appearance of 
inward currents characteristic of excitable cells, such as voltage- 
gated Na+ currents. Indeed, in several cancer epithelial 
cells, the expression of voltage-gated Na+ channels (VGSCs) 
on functional, protein and mRNA levels has been firmly 
established (1, 5, 18). Though the role of VGSCs for the cancer 
cell proliferation, migration, and invasion has already been 
demonstrated (4, 5, 24, 28, 58), very little is known for their 
role in apoptosis.

The involvement of VGSCs in cell death by apoptosis has 
been shown in Jurkat cell line (71). The real-time PCR analysis 
of neoplastic mesothelial cells showed significant expression of 
the mRNAs encoding for Na(V)1.2, Na(V)1.6, and Na(V)1.7 
[and less so for Na(V)1.3, Na(V)1.4, and Na(V)1.5] main 
voltage-gated sodium channel (VGSC) α-subunit(s). However, 
blockade of VGSCs with tetrodotoxin decreased mesothelioma 
cell migration in in vitro motility assays and failed to interfere
with cell viability, proliferation, and apoptosis progression triggered by UV exposure (25). These data on the involvement of VGSC in apoptosis may be confirmed by other indirect evidence. For instance, the progression of the hormone-responsive cancer, as prostate cancer, to the androgen-insensitivity stage is accompanied by the appearance of new apoptosis-resistant cell phenotypes. The enrichment of androgen-independent tumors with malignant neuroendocrine cells should especially be noted. Fully differentiated, nonproliferating, neuron-like apoptosis-resistant neuroendocrine cells are a normal component of the prostate epithelium which, by releasing a variety of neurosecretory products, regulate the development and secretory activity of the prostate in the endocrine/paracrine manner (2, 17). Generally, prostatic neuroendocrine cells are apoptosis-resistant cells and express a variety of membrane ion channels characteristic of neurons, like TTX-resistant VGSCs, high-voltage-activated (HVA) Ca\(^{2+}\)-channels of L- and N-type, and are also able to generate action potentials. An expanding population of neuroendocrine cells beyond normal proportions due to the malignant transformation of epithelial cells is a common characteristic of prostate cancer progression (2). Neuroendocrine cells lack nuclear androgen receptor (AR), thereby representing an androgen-insensitive cell phenotype in the prostate (6). They also exhibit high apoptosis resistance (22) which, according to existing evidence, is unrelated to the common antiapoptotic Bcl-2 protein (90) and is conferred instead by new investigations are needed to precisely characterize the role of VGSCs in the cancer-related apoptosis mechanisms whether related to misregulation of membrane potential mechanisms or other not yet identified mechanisms.

**Cl\(^{-}\) Channels**

Activation of the chloride current through specialized volume-regulated anion channels (VRACs) in response to cell swelling (I\(_{\text{Cl,swell}}\)) is one of the major mechanisms by which cells tend to restore their volume following hypo-osmotic stress [a process known as regulatory volume decrease (RVD)] (26, 57). Extracellular osmotic perturbations are not the only reason for alterations in cell volume. Effectively countering abrupt volume changes and maintaining relative volume constancy during active solute uptake, exocytosis, proliferation, and differentiation are major prerequisites for cell survival. Indeed, there is strong evidence that disordered or altered cell volume regulation is associated with apoptosis (57). Compelling support for such an association has been provided by demonstrating the direct link between apoptotic resistance conferred by antiapoptotic Bcl-2 protein and the strengthening of RVD capability due to upregulation of I\(_{\text{Cl,swell}}\) (48, 73).

The molecular nature of native I\(_{\text{Cl,swell}}\)-carrying VRACs has been identified, and several membrane proteins are considered as potential candidates (26, 57); these data are also consistent with CIC-3 protein involvement in prostate-specific VRAC, as well as with its upregulation in androgen-independent prostate cancer cell phenotypes (48).

When breast tumor cells were transfected with plasmids encoding either murine calcium-sensitive chloride channels 1 or 2 (mCLCA1 or mCLCA2), colony formation was greatly reduced relative to a vector-transfected control, demonstrating that calcium-sensitive chloride channel (CLCA) expression is deleterious to tumor cell survival (19). Furthermore, mammary epithelial cells overexpressing mCLCA2 had twice the rate of apoptosis of normal cells when subjected to serum starvation and formed multinuclear giants at a high frequency in normal culture, suggesting that mCLCA2 can promote either apoptosis or senescence (19).

**BCL-2 overexpression enhances the capability of RVD,** a cellular defensive process against hypotonic stress. In various clones of kidney cancer Madin-Darby canine kidney (MDCK) cells, hypotonic stress induced an outwardly rectified Cl\(^{-}\) current that was significantly upregulated by BCL-2 overexpression (73). Other fundamental characteristics of this channel were similar among different MDCK clones, such as sensitivity to Cl\(^{-}\) channel inhibitor, anion permeability, and time-dependent inactivation at more positive potential. Moreover, neutralization of endogenous BCL-2 by antibody blocks the normal RVD response and the activation of swelling-activated Cl\(^{-}\) channel in human cervical cancer HT-3 cells (73).

mtCLIC/CLIC4 is a p53- and tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\))-regulated cytoplasmic and mitochondrial protein that belongs to the CLIC family of intracellular chloride channels. mtCLIC associates with the inner mitochondrial membrane. Dual regulation of mtCLIC by two stress response pathways suggested that this chloride channel protein might contribute to the cellular response to cytotoxic stimuli. DNA damage or overexpression of p53 upregulates mtCLIC and induces apoptosis. Overexpression of mtCLIC by transient transfection reduces mitochondrial membrane potential, releases cytochrome c into the cytoplasm, activates caspases, and induces apoptosis (20). These studies indicate that mtCLIC, like Bax, Noxa, p53AIP1, and PUMA, participates in a stress-induced death pathway converging on mitochondria. In the same way, in human osteosarcoma cell lines SaOS and U2OS, CLIC4-antisense induction increased TNF-\(\alpha\)-mediated apoptosis without altering TNF-\(\alpha\)-induced nuclear factor-kB activity (75). Also, reducing CLIC proteins in tumor grafts of SP1 cells expressing a tetracycline-regulated CLIC4-antisense substantially inhibited tumor growth and induced tumor apoptosis. It has also been shown that in KCP-4 human epidermoid cancer cell line, which serves as a model of acquired resistance to cisplatin, has virtually no volume-sensitive, outwardly rectifying (VSOR) chloride channel activity (45). It was found that treatment with trichostatin A (TSA), a histone deacetylase inhibitor, caused VSOR chloride channel function to be partially restored. Treatment of the cells with both TSA and cisplatin resulted in an increase in caspase-3 activity. These effects were blocked by simultaneous treatment of the cells with a VSOR chloride channel blocker. These results indicate that restoration of the channel’s functional expression by TSA treatment leads to a decrease in the cisplatin resistance of KCP-4 cells suggesting the involvement of VSOR chloride channel in the cisplatin resistance of KCP-4 cancer cells (45). The same data have been obtained for nonsmall cell lung cancer (54). Cisplatin treatment induced an AVD and activated a Cl\(^{-}\) current that showed properties similar to the VSOR Cl\(^{-}\) current in wild-type A549 cells. Both the AVD process and VSOR Cl\(^{-}\) current were blocked by the chloride channel blocker 4,4’-disothiocyanostilbene-2,2’ disulfonic acid. However, the A549/CDDP cells, a model of acquired cisplatin...
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resistance cells, on the other hand, had almost no AVD process and VSOR Cl− current when treated with cisplatin. Treatment of A549/CDDP cells with TSA, partially restored the VSOR Cl− current and increased cisplatin-induced apoptosis rate. These results suggest that impaired activity of VSOR Cl− channels contributes to the cisplatin resistance in lung cancer.

Furthermore, cyclosporin A (CsA) induces apoptosis in a dose- and time-dependent manner in HepG2 human hepatoma cells (42). It induced also Cl− efflux, which was significantly blocked by niflumic acid (NA), a specific inhibitor. CsA increased intracellular Ca2+ concentration, and treatment with BAPTA/AM, an intracellular Ca2+ chelator, significantly inhibited the CsA-induced Cl− efflux, indicating that CsA induced Cl− efflux through the activation of calcium-activated chloride channels. Their inhibition with niflumic acid (NA), flufenamic acid (FA), 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), and DIDS markedly prevented the CsA-induced apoptosis suggesting that these channels may mediate apoptosis induced by CsA in HepG2 cells (42).

The transition of cancer cells to apoptosis-resistant phenotypes is associated with an increased capability for RVD, or the restoration of cell volume in response to hypoosmotic stress, because of the enhanced expression of VRACs. The latter, at least in some cell types, can involve CIC-3 (47, 48), a member of the CIC family, which is mostly known to function as an endosomal Cl−/H+ exchanger but can also function as PM Cl− channel. Consistent with this, the overexpression of CIC-3 in human bronchial epithelial cells (HBECs) has been reported to inhibit transforming growth factor-β (TGF-β)-induced apoptosis (12). Owing to its preferential targeting to intracellular compartments and Cl−/H+ exchanger function, the excess of CIC-3 was also found to increase the acidity of intracellular vesicles in NE tumor cell lines (BON, LCC-18, and QGP-1), thereby enhancing their resistance to the chemotherapeutic drug etoposide by almost twofold (85). Because these cells seemed to be deficient in common multidrug resistance transporters, the mechanism of enhanced drug resistance to etoposide was attributed to the CIC-3-mediated vesicular acidification, which represents a facilitating factor in vesicular drug sequestration.

Ca2+ Homeostasis and Store-Operated Ca2+ Entry Channels

The role of Ca2+ in the majority of cell-signaling pathways involved in carcinogenesis is well established. Calcium homeostasis, the consequences of calcium signaling, is an equilibrium between influx, efflux, and storage of Ca2+. From a physiological point of view, Ca2+ signaling is involved in the manifestation of cell phenotype, proliferation, differentiation, apoptosis, and in cellular activities such as contraction or secretion or cell excitability. Thus each cellular phenotype, whether normal or pathological, is characterized by a particular “calcium signature” reflecting its kinetics, amplitude, and subcellular localization of the calcium signals.

In cancer epithelial cells, as in other nonexcitable cell types, Ca2+ entry from extracellular space is mainly supported by the “capacitative calcium entry” mechanism, also known as “store-operated calcium entry” (SOCE) (62). This mechanism is capable of monitoring ER Ca2+ filling, enabling influx only when ER content is essentially decreased. It is mediated via specialized plasma membrane store-operated Ca2+-permeable channels (SOC). The common physiological trigger for the activation of these channels is inositol trisphosphate-(IP3)-induced Ca2+ release from the ER in response to the stimulation of surface receptors coupled to the phospholipase C-(PLC)-catalyzed inositol phospholipid breakdown signaling pathway. This is why, when these channels have been identified for the first time by patch-clamp experiments, they were termed “Ca2+ release-activated channels” (CRAC) (38).

To effectively avoid apoptosis, cancer cells must utilize mechanisms that substantially reduce or even prevent Ca2+ influx, for example by downregulating the expression of Ca2+-permeable channels or the signaling pathways that lead to their activation. Consistent with this, hormone-refractory apoptosis-resistant phenotypes of hormone refractory cancer cells are characterized by markedly reduced levels of store-operated calcium entry (67, 80, 81), which prevents Ca2+ overload in response to pro-apoptotic stimuli, thereby reducing the effectiveness of mitochondrial and cytoplasmic apoptotic pathways. In this respect, it is important to assess the role of STIM1 and CRACM1 (Orai1) proteins in cancer cell SOCE. The first work on Orai1 involvement in apoptosis has shown that Orai1 protein represents the major molecular component of endogenous store-operated Ca2+ entry in human prostate cancer cells and constitutes the principal source of Ca2+ influx used by the cell to trigger apoptosis (23). The downregulation of Orai1, and consequently SOCE, protects the cells from diverse apoptosis-inducing pathways, such as those induced by thapsigargin (Tg), TNF-α, and cisplatin/oxaliplatin. The transfection of Orai1 mutants such as R91W, a selectivity mutant, and L273S, a coiled-coil mutant, into the cells significantly decreased both SOCE and the rate of Tg-induced apoptosis. This suggests that the functional coupling of STIM1 to Orai1, as well as Orai1 Ca2+ selectivity as a channel, is required for its pro-apoptotic effects. Thus Orai1 plays a pivotal role in the triggering of apoptosis, irrespective of apoptosis-inducing stimuli, and in the establishment of an apoptosis-resistant phenotype in prostate cancer cells (23). Thus the decrease in SOCE may be responsible for the acquiring of apoptosis-resistant phenotype an important feature of all cancers.

Role of TRP Superfamily of Channels in the Control of Apoptosis

In recent years, some members of the widely investigated family of mammalian homologs of the Drosophila transient receptor potential (TRP) channel were viewed as being involved in SOC formation (for recent reviews see Refs. 62 and 68). In the studies conducted on prostate cancer LNCaP cells the involvement of the members of the “canonical” TRP subfamily TRPC1 and TRPC4 in prostate-specific endogenous SOCs has been suggested (78, 79). However, the expression pattern of TRPC1 and TRPC4 was not modified in androgen-independent apoptosis-resistant prostate cancer cells (79).

Interestingly, the endogenous expression of TRPC1, TRPC3, and TRPV6 proteins in prostate cancer cells was shown to be controlled by the ER Ca2+ filling: after a prolonged (24–48 h) depletion of the stores with Tg, a potent proapoptotic agent, their expression increased (64). Enhanced expression of apparently store-dependent TRP members after prolonged ER store depletion is difficult to reconcile with the findings that androgen-independent, apoptosis-resistant prostate cancer cell phe-
notypes, for which chronic underfilling of the ER Ca\(^{2+}\) pool represents a new level of equilibrium helping them to withstand ER stress-mediated apoptosis, are characterized by reduced SOCE (80, 81). It is, therefore, likely that native SOC in cancer cells is a much more complex entity, whose functional expression cannot be directly correlated with any of the implicated TRP members.

Cold/menthol-sensitive TRPM8 of the “melastatin” TRP subfamily is yet another TRP member that has recently emerged as an important player in normal and pathological development of different tissues. TRPM8 is expressed not only in the plasma membrane of prostate cells, as initially anticipated, but also in the ER membrane, where it operates as an ER in the plasma membrane of prostate cells, as initially anticipated, also in the ER membrane, where it operates as an ER in the plasma membrane of prostate cells, as initially anticipated, TRP members.

Cold/menthol-sensitive TRPM8 of the “melastatin” TRP subfamily is yet another TRP member that has recently emerged as an important player in normal and pathological development of different tissues. TRPM8 is expressed not only in the plasma membrane of prostate cells, as initially anticipated, but also in the ER membrane, where it operates as an ER Ca\(^{2+}\) release channel involved in the activation of SOCE in response to cold/menthol stimulus also known to induce apoptosis (76, 93). Moreover, whereas remaining at moderate levels in a normal prostate, TRPM8 expression strongly increases in prostate cancer, suggesting that the channel is a pro-oncogenic actor in these cells (77). Other nonprostatic primary human tumors (breast, colon, lung, and skin) are also known to become highly enriched in TRPM8, although it is virtually undetectable in corresponding normal tissues (77). Thus even this initial information strongly pointed to much broader roles of TRPM8 beyond cold sensation, especially during carcinogenesis. In another cancer model, it was shown that menthol can induce mitochondrial membrane depolarization via the TRPM8 channel in cells of the human bladder cancer cell line T24, resulting in cell death; however, the precise mechanism of action of menthol in bladder cancer remains unknown (49).

The activity of a member of the “vanilloid” TRP subfamily, TRPV6, may also have some relation to the sequence of events following the ER store depletion in LNCaP cells, as its antisense knockdown decreases endogenous store-operated membrane current (ISOC) (79), but the mechanisms underlying such TRPV6 activation in LNCaP cells remain elusive. In 2007 Lehen’kyi et al. (46) published the direct implication of TRPV6 channel in apoptosis resistance of hormone-sensitive prostate cancer cells that may be mediated by activation of NF\(\text{aT}\) transcription factor. However, the precise mechanism of TRPV6 contribution to apoptosis resistance remains unknown. Another member of vanilloid family TRPV2 has also been suggested to contribute to apoptosis resistance of androgen-independent prostate cancer cell lines, likely by augmenting Ca\(^{2+}\)-influx into these cells (55). Although, such its regulatory effect is not universal, since in human urothelial carcinoma cells the regulation of calcium influx through these channels leads directly to the death of these cells (91).

Decreased levels of the expression of Ca\(^{2+}\)-permeable channels with activation mechanisms other than store depletion also contribute to the ability of cancer cells to escape apoptosis. For instance, the antisense knockdown of TRPM2 (an endogenous ADP-ribose-sensitive, cADP-ribose-sensitive, and H\(_2\)O\(_2\)-sensitive TRP member) in rat insulinoma RIN-5F cells and the U937 monocyte cell line has been shown to significantly suppress Ca\(^{2+}\) influx and cell death induced by H\(_2\)O\(_2\), whereas the heterologous overexpression of this channel enhanced H\(_2\)O\(_2\)-induced apoptosis (35). Unexpectedly, the lack of the TRPV6 channel, rather than of the capsaicin receptor TRPV1, was found to suppress apoptosis of gastric cancer cells under capsaicin treatment (13). Although in urothelial cancer cells activation of TRPV1 channel directly triggers apoptosis via Fas/CD95-mediated intrinsic and extrinsic apoptotic pathways (3). At the same time, the functional knockout of TRPM2, as well as overexpression of wild-type TRPM2, increased melanoma susceptibility to apoptosis and necrosis (59).

The superfamily of TRP channels represents a relatively new division of Ca\(^{2+}\)-permeable channels particularly involved in the control of calcium influx participating in apoptosis machinery. Sufficient evidence has been collected to date to definitively implicate these channels in the apoptosis control performed by cancer cell. TRP channels, therefore, represent a substantial field of study of apoptosis presenting TRP channels as prospective pharmaceutical targets.

![Diagram](http://ajpcell.physiology.org.org/ by 10.220.32.246 on July 24, 2017)
Voltage-Gated Calcium Channels

The involvement of voltage-gated Ca\(^{2+}\) channels (VGCC) in regulating balance between proliferation and apoptosis in the cell has been known since at least the 1980s. Thus it was known that the percentage of epithelial rat ventral prostate cells undergoing apoptosis in response to androgen ablation is reduced by administering voltage-gated Ca\(^{2+}\) channel VGCC blockers such as nifedipine and verapamil (14, 52). These observations gave rise to the hypothesis that calcium channel blockers may increase the risk of prostate cancer by inhibiting calcium signal-mediated apoptosis (4).

Despite this evidence, the presence of VGCC activity has not been detected in cancer epithelial cells by means of electrophysiology. On the other hand, evidence has been presented showing that the small proportion of undifferentiated LNCaP cells display an LVA Ca\(^{2+}\) current carried by T-type Ca\(^{2+}\) channels, as well as the significantly increased current density in the neuroendocrine differentiation of LNCaP cells induced by either long-term treatments with membrane-permeable cAMP analogs or by steroid-deprived culture medium (51). RT-PCR experiments demonstrated that only mRNA for Ca\(^{3.2}\) isoform of T-type Ca\(^{2+}\) channel 1 subunit is expressed in LNCaP cells and becomes highly elevated during NE differentiation (51). It was also shown that basal Ca\(^{2+}\) entry through this channel at resting membrane potential due to the presence of a prominent “window current” is likely to facilitate neurite elongation, thereby promoting neuroendocrine differentiation. It was suggested that this channel could be also involved in the stimulation of mitogenic factor secretion, thus representing an attractive potential target for future therapeutic strategies (27, 51). However, whether or not these channels contribute to the enhanced antiapoptotic potential of apoptosis-resistant cells is not yet clear.

Metabotropic Channels and Cancer Cell Apoptosis

In 1990, Maneckjee and Minna (50) described the presence of \(\alpha7\)-nAChR (nicotinic acetylcholine receptor) on small and nonsmall lung cancer cell lines. More recently, Lam et al. (43) suggested that nAChRs play a significant role in lung cancer predisposition and natural history. Furthermore, an important study of Song et al. (74) presented data that small cell lung cancer express a cholinergic autocrine loop that can regulate

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### Table 1. Ion channel involved in apoptosis

<table>
<thead>
<tr>
<th>Family, Ion Selectivity</th>
<th>Subfamily</th>
<th>IUPHAR Name, Other Names</th>
<th>Role in Apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cys-loop, cationic Ca(^{2+})-permeable</td>
<td>nAChR</td>
<td>(\alpha7)</td>
<td>ND</td>
</tr>
<tr>
<td>Purinergic, cationic Ca(^{2+})-permeable</td>
<td>P2X</td>
<td>P2X2, P2X4, P2X7</td>
<td>Apoptosis resistance</td>
</tr>
<tr>
<td>Voltage-gated Na(^{+}) (Na(_v))</td>
<td>Na(_v),1</td>
<td>Na(_v),1.5, Na(_v),1.6, h1, sm2, cardiac sodium channel</td>
<td>Apoptosis resistance</td>
</tr>
<tr>
<td>Voltage-gated Ca(^{2+}) (Ca(_v))</td>
<td>Ca(_v),1 (L-type)</td>
<td>Subunits are ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ca(^{2+})-permeable</td>
<td>Ca(_v),1, Ca(_v),2, Ca(_v),3 (T-type)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Voltage-gated K(^{+}) (K(_v))</td>
<td>K(_v),1</td>
<td>K(_v),1.5, K(_v),2, K(_v),3, K(_v),4</td>
<td>Apoptosis resistance</td>
</tr>
<tr>
<td>Background K(^{+}) (K(_b))</td>
<td>K(_b),10, K(_b),11, K(_b),11.1, K(_b),11.2, K(_b),11.3</td>
<td>(\alpha7)-related gene</td>
<td>Apoptosis resistance</td>
</tr>
<tr>
<td>Ca(^{2+})-activated K(^{+}) (K(_{ca}))</td>
<td>K(<em>{ca}),1 (BK(</em>{ca}))</td>
<td>K(_{ca}),1, BK, Slo, Slo1, maxi K</td>
<td>Apoptosis resistance</td>
</tr>
<tr>
<td>Inwardly rectifying K(^{+}) (K(_{ir}))</td>
<td>K(_{ir}),2 (GIRK, G protein-acti-vated)</td>
<td>K(<em>{ir}),2.1, K(</em>{ir}),2.3, K(<em>{ir}),2.4, K(</em>{ir}),2.5</td>
<td>Apoptosis resistance</td>
</tr>
<tr>
<td>Background K(^{+}) (K(_{b}))</td>
<td>K(_{b}),2</td>
<td>K(<em>{b}),2.1, TREK, K(</em>{b}),2.2, TASK-3, KCNK9</td>
<td>Increased survival</td>
</tr>
<tr>
<td>SOC, Ca(^{2+})-selective</td>
<td>Orai/STIM</td>
<td>Orai1/STIM1, Orai2/STIM2</td>
<td>Apoptosis resistance</td>
</tr>
<tr>
<td>TRP, cationic Ca(^{2+})-permeable</td>
<td>TRPC</td>
<td>TRPC1, TRPC2, TRPC3, TRPC4, TRPC6</td>
<td>Apoptosis resistance</td>
</tr>
<tr>
<td>TRPV</td>
<td>TRPV1, TRPV2, TRPV3, TRPV4, TRPV5</td>
<td>TRPV1, TRPV2, TRPV3</td>
<td>Apoptosis resistance</td>
</tr>
<tr>
<td>TRPM</td>
<td>TRPM1, TRPM2, TRPM3, TRPM4, TRPM5, TRPM6, TRPM7, TRPM8</td>
<td>TRPM1, TRPM2, TRPM3, TRPM4, TRPM5, TRPM6, TRPM7, TRPM8</td>
<td>Apoptosis resistance</td>
</tr>
<tr>
<td>Na(^{+}) nonvoltage-gated, DEG-related</td>
<td>ENaC</td>
<td>ENaC, ENaC</td>
<td>ND</td>
</tr>
<tr>
<td>Cl(-) channels</td>
<td>CIC</td>
<td>CIC-3</td>
<td>Apoptosis resistance</td>
</tr>
</tbody>
</table>

The table lists ion channel subunits, for which the involvement in apoptosis has been established. IUPHAR names of subunits along with other names found in the literature are underlined. Upward arrow and downward arrow indicate increase or decrease in channel expression or functional activity and the acquired specified effect, respectfully; ND, not determined. Question mark depicts controversial results. See text for additional information and definition of abbreviations.
cell growth and lead to the inhibition of drug-induced apoptosis. The results of other studies showed that α-CbT, a powerful high affinity α7-nACHR inhibitor, induces antitumor effects against nonsmall cell lung cancer and malignant pleural mesothelioma by triggering apoptosis (61). The probable mechanism of nACHRs-induced apoptosis resistance likely lies in the activation of PI3K/AKT that directly phosphorylates and inactivates the pro-apoptotic function of Bax (88) and upregulation of nuclear factor-κB (16).

It has also been demonstrated that the activation of purinoceptors, such as P2X, by ATP leads to apoptosis of hormone-refractory PC3 cells (10), though the mechanisms remain obscure. The reason may be that extracellular ATP induces the activation of multiple caspases including caspase-1, -3, and -8, required for apoptotic but not necrotic alterations of ATP-induced cell death shown for myeloma (21).

Another metabotropic receptor, glycine receptor (GlyR) α1 subunit GLRA1 contains in its gene a sequence motif for neuron-restrictive silencer factor (NRSF) binding protein within its 5′-UTR (56). While no GLRA1 transcripts were found in the majority of nonmalignant biopsies, the GLRA1 transcripts were detected in the majority of small lung cancer cells (31). The authors showed that the reconstitution of NRSF expression induced apoptosis in lung cancer cells via the inhibition of GlyR receptor suggesting its role in apoptosis resistance.

Conclusions

Ion channels are known to play an important role in regulation of balance between apoptosis and proliferation in the cells. Recent reports clearly show their involvement in triggering the onset of apoptosis or even progression of cancer cell toward apoptosis-resistant phenotype. The dual role that some of them play due to their permeability to both calcium and potassium is especially interesting and is often explained as a part of the particular cancer model and the triggered downstream events. Generally, the emergence of more excitable phenotype by the reexpression of voltage-gated sodium channels and decrease in potassium efflux is a characteristic feature of advanced stage of cancer. As for the calcium, its dual role strongly depends on particular calcium signature within the cell: the transit oscillations or sustained increase, the involvement of ER or mitochondria, and the constitutive or store-operated entry. The increased expression of chloride channels is accompanied by the enhanced ability of cancer cell to regulate its volume and resist to apoptosis and even to contribute to chemoresistance of some cancers. General interplay between the ion channels and their effectors is briefly summarized in Fig. 2, and the overview of the involved ion channels can be found in Table 1. Given the obviously important role of ion channels in cancer development and progression and the growing evidences on their role therein, further investigations are still needed to better elucidate their role in apoptosis regulating mechanisms and to elaborate the ion channels as effective discriminative markers and therapeutic targets in foreseeable future.

DISCLOSURES

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REFERENCES


