ISSN 1669-5402 (Print) ISSN 1669-5410 (Online)

Physiological Mini-Reviews

Edited by the Argentine Physiological Society.

Vol. 3, N° 6, June - July 2008.

http://www.mini.reviews.safisiol.org.ar

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[ISSN 1669-5402 (Print); ISSN 1669-5410 (Online)]

Edited by the **Argentine Physiological Society**

Journal address: Sociedad Argentina de Fisiología, Universidad Favaloro, Solís 453 (1078), Ciudad de Buenos Aires Argentina.
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http://www.mini.reviews.safisiol.org.ar

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(Resol. IGJ 763-04)

ION CHANNELS IN K562 HUMAN LEUKEMIC CELLS AND THE RELATION WITH THE MULTIDRUG RESISTANCE.

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Enhanced proliferation, aberrant differentiation, and impaired ability to die are the prime reasons for abnormal tissue growth, which can eventually turn into uncontrolled expansion and invasion, characteristic of cancer. Ion channels contribute to virtually all basic cellular processes, including those for maintaining tissue homeostasis as proliferation, differentiation, and apoptosis (1). Changes in cell volume are crucial events during both cell proliferation and apoptosis and thus on tumor development and growth (2). Cell proliferation depends on an increased cell volume and cell shrinkage is a characteristic of apoptosis. Potassium, calcium, chloride channels and others are abnormally expressed in the membrane of tumor cells. By the regulation of membrane voltage, cell volume, Ca^{2+} signaling, cytosolic pH and cell cycle, they can adjust the cell proliferation and apoptosis.

The specific blockade of ion channels may not only allow the dissection of the channel role in distinct physiologic processes, but because of the implication of them in tumor development, it may also offer an opportunity for the treatment of cancer. However, many ion channels are structurally similar to one another, and it has been notoriously difficult to obtain specific blockers for any given channel, thus it is essential to generate more potent and specific inhibitors of ion channels before using them in human therapeutics.

MULTIDRUG RESISTANCE.

Patients who receive chemotherapy often experience resistance to a broad spectrum of chemotherapeutic agents (3). The phenomenon, termed multidrug resistance (MDR) is one of the major obstacles to treatment of malignancies and is often associated with the over-expression of P-glycoprotein (P-gp or MDR1), a membrane protein expressed in numerous multidrug-resistant tumor cells. This protein interacts with many chemically unrelated compounds including chemotherapeutic agents, and uses the energy from ATP hydrolysis to transport these drugs out of the cells, limiting its intracellular accumulation and hence reducing the efficacy (4,5). P-gp is also expressed on the apical membranes of various normal epithelia and at the blood-tissue barrier in endothelia, but its physiological function is still under debate (4) The over-expression of P-gp has been also observed in different hematological malignances and is associated with poor prognosis in patients with acute myeloid leukemia (6).

The human myeloid cell line K562 (K562-WT) was established from a patient in blastic transformation. These cells are arrested in very early stages of development and they can be induced to differentiate along with erythroid, monocyte-macrophage and megakaryocytic lineages in response to various stimuli (7). In addition, the interest in these cells is based on they can be exposed to increasing amounts of vincristine and selecting

by this way a vincristine-resistant culture (K562-Vinc) in order to study the multidrug resistance phenomenon (Fig. 1).

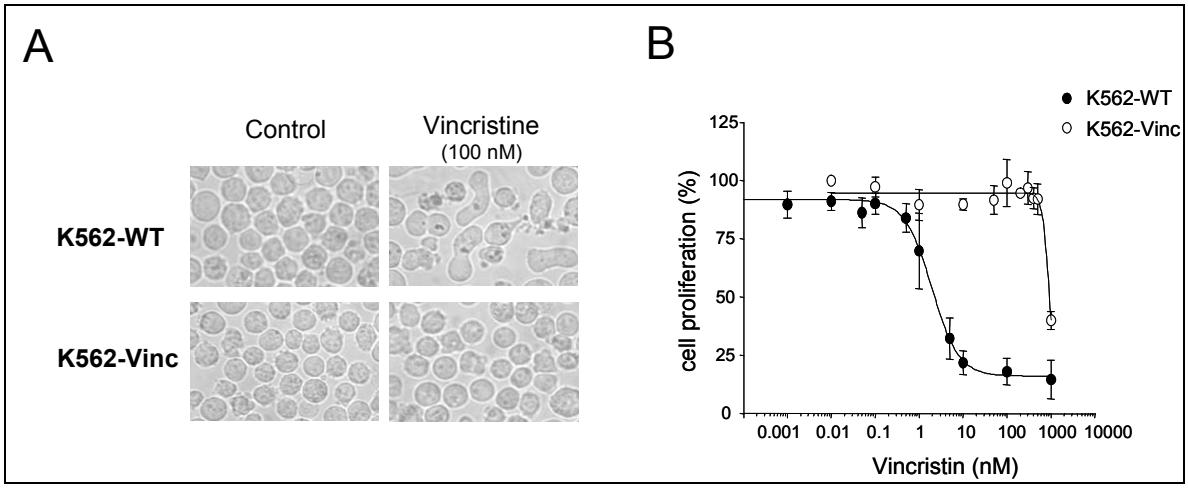

Figure 1: *Cytotoxic effect of vincristine in K562-WT and K562-Vinc.* ***A.*** *Morphological changes in both cell lines after the incubation with 100 nM of vincristine.* ***B.*** *Cell proliferation was studied by MTT assay after 72 h of incubation at the indicated vincristine concentration. Data represent mean ± SD from four separate experiments and curve lines are the dose-response best fits.*

ABC FAMILY.

P-gp belongs to the ABC superfamily of transporters, multidomain proteins that may be encoded by different genes. A common minimum functional unit seems to consist of two transmembrane domains (TMDs), each of which contains multiple a-helices, and two cytoplasmic nucleotide binding domains (NBDs). The TMDs form substrate binding sites and provide a translocation path across the membrane, whilst the NBDs bind and hydrolyze ATP, the energy of which powers the drug export process in the case of the P-gp (4,5). At least three human ABC transporters, namely P-glycoprotein (MDR1; ABCB1), multidrug resistance associated protein 1(MRP1; ABCC1) and breast cancer resistance protein (BCRP; ABCG2), have been shown in vitro to have the capability to efflux a structurally and mechanistically diverse range of anti-cancer agents (8).

Other members of the ABC transporter superfamily are the sulfonylurea receptor (SUR) and the cystic fibrosis transmembrane regulator (CFTR). SUR binds sulfonylurea compounds such as glibenclamide and confers sulfonylurea inhibition upon a separate ATP-gated K^+ channel protein in pancreatic cells. CFTR is a Cl^- channel expressed in epithelia and other cells (9). Malfunction of the CFTR leads to cystic fibrosis, an autosomal recessive disease caused by mutations in the CFTR gene. The commonest mutation (70 %) is the deletion of phenylalanine at codon 508 (ΔF508) (10). Modulation of normal CFTR channel function involves ATP binding and hydrolysis at the NBDs in combination with phosphorylation by protein kinase A (PKA) at the cytoplasmic regulatory region of the channel named R domain (11). Forskolin, an adenylate cyclase activator, is used to induce channel activation.

In addition, a switch in expression from one gene to the other was observed in epithelial intestine where the expression changes from CFTR to MDR1 as the cells migrate across the crypt-villus boundary (12); a similar phenomenon occurred in human placenta (13). On the other hand, Dragomir and Roomans (14) found that colchicine-resistant air-

way epithelial cell lines had higher expression of multidrug resistance protein compared to untreated cells whereas CFTR showed no significant changes.

A dual function is not unusual in ABC proteins. P-gp was proposed as a chloride channel per se and also as a regulator of others Cl^- channels (15). Moreover, CFTR not only acts as a Cl^- channel, but may fulfill several other cellular functions, particularly by regulating others membrane conductances such as the outward rectifying anion channel (ORCC) and the epithelial sodium channel (ENaC) (10).

P-gp AND CFTR IN K562 CELLS.

P-glycoprotein (Pgp) product is an obstacle in the treatment of hematological malignancies (6). This protein is over-expressed in the vincristine-resistant leukemic K562 cells (K562-Vinc) which were obtained by culturing the cells in the presence of 150 nM vincristine sulphate. As P-gp belongs to the ABC superfamily we found of interest to evaluate if the Cl^- channel CFTR, other member of this family, is functionally active in both, the wild-type K562 cells and in its resistant counterpart.

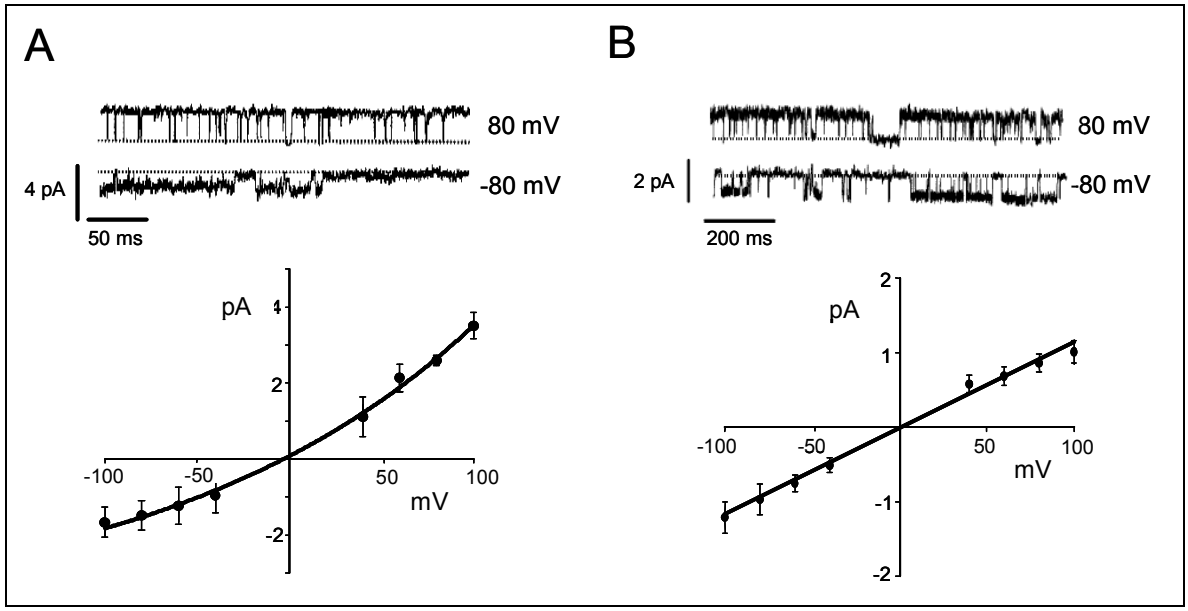

Figure 2: *cAMP-activated chloride channels in K562-WT. Representative single channel recordings in excised inside-out patches held at 80 and -80 mV in symmetrical 140 mM NaCl solution showing the activity of two types of channels, ORCC (**A**) and CFTR (**B**). Dotted lines indicate the closed state of the channels. The current-voltage (I-V) relationship of single-channel currents allowed an estimation of the conductance being ~40 pS (at depolarizing pulses) for ORCC and ~12 pS for CFTR.*

Patch clamp experiments revealed two types of Cl^- channels activated by cAMP in wild-type K562 cells, one consistent with ORCC type channel, with an outwardly rectification, 40 picoSiemens (pS) of single channel conductance at depolarizing voltages and blocked by glibenclamide or diphenylamine-2-carboxylate, DPC (Fig. 2 A) (16). The other, with non-rectifying 11 pS conductance, inhibited by glibenclamide and anti-CFTR antibodies, with perm-selectivity and kinetic parameters, was consistent with CFTR channel (Fig. 2 B) (17). Further, RT-PCR analysis performed in K562-WT and K562-Vinc cells using specific primers showed expected bands of ~295 and ~167 bp corresponding to human CFTR and MDR1 products. The semi-quantification using $ß_2$-microglobulin as internal con-

trol showed no differences between CFTR mRNA levels in both cells types whereas a 20-fold increase in MDR1 mRNA in comparison with the K562-WT levels was observed in the vincristine-resistant cells. In addition, the presence of the CFTR protein in both K562 cells was detected by western blot and immunocytochemistry. The expression of P-gp was also confirmed by western blot detecting a ~170 kDa expected band in resistant K562 cells while this protein was not observed in the K562-WT cells, indicating an over-expression of P-gp in a vincritine-resistant variant.

In order to study if the CFTR activity is modified by P-gp expression, whole cell current recordings were obtained in both K562 and K562-Vinc cells. For these experiments we used pipette and bath solutions suitable for studying chloride currents. In view of the fact that two types of cAMP-activated chloride channels are expressed in K562 cells, we tried to differentiate the currents through ORCC and CFTR channels by exploiting their dissimilar sensitivity to Cl^- channel blockers (18) CFTR channels are characterized by their sensitivity to block in an irreversible manner by intracellular but not extracellular disulfonic stilbenes such as DIDS and their reversible blockade by DPC applied to either extra or intracellular sides. On the other hand, ORCC are blocked by extracellular DIDS and DPC (19). Thus, the effect of DIDS applied from the extracellular side after AMPc stimulation (with forskolin), is an appropriate tool to differentiate the ORCC and CFTR contributions to the whole current. Fig. 3 shows the increase in current by forskolin in K562-WT and the effects of DIDS and DPC added in a sequential way. It is clear that a fraction of the current can be inhibited by 500 µM DIDS and a subsequent addition of 500 µM DPC inhibited the remaining fraction of cAMP-activated current in K562-WT. From these experiments we concluded that about 70% of the cAMP-stimulated current in K562-WT cell line may be conducted through ORCC channels and the remaining through CFTR channels. The activities of both CFTR and ORCC were also unaffected in the vincristine-resistant cells (20).

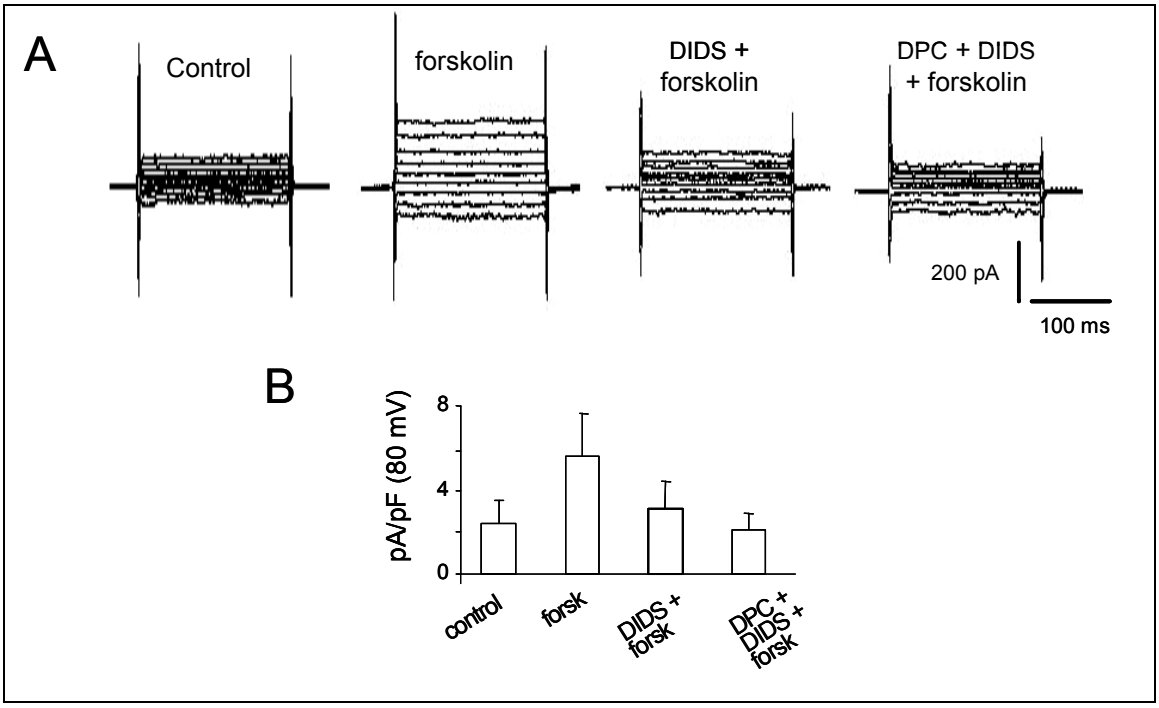

Figure 3: *ORCC and CFTR contribution to the whole cell currents activated by forskolin.* ***A****. Representative whole cell currents recordings in K562-WT cell after the indicated treatments.* ***B.*** *The histogram illustrates the current density (pA/pF; normalized by the cell capacitance) at 80 mV for the same treatments shown above. Data represent mean ± SD from five separate experiments.*

CONTROL OF P-gp EXPRESSION.

The molecular mechanisms associated to the expression control of P-gp are not complete elucidated, even though evidences suggest that NF-?B may be one of the transcription factors involved in this process (21). NF-?B is the prototype of a family of transcription factor made by dimerization of five subunits: p65, c-Rel, RelB, p50, and p52. Although many dimeric forms of NF-?B have been detected, the more ubiquitous heterodimer is the p65 and p50. Before cell stimulation, most of NF-?B is present in the cytoplasm as an inactive complex consisting of Rel hetero-dimer and the inhibitor subunit (I?B). After stimulation, I?B undergoes phosphorylation by I?B Kinases (IKK) and ubiquitination-dependent degradation by the proteosome, thus NF-?B dimers translocate to the nucleus where they bind to a specific consensus sequence in the DNA resulting in the activation of target genes transcription (22).

NF-?B is a mediator of inducible gene expression in response to inflammatory cytokines, pathogens and several stress signals, and is known for its crucial roles in the immune system, cell proliferation and transformation, apoptosis and tumor development (23). A constitutive NF-?B activity has been observed in several haematological malignancies and moreover, antineoplastic agents promote the activation of NF-?B when they are used in acute conditions, even though the information originated from the use of those drugs in the long term is scarce (see 24). However, vincristine did not increase NF-?B activity in our conditions because we found that NF-?B was constitutively activated in both cell lines but wild type K562 cells exhibited a higher activity compared to resistant cells. This result could indicate that this factor was not likely directly involved in the over expression of P-gp in K562-Vinc and also that its participation in the regulation of the human MDR1 gene is cell type specific.

It is well known that most of the NF-?B target genes have anti-apoptotic functions and it has been suggested that inhibition of NF-?B activation may comprise a useful tool to increase apoptosis sensitivity in the cancer therapy (25, 26). With the aim of investigate if the inhibition of NF-kB may overcome multidrug resistance we incubated both cell lines with BAY 11-7082, an inhibitor of IKK which leads to reduced I?Ba proteasomal degradation, thereby resulting in a decreased nuclear NF-?B (27). First, we demonstrate that P-gp over-expression in K562-Vinc cells could be associated to a MDR phenotype from their resistance to the cytotoxic effect of structurally unrelated drugs including imatinib. Preliminary studies indicate that the inhibition of NF-?B by BAY 11-7082 sensitized multidrug resistant human leukemic cells rather than parental ones to cell death, and combinations of this drug with imatinib for 24 h also increased this effect (Figure 4).

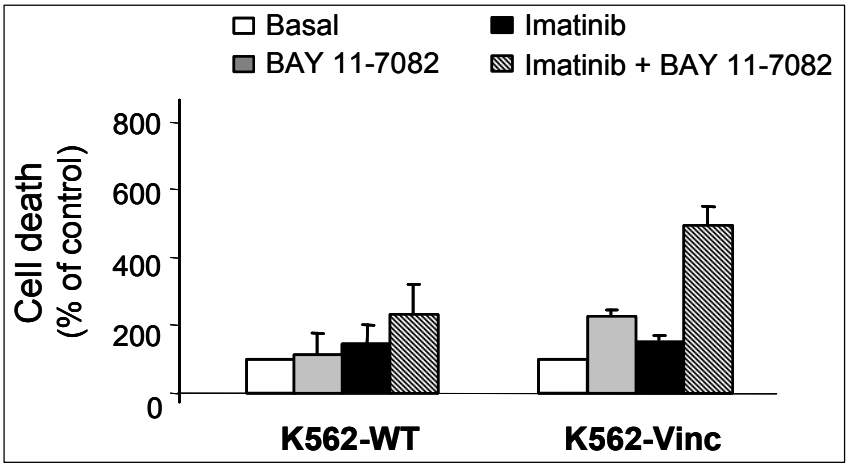

Figure 4: *Effect of NF-?B inhibition over cell death. Preliminary studies showed that the incubation with BAY 11-7082 (2.5 μM, 24 h) increased the percentage of annexin V positive cells in K562-Vinc but not in K562-WT. Combinations of BAY 11-7082 with imatinib also increased this cytotoxic effect in resistant cells (n=3). Both cells were exposed to equipotent concentration of imatinib (corresponding to their IC_{50}); 0.15 μM and 1.5 μM for K562-WT and K562-Vinc, respectively.*

CONCLUSIONS.

1) K562 cells are a useful model for studying the expression and function of ion channels in hematological malignancies. The presence of CFTR in this cell line confirms the housekeeping character of the gene involved in a great range of functions such as secretion of antibodies and cytokines by lymphocytes and bronchial epithelial cells, transmembrane ATP release in red blood cells, macrophage activation, apoptosis in fibroblasts and cell differentiation and proliferation.

2) Contrarily to previous reports in other cells, the expression of CFTR in K562 cells was not affected by the over-expression of P-glycoprotein. Both, P-gp and CFTR exhibit complementary patterns of expression in most of the examined epithelial cells, that is cells expressing CFTR do not express P-gp and vice versa leading to the speculation that these two proteins have a common function (12,13). This regulation of Cl^- channels could make their co-expression redundant and unnecessary. The results presented for us indicate that MDR1 over-expression in human leukemia cells does not repress CFTR expression. It has been suggested that a selective down-regulation of CFTR is elicited by acquisition of the multidrug resistance phenotype and that induction of P-gp expression leads to a reversible repression of CFTR biosynthesis (28). Although the physiological function of such regulation or the nature of these signals is still unknown, the expression of CFTR and MDR1 is cell-specific and subject to hormonal, humoral and temporal regulation. The overlap between CFTR and MDR1 found in K562 cells could be of particular interest since it has been suggested that in cystic fibrosis, patients with deficient CFTR protein could be complemented by other ABC proteins activity (29).

3) We present evidence that the development of multidrug resistance (MDR) in human chronic myeloid leukemia cell line is accompanied with the over-expression of P-gp. This MDR phenotype conferred imatinib resistance which could be reversed by inhibition of NF-?B pathway. These results suggest that NF-?B could be playing a key role as an anti-apoptotic factor in cells with an MDR phenotype, which is independent of the resistance mechanism developed (30). Moreover, this knowledge could be particularly useful for the development in the future of more effective therapies anti-cancer.

Acknowledgments: YA and BAK are members of the National Council of Research. This work was supported by grants from the CONICET (PIP 5065) and Agencia de Promoción Científica (PICT 2006, 090).

REFERENCES.

1. **Ashcroft F.** Ion channels and disease. Elsevier, NY, 2000.
2. **Lang F, Gulbins E, Szabo I et al.** Cell volume and the regulation of apoptotic cell death. J Mol Recognit 17: 473-480, 2004.
3. **Di Nicolantonio F, Mercer SJ, Knight LA et al.** Cancer cell adaptation to chemotherapy. BMC Cancer 5:78, 2005.
4. **Stein WD.** Kinetics of the multidrug transporter (P-glycoprotein) and its reversal. Physiol Rev 77:545-590, 1997.
5. **Loo TW, Clarke DM.** Recent progress in understanding the mechanism of P-glycoprotein-mediated drug efflux. J Membr Biol 206:173-185, 2005.
6. **Trnková Z, Bedrlíková R, Marková J, Michalová K, Stöckbauer P, Schwarz J.** Semiquantitative RT-PCR evaluation of the MDR1 gene expression in patients with acute myeloid leukaemia. Neoplasma 54:383-390, 2007.

7. **Kubohara Y.** Effects of differentiation-inducing factors of Dictyostelium discoideum on human leukemia K562 cells: DIF-3 is the most potent anti-leukemic agent. Eur J Pharmacol 381:57–62, 1999.
8. **Dean M, Rzhetsky A, Allikmets R.** The human ATP-binding cassette (ABC) transporter superfamily. Genome Res 11: 1156-1166, 2001.
9. **Gadsby D, Nagel G, Hwang T-C.** The CFTR chloride channel of mammalian heart. Ann Rev Physiol 57: 387-441, 1995.
10. **Kunzelmann K, Schreiber R.** CFTR, a regulator of channels. J Membr Biol 168: 1–8, 1999.
11. **Sheppard D, Welsh M.** Structure and function of the CFTR chloride channel. Physiol Rev 79: S23-S45, 1999.
12. **Trezise AE, Romano PR, Gill DR et al.** The multidrug resistance and cystic fibrosis genes have complementary patterns of epithelial expression. EMBO J 11: 4291-4303, 1992.
13. **Mylona P, Glazier JD, Greenwood SL, Sides MK, Sibley CP**. Expression of the cystic fibrosis (CF) and multidrug resistance (MDR1) genes during development and differentiation in the human placenta. Mol Hum Reprod 2:693-698, 1996.
14. **Dragomir A, Roomans G.** Increased chloride efflux in colchicine-resistant airway epithelial cell lines. Biochem Pharmacol 68: 253-261, 2004.
15. **Vanoye C, Castro A, Pourcher T, Reuss L, Altenberg G.** Phosphorylation of P-glycoprotein by PKA and PKC modulates swelling-activated Cl- currents. Am J Physiol 276: C370-C378, 1999.
16. **Assef YA, Kotsias BA.** An outwardly rectifying anion channel in human leukaemic K562 cells. Pflugers Arch 444:816-820, 2002.
17. **Assef YA, Damiano AE, Zotta E, Ibarra C, Kotsias BA.** CFTR in K562 human leukemic cells. Am J Physiol Cell Physiol 285:C480-488, 2003.
18. **Schwiebert E, Flotte T, Cutting G, Guggino W.** Both CFTR and outwardly rectifying chloride channels contribute to cAMP-stimulated whole cell chloride currents. Am J Physiol 266: C1464-C1477, 1994.
19. **Zhang Z, Zeltwanger S, McCarty N.** Direct comparison of NPPB and DPC as probes of CFTR expressed in Xenopus oocytes. J Membr Biol 175: 35-52, 2000.
20. **Assef YA, Cavarra SM, Damiano AE, Ibarra C, Kotsias BA.** Ionic currents in multidrug resistant K562 human leukemic cells. Leuk Res 29:1039-1047, 2005.
21. **Cusack JC Jr, Liu R, Baldwin AS Jr.** Inducible chemoresistance to 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothe cin (CPT-11) in colorectal cancer cells and a xenograft model is overcome by inhibition of nuclear factor-kappaB activation. Cancer Res 60:2323-2330, 2000.
22. **Baldwin AS.** Series introduction: the transcription factor NF-kappaB and human disease. J Clin Invest 107:3-6, 2001.
23. **Karin M.** Nuclear factor-kB in cancer development and progression. Nature 44:431-436, 2006.
24. **Kordes U, Krappmann D, Heissmeyer V, Ludwig WD, Scheidereit C.** Transcription factor NF-kappaB is constitutively activated in acute lymphoblastic leukemia cells. Leukemia 14:399-402, 2000.
25. **García MG, Alaniz L, Lopes EC, Blanco G, Hajos SE, Alvarez E.** Inhibition of NF-kappaB activity by BAY 11-7082 increases apoptosis in multidrug resistant leukemic T-cell lines. Leukemia Res 29:1425-1434, 2005.
26. **Kim HJ, Hawke N, Baldwin AS.** NF-kappaB and IKK as therapeutic targets in cancer. Cell Death Differ 13:738-747, 2006.
27. **Pierce, J W, Schoenleber R, Jesmok G, et al.** Novel inhibitors of cytokine-induced

IkappaBalpha phosphorylation and endothelial cell adhesion molecule expression show anti-inflammatory effects in vivo. J Biol Chem 272: 21096–21103, 1997.

28. **Breuer W, Slotki IN, Ausiello DA, Cabantchik IZ.** Induction of multidrug resistance downregulates the expression of CFTR in colon epithelial cells. Am J Physiol 265:C1711-C1715, 1993.
29. **Lallemand J, Stoven V, Annereau J et al.** Induction by antitumoral drugs of proteins that functionally complement CFTR: a novel therapy for cystic fibrosis? Lancet 350: 711-712, 1997.
30. **Cilloni D, Messa F, Arruga F, et al.** The NF-kappaB pathway blockade by the IKK inhibitor PS1145 can overcome imatinib resistance. Leukemia 20:61-67, 2006.


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