

Cell volume and cell volume sensitive transporters in control of proliferation and apoptosis

Else K. Hoffmann

Biochemical Department, Institute of Molecular Biology and Physiology, University of Copenhagen, Denmark

Abstract. The importance of the volume regulated anion channel (VRAC) in maintaining cellular volume and in the control of cell proliferation and apoptosis will be discussed. Two signalling pathway from cell shrinkage to apoptosis will be described. (www.actabiomedica.it)

Key words: Volume regulation, volume regulated anion channels, proliferation, apoptosis.

Introduction

(i) *The Volume Regulated Anion Current (VRAC)* is a major player in the regulatory volume decrease (RVD) process and in the control of cell cycle progression. The possible relation between VRAC current and cell cycle progression is however still controversial. The aim of this study was to investigate the pattern of maximal swelling-activated and basal isotonic Cl⁻ currents, respectively, during the cell cycle, and the effect of a high affinity Cl⁻ channel inhibitor on cell growth.

(ii) The opening of VRAC is also demonstrated to be involved in *apoptotic volume decrease (AVD)*. The acquired tolerance of cancer cells to a variety of chemotherapeutic drugs (Multidrug-resistance, MDR) has been reported to include resistance to apoptosis, indicating that regulation of VRAC could contribute to chemotherapeutic resistance. The aim of the present study is to investigate whether VRAC down regulation contributes to development of Multidrug resistance.

(iii) *Cell shrinkage to apoptosis*. Cell shrinkage is a hallmark of apoptosis, but it is as yet unclear whether a reduction in cell volume is a primary signal of apoptosis. In the present investigation we study the se-

quential signaling events leading from cell shrinkage to apoptosis.

Materials and methods

The model systems used were Ehrlich Ascites Tumour cells (EAT) cells, Ehrlich Lettre Ascites (ELA) cells and NIH-3T3 cells. ELA cells were temporarily arrested in the G₀-stage of the cell cycle using serum starvation for 24h. The addition of serum and subsequent incubation for 5h and 15h forced the G₀ phase cells to re-enter the proliferation cycle and the G₁- and S-phase, respectively. VRAC and the steady state isotonic current were studied by the whole cell patch clamp. Antibodies against phosphorylated Kinases were used in Western blotting to evaluate kinase activation.

Outcomes and discussion

(i) Maximal swelling-activated VRAC decreased significantly in G₁ and increased significantly in early S phase. The isotonic steady state Cl⁻ current, behaved like VRAC (1). The Cl⁻ channel blocker an acidic di-

aryl-urea NS3728 inhibited ELA cell growth suggesting a possible mechanistic link between cell cycle progression and cell cycle dependent changes in the capacity for Cl⁻ current. The large significant differences between the currents during cell cycle suggest that the ELA cell S phase requires an increase in VRAC activity and/or an increased potential for RVD. These results indicate that VRAC plays an important role during cell cycle advancement making it potential therapeutic targets in cancer treatment.

(ii) MDR EAT cells, selected for daunorubicin resistance are more resistant to cell death (evaluated from a MTT assay) and to Caspase-3 induction induced by 5 μ M of cisplatin for 18 h compared to non-selected cells demonstrating that resistant EAT cells have acquired a drug tolerance that is independent of drug efflux mediated by P-glycoprotein (cisplatin is not a substrate for P-glycoprotein). The presence of NS3728 increases the cell viability and decreases the Caspase-3 activation in the cisplatin-treated wild-type cells which in the presence of the inhibitor reach a level comparable to that of cisplatin treated resistant cells. The maximal VRAC current was reduced markedly >50% in resistant cells compared to drug-sensitive cells and by cell volume measurements it was shown that the AVD seen after addition of Cisplatin is essentially absent in the resistant cells. Thus, down regulation of volume-sensitive anion permeability seems to protect against apoptosis and to contribute to the increased tolerance to cisplatin of MDR EAT cells.

(iii) Using NIH3T3 cells we have found that the following signalling events are associated with hyper osmotic stress induced apoptosis: cellular shrinkage activates Rac, with activation of p38, followed by pho-

sphorylation at S15 and nuclear translocation of p53 and finally in Caspase 3 activation (2). Since high cellular ion concentrations damage DNA, DNA repair enzymes could also be involved in the activation of p53. The PI3K super family is the major p53 N-terminal Kinases upon DNA damage and one of these ATM (ataxia-telangiectasia mutated) is known to phosphorylate p53 at serine 15. We therefore investigated the NaCl dose response dependencies of p53 and ATM activities and found that they follow a similar activation pattern, suggesting a possible role for ATM in p53 phosphorylation. Inhibition of ATM abolished p53 (S15) phosphorylation at 600 mOsm NaCl.

Conclusion

Cell volume regulation and regulation of cell proliferation or apoptosis use closely related signalling sequences and cell volume acts as a signal in the cells choice between proliferation and apoptosis

References

1. Klausen TK, et al. *J Cell Physiol* (Submitted)
2. Friis M, et al. *J Physiology* 2005; 567: 427-43.

Correspondence: Else K Hoffmann
Biochemical Department, Institute of Molecular Biology
and Physiology, University of Copenhagen,
13 Universitetsparken, 2100 Copenhagen Ø, Denmark.
Tel. 45 353216095
Fax: 4535321567
E-mail: ekhoffmann@aki.ku.dk