Cancer Biology & Therapy
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/kcbt20

Multidrug resistance phenotypes and MRS2 mitochondrial magnesium channel: Two players from one stemness?

Federica I. Wolf & Valentina Trapani
Published online: 01 Apr 2009.

To cite this article: Federica I. Wolf & Valentina Trapani (2009) Multidrug resistance phenotypes and MRS2 mitochondrial magnesium channel: Two players from one stemness?, Cancer Biology & Therapy, 8:7, 615-617, DOI: 10.4161/cbt.8.7.8152

To link to this article: http://dx.doi.org/10.4161/cbt.8.7.8152

[Cancer Biology & Therapy 8:7, 1-3; July 2009]; ©2008 Landes Bioscience

Commentary

Multidrug resistance phenotypes and MRS2 mitochondrial magnesium channel

Two players from one stemness?

Federica I. Wolf* and Valentina Trapani

Istituto di Patologia generale e Centro di Ricerche Oncologiche Giovanni XXIII; Università Cattolica del Sacro Cuore; Facoltà di Medicina "A. Gemelli"; Roma, Italy

Abbreviations: ABC, ATP binding cassette; CSC, cancer stem cell; hESC, human embrionic stem cells; MDR, multidrug resistance; MRP, multidrug resistance protein; P-gp, permeability-glycoprotein; OXPHOS, oxidative phosphorylation

Key words: adriamycin resistance, apoptosis, cancer stem cells, cytochrome *c* release, CorA, mrs2, P-gp, proliferation

The development of multidrug resistance (MDR), that is, the ability to resist the action of structurally and mechanistically unrelated drugs, hampers the treatment of many human tumors. Multiple mechanisms contribute to this phenomenon, but only few of them have been identified. The first factor found to play a role in MDR is the well-known membrane-embedded transporter MDR1/P-glycoprotein (MDR1/P-gp or ABCB1), which acts as a drug efflux pump that decreases the intracellular drug concentration. P-gp is the most prominent and best characterized member of the superfamily of adenosine triphosphate (ATP)-binding cassette (ABC) transporters. Subsequently, many other human ABC transporters have been associated with drug transport in vitro, including the MDR associated-protein 1 (MRP1 or ABCC1) and the breast cancer resistance-protein (BCRP or ABCG2).[1] Drug antiporters, and especially P-gp, have been brought into a focus by many researchers with the aim of designing strategies that circumvented MDR.[2] However, it has become increasingly evident that many other corollary mechanisms contribute to making tumor cells resistant to anti-tumor drugs. These mechanisms include alterations in target molecules, enhanced DNA repair, increased drug detoxification, alterations in genes that control cell cycle and apoptosis (reviewed in ref. 3).

In recent years new techniques for the high-throughput characterization of gene expression have been developed and used to detect molecular signatures that distinguish MDR tumor cells from their drug-sensitive counterparts. Researchers hoped that these studies would identify new determinants of drug resistance, and combining genomic and proteomic approaches did prove useful in uncovering unexpected molecular players and circuits.

The paper by Chen et al.[4] in this issue of Cancer Biology & Therapy explores new paths in such intriguing scenario. A previous genomics-based study by the same group found that the MRS2 gene (GenBank number AF288288) was upregulated in a gastric cell line with acquired resistance to adriamycin and other common chemotherapeutics.[5] The human gene MRS2, located at chromosome 6 (6p22.1-p22.3), encodes a mitochondrial protein distantly related to the CorA family of Mg^{2+} transport proteins.[6] The CorA family is a group of well-characterized prokaryotic ion transporters that mediate the transport of divalent metal ions across biological membranes.[7] Metal ions are essential elements in most cellular processes; hence, the concentrations of ions in cells and organelles must be kept at appropriate levels. In fact, an impairment of transport systems has been implicated in a number of pathological conditions, collectively referred to as channelopathies.[8] The protein encoded by the MRS2 gene, hsaMrs2p or mrs2, has been the first metal ion channel protein to be identified in the inner mitochondrial membrane. The expression of mrs2 has been associated with the maintenance of proper steady-state concentrations of mitochondrial Mg^{2+}.[6]

Magnesium is an essential component of many crucial enzyme activities, that include also transphosphorylation reactions; in mitochondria, magnesium is a key factor of the ATP-synthesizing machinery.[9] Proliferating cells require and contain more magnesium than resting ones, and the lack of magnesium availability or defects in magnesium transport systems affect the proliferation rate.[10,11] Mrs2-1 knock-out yeast strains show a significantly reduced concentration of magnesium, but hsaMrs2p transfection in the same strains partly restores magnesium levels and alleviates other defects secondary to low magnesium availability.[6] Mrs2 is a conserved protein, with two transmembrane domains that form a Mg^{2+} selective, high-conductance channel which controls Mg^{2+} influx into mitochondria via an intrinsic negative feedback mechanism.[12] The available data support the notion that the MRS2 gene is essential for the survival of eukaryotic cells.[13] The central role of mitochondria in such a survival mechanism may be well reconciled with the following: (i) mitochondria are the main energy-producing organelles of the cell and thus support Mg-ATP-requiring functions such as membrane permeability and proliferative potential, (ii) mitochondria are crucial targets of the apoptotic cascade, eventually governing

*Correspondence to: Federica I. Wolf; Istituto di Patologia generale; Università Cattolica del Sacro Cuore; Largo F. Vito 1; Roma 00168 Italy; Tel.: +39.06.3016619; Fax: +39.06.3012753; Email: fwolf@rm.unicatt.it

Submitted: 01/23/09; Accepted: 02/11/09

Previously published online as a *Cancer Biology & Therapy* E-publication: http://www.landesbioscience.com/journals/cbt/article/8152

the balance of cell survival versus death. In regard to energy metabolism, five multienzymatic complexes embedded in the mitochondrial inner membrane and constituting the oxidative phosphorylation (OXPHOS) system represent the final pathway to cellular energy production. In vitro, there seems to be a relation between the expression of mrs2 and a proper functioning of respiratory complex I.[13] Patients with a mitochondrial defect suffer from a so-called combined deficiency, meaning that the enzymatic activities of two or more complexes of the OXPHOS system are decreased. Among the nuclear genes that might be involved in the pathology of these combined enzymatic deficiencies, there is also MRS2.[14] At present, however, we do not know how MRS2 could be involved in the pathogenesis of human OXPHOS deficiencies. It follows that many questions about the actual role of the MRS2-transcribed channel, the molecular basis of its Mg^{2+} selectivity, and its role(s) in eukaryotic cells, remain unanswered.

Following their previous observation of MRS2 upregulation in adriamycin-resistant gastric cancer cells,[5] the group led by Fan now investigate on the role of this channel in MDR development and characterize a persuasive relation between mrs2 expression and the MDR phenotype.[4] They show that MRS2 transfection in wild type cells confers an MDR phenotype, as assessed by IC_{50} values for several common chemotherapeutics, intracellular adriamycin retention, cell proliferative potential, apoptosis indices such as annexin V exposure and Bax-induced mitochondrial release of cytochrome *c*; conversely, knocking down of MRS2 partially reverses the MDR phenotype in cells with acquired resistance. The negative regulation of adriamycin-induced apoptosis by mrs2 expression could simply be ascribed to the decreased accumulation of adriamycin in the cells; on the other hand, promotion of cell growth could be easily attributed to the aforesaid role of magnesium in cell proliferation.[11]

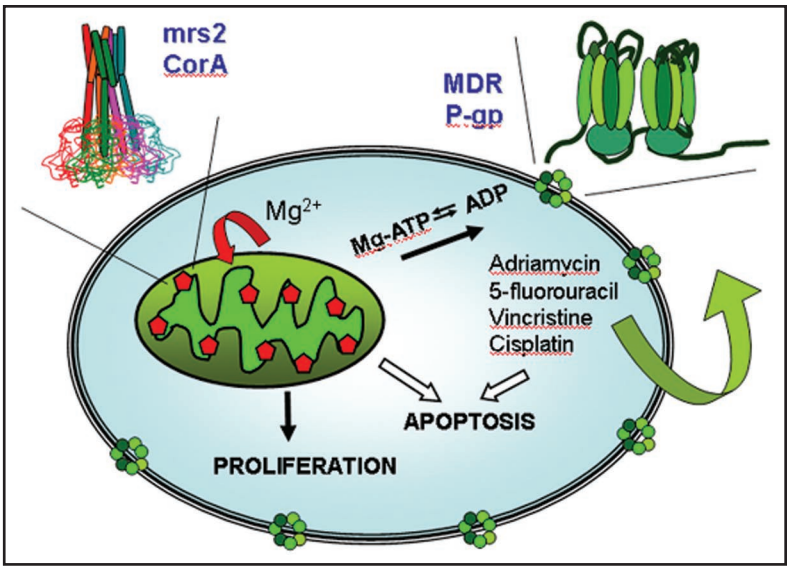

Figure 1. The MDR/MRS2 phenotype suggested by Chen et al.[4] and its possible role in cell survival. The MDR phenotype implies the expression of an ABC transporter, which is an ATP-driven carrier able to extrude a large variety of unrelated chemotherapeutic agents, including adriamycin, vincristine, 5-fluorouracil, cisplatin. Here the best known P-gp (ABCB1) is drawn as an example. The MRS2 gene encodes a magnesium channel on the mitochondrial inner membrane, which is responsible for Mg^{2+} influx into mitochondria. MRS2, encoding a protein with two transmembrane domains, is distantly related to bacterial CorA. The CorA-transcribed channel assembles as a homopentamer resembling a funnel, as depicted in the figure. Both transporters concur to cell survival. The ABC pump, by extruding drug molecules, prevents drug binding to target molecules and thus decreases drug efficacy; mrs2, by promoting Mg^{2+} uptake, potentiates mitochondrial energetic function, which sustains both pump activity, by Mg-ATP, and cell proliferation (black arrows). It is also possible that MDR efflux function and mitochondrial Mg^{2+} uptake both concur in counteracting the apoptotic cascade at various levels (white arrows), thus providing an additional survival advantage to this phenotype.

Decreased drug accumulation and cytochrome *c* release are the most interesting data presented in this paper. The former hints to a direct relationship between mitochondrial activity and efflux pump function, which is more than plausible if one considered that MDR proteins bind ATP with low affinity and thus rely on robust fluxes of ATP synthesis. The latter points to multiple links between mitochondrial function, magnesium and apoptotic responses. Thus, the paper by Chen et al.[4] modifies and extends our appraisal of the MDR phenomenon: it seems that the consequences of an MDR phenotype extend beyond protection against cytotoxic agents but encircle also mitochondrial energy functions which contribute to determining resistance to apoptosis. Figure 1 summarizes the possible role of the MDR/MRs2 phenotype in cell survival.

Generally speaking, the concept of a possible network between drug sensitivity and mitochondrial function, morphology and/or localization is not conceptually new or unprecedented (see for example refs. 15 and 16). Mitochondria were shown to contain P-gp,[17] which probably contributed to blocking the release of cytochrome *c*.[18] On the other hand, a role for magnesium in apoptotic processes has also been postulated. Both intrinsic[19] and extrinsic[20] pathways of apoptosis have been shown to be accompanied by an early increase in cytosolic Mg^{2+}, and Mg^{2+} appears to be required for the release of cytochrome *c* from isolated mitochondria.[21,22] Even though it is not clear whether such an increase in cytosolic Mg^{2+} is derived from mitochondrial or extra-mitochondrial compartments, it is tempting to speculate that mrs2 overexpression counteracted cytochrome *c* release by upregulating Mg^{2+} influx into the mitochondria. We obviously miss a comprehensive picture that accommodated all such findings and described how precisely could magnesium influence apoptosis. This having been acknowledged, there is little, if any, doubt that the most disparate pathways and molecular players might well converge at the crossroads of what appears to be the key problem in cancer treatment, particularly in the late stages of malignancies: the unabated survival capacity of MDR cells.

Until a few years ago MDR was considered the last and most severe consequence of genetic and epigenetic alterations that accompany cancer treatment and progression. The discovery of cancer stem cells (CSCs) clearly changed this scenario. CSCs were discovered first in hematopoietic cancers and then in many solid tumors. CSCs are likely to share many of the properties of normal stem cells, such as (i) a long lifespan, including relative quiescence, (ii) resistance to drugs and toxins through the expression of several ABC transporters, (iii) active DNA-repair capacity, (iv) resistance to apoptosis (reviewed in ref. 23). The MDR-MRS2 phenotype described by Chen et al.[4] seems to fit reasonably well in this context. Tumors might have a built-in population of drug-resistant pluripotent cells that remain

at low frequency among a heterogeneous tumor mass, but that can survive chemotherapy and repopulate the tumour. The CSC hypothesis states that the cancer-initiating cell is a transformed tissue stem cell, which retains the essential property of self-protection through the activity of MDR transporters.[24] Both hereditary and sporadic cancers may develop through dysregulation of stem-cell self-renewal pathways. Obviously, the CSC hypothesis has important implications in the settings of prevention, early detection and successful treatment of many solid tumors.

The last decades have witnessed a remarkable effort to characterize the stemness fingerprint of CSCs in order to identify the Achilles' heel of their silent survival within the tumor mass and their resistance to radio- and chemo-therapy. Again gene expression profiles or protein expression patterns can be extremely useful to investigate on this complex phenotype. A recent paper[25] is quite indicative of such possibility. Comparison of the transcriptome of human mature oocytes and embryonic stem cells highlighted genes that were involved in pluripotency initiation. The identified common oocyte/hESC gene expression profile included a strong cell cycle signature, genes associated with pluripotency, a large chromatin remodelling network, 18 different zinc finger transcription factors, and several still poorly annotated genes, among which MRS2.[25] This observation prompted us to formulate a unifying hypothesis: by having included the MRS2 gene in the transcriptome of the pluripotent phenotype, can we pick this information and that presented by Chen et al.[4] to conclude that stemness and MDR share a magnesium-regulating mechanism such as the overexpression of mrs2 channel? Would this concept pave new avenues to a better understanding of cancer stemness and cancer treatment? The paper by Chen et al.[4] might be credited with the merit of steering research in that direction.

Acknowledgements

Supported by PRIN 2007ZT39FN.

Note

The authors wish to dedicate this paper to the memory of Professor Rudolf Schweyen (1941–2009), the "father" of mrs2 gene.

References

1. Stavrovskaya AA, Stromskaya TP. Transport proteins of the ABC family and multidrug resistance of tumor cells. Biochemistry (Mosc) 2008; 73:592-604.
2. Shukla S, Wu CP, Ambudkar SV. Development of inhibitors of ATP-binding cassette drug transporters: present status and challenges. Expert Opin Drug Metab Toxicol 2008; 4:205-23.
3. Lage H. An overview of cancer multidrug resistance: a still unsolved problem. Cell Mol Life Sci 2008; 65:3145-67.
4. Chen Y, Wei X, Yan P, Han Y, Sun S, Wu K, Fan D. Human mitochondrial Mrs2 protein promotes multidrug resistance in gastric cancer cells by regulating p27, cyclin D1 expression and cytochrome *C* release. Cancer Biol Ther 2009.
5. Zhao Y, You H, Liu F, An H, Shi Y, Yu Q, Fan D. Differentially expressed gene profiles between multidrug resistance gastric adenocarcinoma cells and their parental cells. Cancer Lett 2002; 185:211-8.
6. Zsurka G, Gregán J, Schweyen RJ. The human mitochondrial Mrs2 protein functionally substitutes for its yeast homologue, a candidate magnesium transporter. Genomics 2001; 72:158-68.
7. Niegowski D, Eshaghi S. The CorA family: structure and function revisited. Cell Mol Life Sci 2007; 64:2564-74.
8. Hübner CA, Jentsch TJ. Channelopathies of transepithelial transport and vesicular function. Adv Genet 2008; 63:113-52.
9. Maguire ME, Cowan JA. Magnesium chemistry and biochemistry. Biometals 2002; 15:203-10.
10. Wolf FI, Fasanella S, Tedesco B, Torsello A, Sgambato A, Faraglia B, et al. Regulation of magnesium content during proliferation of mammary epithelial cells (HC-11). Front Biosci 2004; 9:2056-62.
11. Wolf FI, Trapani V. Cell (Patho)physiology of Magnesium. Clin Sci 2008; 114:27-35.
12. Schindl R, Weghuber J, Romanin C, Schweyen RJ. Mrs2p forms a high conductance Mg^{2+} selective channel in mitochondria. Biophys J 2007; 93:3872-83.
13. Piskacek M, Zotova L, Zsurka G, Schweyen RJ. Conditional knock-down of hMRS2 results in loss of mitochondrial Mg^{2+} uptake and cell death. J Cell Mol Med 2008; Epub.
14. Coenen MJ, Smeitink JA, Smeets R, Trijbels FJ, van den Heuvel LP. Mutation detection in four candidate genes (OXA1L, MRS2L, YME1L and MIPEP) for combined deficiencies in the oxidative phosphorylation system. J Inherit Metab Dis 2005; 28:1.
15. Harper ME, Antoniou A, Villalobos-Menuey E, Russo A, Trauger R, Vendemelio M, et al. Characterization of a novel metabolic strategy used by drug-resistant tumor cells. FASEB J 2002; 16:1550-7.
16. Villa AM, Doglia SM. Mitochondria in tumor cells studied by laser scanning confocal microscopy. J Biomed Opt 2004; 9:385-94.
17. Solazzo M, Fantappiè O, Lasagna N, Sassoli C, Nosi D, Mazzanti R. P-gp localization in mitochondria and its functional characterization in multiple drug-resistant cell lines. Exp Cell Res 2006; 312:4070-8.
18. Tainton KM, Smyth MJ, Jackson JT, Tanner JE, Cerruti L, Jane SM, et al. Mutational analysis of P-glycoprotein: suppression of caspase activation in the absence of ATP-dependent drug efflux. Cell Death Differ 2004; 11:1028-37.
19. Patel T, Bronk SF, Gores GJ. Increases of intracellular magnesium promote glycodeoxycholate-induced apoptosis in rat hepatocytes. J Clin Invest 1994; 94:2183-92.
20. Chien MM, Zahradka KE, Newell MK, Freed JH. Fas-induced B cell apoptosis requires an increase in free cytosolic magnesium as an early event. J Biol Chem 1999; 274:7059-66.
21. Eskes R, Antonsson B, Osen-Sand A, Montessuit S, Richter C, Sadoul R, et al. Bax-induced cytochrome *C* release from mitochondria is independent of the permeability transition pore but highly dependent on Mg^{2+} ions. J Cell Biol 1998; 143:217-24.
22. Kim TH, Zhao Y, Barber MJ, Kuharsky DK, Yin XM. Bid-induced cytochrome *C* release is mediated by a pathway independent of mitochondrial permeability transition pore and Bax. J Biol Chem 2000; 275:39474-81.
23. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer 2005; 5:275-84.
24. Donnenberg VS, Donnenberg AD. Multiple drug resistance in cancer revisited: the cancer stem cell hypothesis. J Clin Pharmacol 2005; 45:872-7.
25. Assou S, Cerecedo D, Tondeur S, Pantesco V, Hovatta O, Klein B, et al. A gene expression signature shared by human mature oocytes and embryonic stem cells. BMC Genomics 2009; 10:10.