Expression of ABCB1 transporters is regulated by several different mechanisms in drug-resistant rat hepatoma cells

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Introduction

MDR1 (ABCB1) is an energy-dependent transporter that is able to extrude cytotoxic agents from the cell. MDR1 expression is regulated by different mechanisms, though the molecular background of increased MDR expression is mostly unknown. The aim of our study was to reveal epigenetic modifications responsible for the increased MDR1 level in multidrug resistant cell lines. We used a drug sensitive parental rat hepatoma cell line (D12), a medium (col500) and a highly (col1000) drug-resistant variant of it.

Results

Rodents have two MDR1 isoforms: ABCB1a and ABCB1b. In the drug-resistant cell lines we detected an increased ABCB1 activity, while the activity of ABCC1 was decreased significantly in the colchicine-selected cells (data not shown). Next, we distinguished between ABCB1a and ABCB1b activities and found that the elevation of ABCB1b activity is responsible for the MDR phenotype in the resistant col500 and col1000 cells **(Figure 1.)**.
Since high drug efflux activity may result by several mechanisms affecting *Abcb1* gene expression and/or ABCB1 activity, we next determined the mRNA levels of *Abcb1a* and *Abcb1b* by quantitative RT-PCR. We found that in the parental D12 cell line *Abcb1a* and *Abcb1b* mRNA levels differed significantly with the level of *Abcb1a*-specific mRNA nearly thirty-fold higher than that of *Abcb1b*. In drug-resistant col500 and col1000 cell lines both genes were upregulated. **(Figure 2.)**.

On the other hand, neither the copy number of these genes, nor the half life of their mRNA product showed a significant change in resistant versus sensitive cells (data not shown).

In order to test the regulatory potential of histone acetylation on the expression of *Abcb1* genes, we compared *Abcb1* expression in the absence and presence of histone deacetylase inhibitor trichostatin-A (TSA). The efficacy of histone deacetylase inhibition was verified by western blots showing that acetylated histone H3 and H4 levels were increased in both drug-sensitive and drug-resistant cell lines (data not shown). Results of chromatin immunoprecipitation experiments demonstrated that TSA treatment resulted in elevated H3K9ac (and also K14) levels at the *Abcb1a* and *Abcb1b* genes as well, at both tested 5' regions and significantly, in both the drug-sensitive and drug-resistant cell lines **(Figure 3.)**.

However, when we examined the effect of TSA on the expression of *Abcb1* genes, we found that surprisingly the two genes responded differently to elevated acetylated histone levels in D12, col500 and col1000 cells as well: the mRNA level of *Abcb1a* decreased, while that of *Abcb1b* increased **(Figure 4.A. B.)**. A comparison of *Abcb1* pre-mRNA levels in TSA treated versus untreated D12 parental and col500 and col1000 drug-resistant cells indicated similar changes in *Abcb1* expression upon TSA treatment, demonstrating that the histone deacetylase inhibitor affected the transcription of the two *Abcb1* genes differently **(Figure 4.C. D.)**.

Since HDAC inhibitors changed the expression levels of *Abcb1* genes, we wondered whether this treatment affected the drug efflux capacity of the cells. We found that HDACi-treatment had no effect on the drug-efflux capacity either in the sensitive or in the resistant cells (data not shown).

Conclusions

Our data suggest that elevated *Abcb1* gene expression is not always coupled to histone acetylation changes, and conversely, the H3K9 and H3K14 acetylation levels do not necessarily determine the expression level of *Abcb1* genes. Thus, further histone acetylation sites and other histone modifications need to be examined to understand the complex regulation of MDR1 by chromatin modifications.

Acknowledgement

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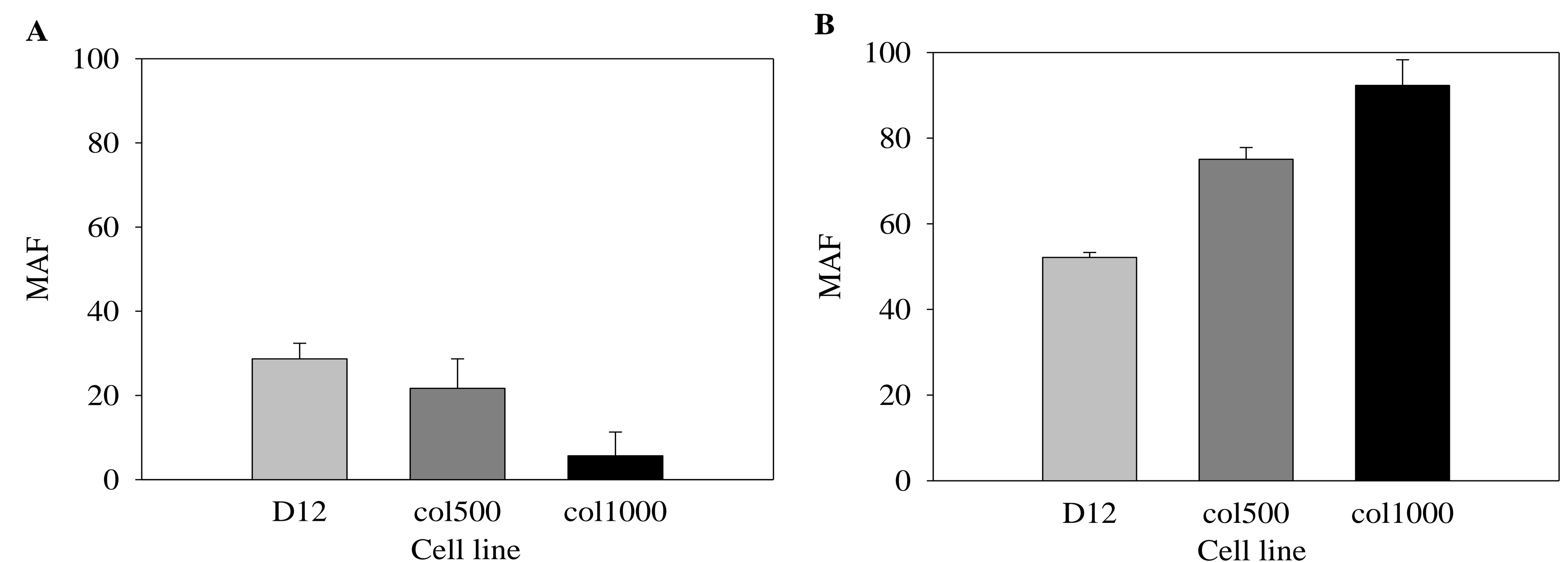

Figure 1. : Activity of drug transporters in drug-sensitive and drug-resistant cell lines. MAF values reflecting ABCB1a **(A)** and ABCB1b **(B)** transporter activities.

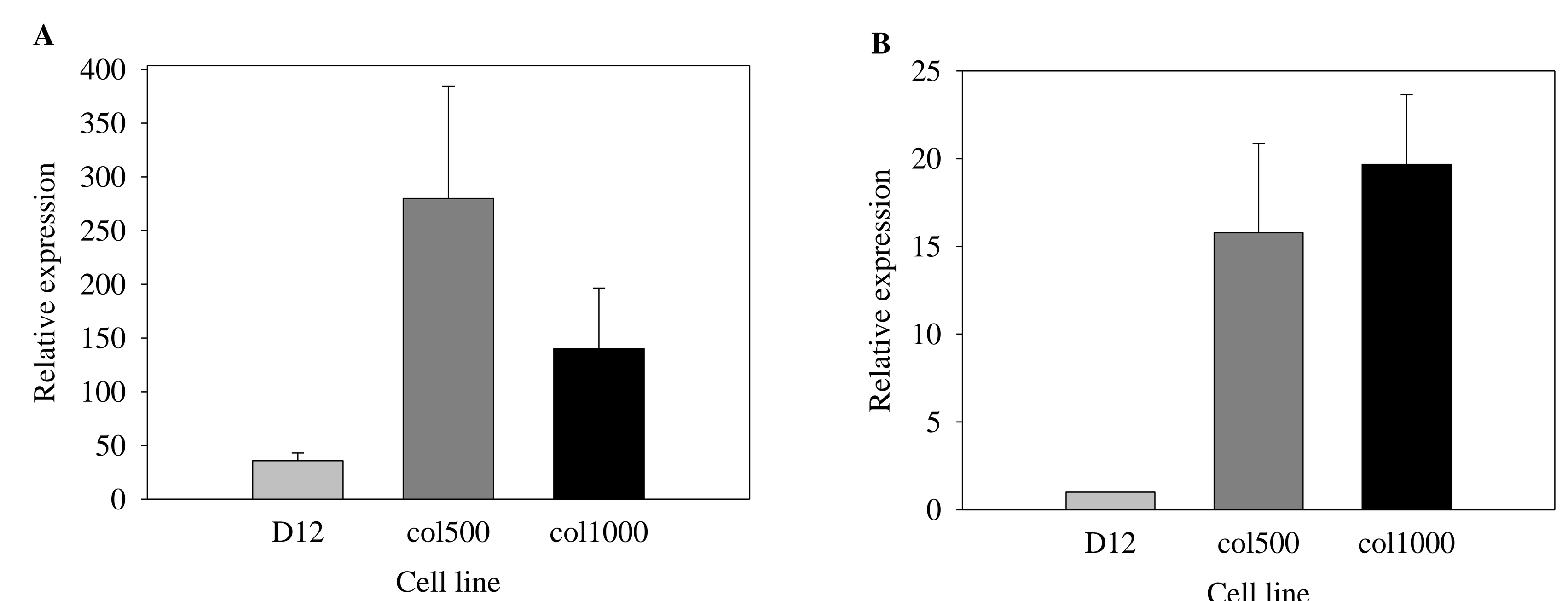

Figure 2. : Cells were grown for 3 days without colchicine, then the mRNA levels of *Abcb1a* and *Abcb1b* were determined by Q-PCR and normalized to 18S RNA. **A**: Expression of *Abcb1a*. **B**: Expression of *Abcb1b*.

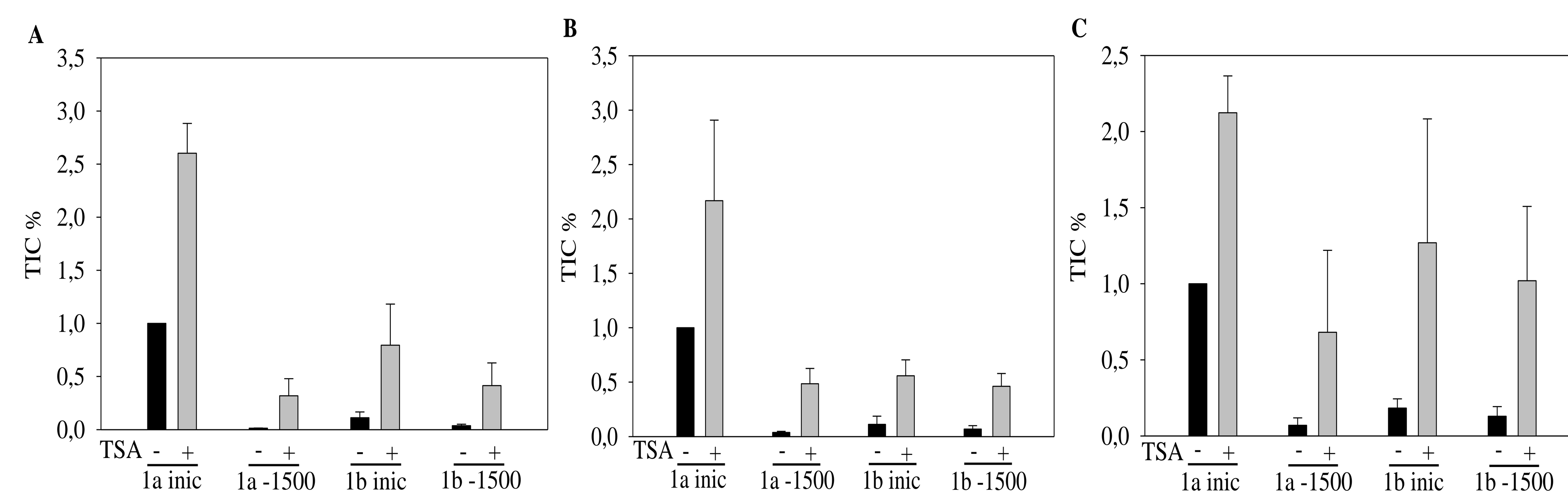

Figure 3. : Cells were grown for three days without colchicine then treated with 50 ng/ml TSA for 6 hours. The sonicated chromatins were immunoprecipitated with anti-H3K9ac antibody and the quantity of *Abcb1a* and *1b* transcriptional iniciation regions and -1500 regions were measured by Q-PCR. **A**: ChIP on D12. **B**: ChIP on col500. **C**: ChIP on col1000.

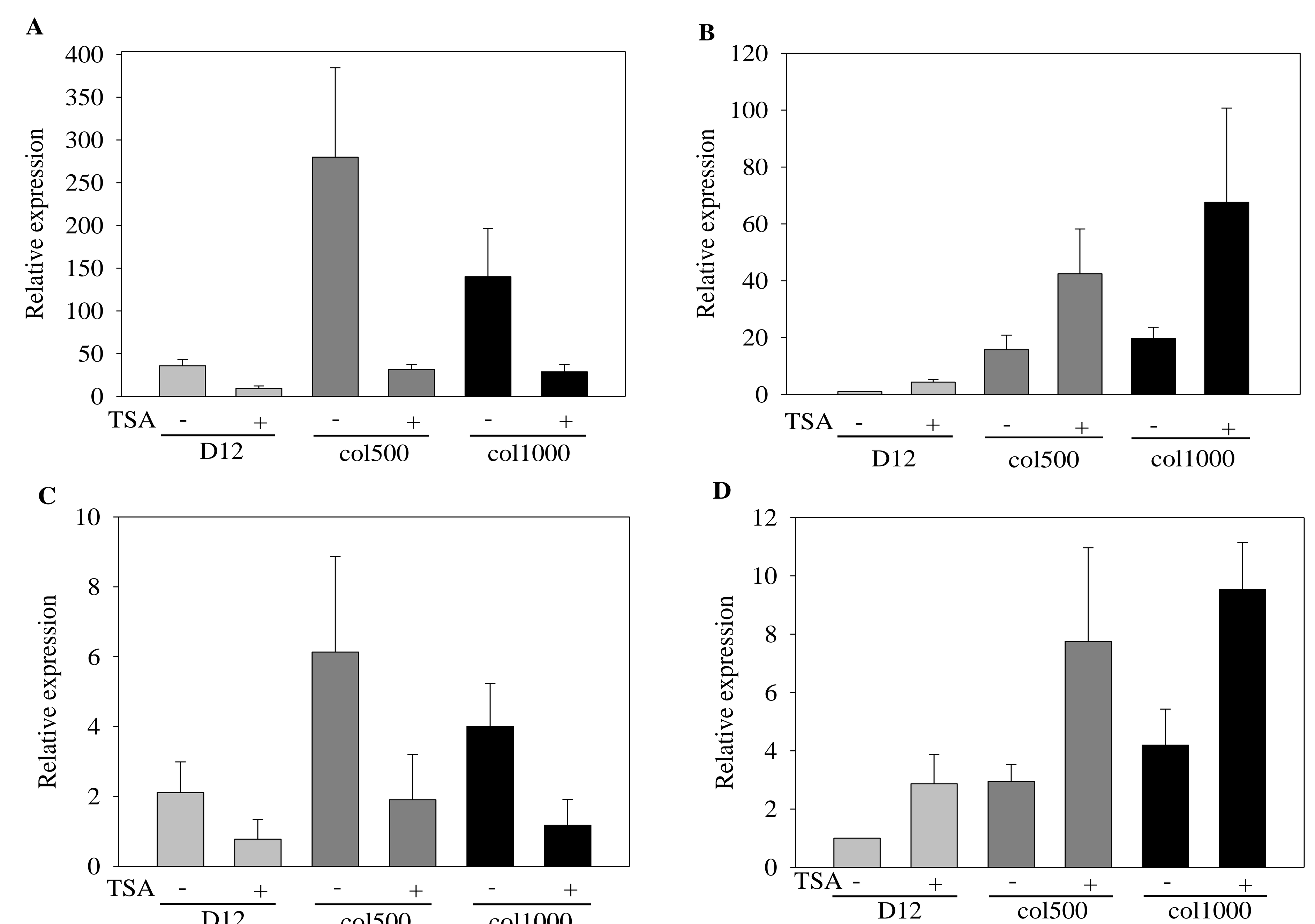

Figure 4. : Cells were grown for three days without colchicine then treated with 50 ng/ml TSA for 6 hours. The mRNA levels of *Abcb1a* **(A)**, *Abcb1b* **(B)** and the pre-mRNA levels of *Abcb1a* **(C)** and *Abcb1b* **(D)** were determined by Q-PCR and normalized to 18S RNA.