

Ph.D. THESIS

**Analysis of the chromatin structure of MDR1 gene in drug
sensitive and drug resistant human cells**

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INTRODUCTION

Chromatin consists of DNA, non-histone and histone proteins; its structure can be regulated by DNA methylation and covalent modifications of histones to allow access to DNA during transcription. The combination of specific posttranslational modifications of histones is required to regulate transcription. Acetylation of lysine residues on histone N terminus usually correlates with transcription. Histone acetylation is regulated by the opposing actions of histone acetyl transferases (HATs) and histone deacetylases (HDACs).

We have studied the epigenetic regulation of multidrug resistance. The development of multidrug resistance in cancer cells is often caused by increased energy-dependent efflux of hydrophobic drugs that can enter the cell through diffusion. The drug efflux is generally mediated by the increased activity of a transporter, encoded by ABCB1/MDR1 (multidrug resistance 1) gene, a member of the family of ABC (ATP binding cassette) transporters. Clinical studies have described that MDR1 gene is often overexpressed during chemotherapy. Histone acetylation and deacetylation have an important role in the transcriptional regulation of MDR1. PCAF (p300/CBP associated factor), p300 and CBP (CREB binding protein) HATs co-activate MDR1 promoter; binding of these HATs to the MDR1 promoter has been demonstrated in vivo as well, but their function is not fully understood.

As MDR1 transporter protein expression is correlated with shorter survival of patients, several attempts have been made to combat multidrug resistance during the past decades. Since histone acetylation is implicated in the transcriptional regulation of MDR1 gene, alteration of activity of HDACs or HATs can contribute to the inhibition of MDR1 expression.

AIMS

In order to study the epigenetic regulation of MDR1 gene expression in the MCF7 breast carcinoma cell line and in its drug resistant derivative we aimed to answer the following questions:

1. Does drug resistance correlate with elevated MDR1 expression? Is the elevated level of MDR1 mRNA in the drug resistant cell line attributable to changed promoter activity or to other alterations (gene amplification, alteration in mRNA stability)?
2. If MDR1 promoter is actively transcribed in the drug resistant cells, does histone acetylation have a role in the maintenance of gene expression? By which specific acetyl lysine residue modifications can the chromatin of MDR1 gene be characterized?
3. Can MDR1 expression be altered by modification of histone acetylation pattern using HDAC and HAT inhibitors? Can this treatment decrease the high MDR1 expression in the drug resistant cell line?

4. Do p300, GCN5 or PCAF histone acetyl transferases participate in the regulation of histone acetylation in the chromatin of MDR1 gene? In what HAT complexes may they exert their function?

METHODS

- human cell culture, drug treatment
- MTT assay to measure cell viability
- FACS analysis to measure drug accumulation
- mRNA isolation and reverse transcription
- quantitative PCR (QPCR)
- MDR1 gene copy number determination using QPCR
- Western blot
- chromatin immunoprecipitation (ChIP)
- gene silencing using small interfering RNA (siRNA)

RESULTS AND DISCUSSION

Multiple mechanisms contribute to increased expression of MDR1 in the MCF7-KCR cell line. MDR1 mRNA level is largely elevated in the drug resistant MCF7-KCR cells compared to the drug sensitive MCF7 cell line. Gene amplification contributes to the elevated mRNA level, the MDR1 gene copy number is increased by one order of magnitude in the drug resistant cell line. The mRNA level is increased by four orders of magnitude that suggests high MDR1 promoter activity in MCF7-KCR cells. The MDR1 gene can

be transcribed from two promoters. We found in the MCF7-KCR cells, that in contrast to the downstream promoter (DSP), the activity of the upstream promoter (USP) is unchanged. The stability of MDR1 mRNA in MCF7-KCR cells is not increased.

Epigenetic alterations contribute to MDR1 gene activity.

To study the epigenetic regulation of MDR1 expression, we examined the acetylation level of H3K4, H3K9, H3K14, H4K8 and H4K12 lysine residues in the chromatin of the regulatory and transcribed regions of the gene using chromatin immunoprecipitation. The level of acetyl H3K9 is a hundred fold increased on the downstream promoter and the first exon of MDR1 in the MDR1 overexpressing cell line. In the region of the transcription start site the acetylation level of H3K4 is slightly elevated, at the chromatin of the gene body H3K4 and H4K8 acetylation is mildly increased in the case of active transcription. Our results confirm the recently described data reporting that not only the methylation of H3K4 lysine but its acetylation also correlates with transcriptional activity of a given gene.

We found that altered genome wide histone acetylation level may accompany the drug resistance, because the levels of all studied histone acetyl lysine residues are higher in the doxorubicin resistant cells than in the drug sensitive counterpart. While treatment with a histone deacetylase inhibitor, trichostatin A (TSA) caused an increase of global histone acetylation level in MCF7, it induced no further increase of that in MCF7-KCR. This data further suggests the

altered regulation of histone acetylation during drug resistance development.

Effect of HDAC inhibition is different in the drug sensitive and drug resistant cells.

TSA sensitivity of MDR1 expression proves the important role of histone acetylation in the transcriptional regulation of MDR1. In drug sensitive cells MDR1 expression increased upon TSA treatment in contrast to MCF7-KCR cells, where it remained unchanged. We found that induction of MDR1 expression in MCF7 cells was accompanied with increases in the levels of H3K4 and H3K9 acetylation in the region of the gene body and in the DSP, first exon, respectively. HDAC inhibition did not affect MDR1 mRNA level in MCF7-KCR cells, but at the same time acetylation of H4K12 increased in every studied region.

Effect of inhibition of HATs is different in the drug sensitive and resistant cells.

To study the effect of lack of GCN5/PCAF containing complexes on MDR1 expression, which can catalyze acetylation of H3K9 lysine according to in vivo data, we used RNAi knockdown. Treatment of the MCF7 cells with siRNAs specific to PCAF HAT and ADA2B, an essential subunit of the PCAF/GCN5 containing HAT complexes, resulted in the reduction of MDR1 mRNA level. These data suggest that a HAT complex containing PCAF and ADA2B has a role in the regulation of basal expression of MDR1. Furthermore, these data also indicate that, although PCAF and GCN5 are homologous, they have distinct functions. In contrast to drug sensitive cells, PCAF,

ADA2B and GCN5 specific siRNA treatment resulted in no change of MDR1 expression in MCF7-KCR cells. However, simultaneous knockdown of PCAF and GCN5 resulted in a reduction of MDR1 mRNA level in the drug resistant cells. This finding suggests that these HATs substitute each other in maintaining the elevated expression of MDR1 and concomitantly the high acetylation level of H3K9.

In order to study the role of p300 in the regulation of histone acetylation and MDR1 gene expression, its function was inhibited by HATi II. In both studied cell lines HATi II treatment resulted in the reduction of genome-wide acetylation on H3K9, H3K14, H3K18 and H4K12 lysine residues. Upon HATi II treatment of MCF7 cells, MDR1 mRNA level slightly decreased, which may indicate the regulatory role of p300 in MDR1 expression in the drug sensitive cells. Despite the reduced genome wide histone acetylation, levels of acetyl lysine residues in the studied chromatin region of MDR1 gene were not affected by HATi II treatment. Interestingly, in MCF7-KCR cells HATi treatment caused a further increase in the expression of MDR1. Higher mRNA level was accompanied with elevated acetylation level of H3K4 at MDR1 specific regions.

Generally, histone acetylation level is positively correlated with transcriptional activity. Our results demonstrate that active gene expression correlates with the increased acetylation level of only specific lysine residues. However, acetylation of specific lysines not certainly results in additional gene activity. Importantly, despite a

change in gene expression, histone acetylation level in the gene chromatin can be unaltered.

Deregulated histone acetylation in the drug resistant cells.

Our results suggest that the generation and maintenance of histone acetylation is deregulated in the drug resistant cells. It can be the consequence of increased HAT activity, but our results did not confirm this possibility in the case of PCAF, GCN5 and p300. Increased acetyl lysine level can also be caused by the reduced activity or the lack of HDACs. We observed an increased expression level of HDAC5 and a reduced mRNA level of SIRT7, and presumably other regulatory mechanisms of HDACs may also be changed in the MCF7-KCR cells compared to MCF7 cells.

SUMMARY

1. Expression level of MDR1 gene is highly elevated in the drug resistant MCF7-KCR cell line. Gene amplification contributes partially to high mRNA level, but changes in stability of MDR1 mRNA in the drug resistant cells is not responsible for it.
2. Global acetylation levels of H3K4, H3K9, H3K14, H3K18, H4K8 and H4K12 are higher in the drug resistant MCF7-KCR cells than in the drug sensitive MCF7 cells.
3. In the drug resistant cells gene expression is associated with highly increased acetylation level of H3K9 lysine residue around the transcriptional start site and slightly increased acetyl H3K4 and H4K8 level at the chromatin of the gene body.

4. Expression levels of some HDAC genes, PCAF and GCN5 HAT genes are altered in the MCF7-KCR cells compared to MCF7 cells.
5. Treatment with a HDAC inhibitor, TSA caused an increase of global histone acetylation level in MCF7. TSA treatment induced MDR1 expression in drug sensitive cells, which was accompanied with increases in the levels of H3K4 and H3K9 acetylation in the some region of the gene.
6. In MCF7-KCR cells TSA treatment resulted in no further increase of global histone acetylation level. MDR1 expression level remained unchanged, but acetylation of H4K12 increased in the chromatin of the gene.
7. In MCF7 cells PCAF and ADA2B specific siRNA treatment inhibited MDR1 transcription, but knockdown of GCN5 resulted in no change in MDR1 mRNA level. PCAF, ADA2B and GCN5 specific siRNA treatment had no effect on MDR1 expression in MCF7-KCR cells. However, simultaneous knockdown of PCAF and GCN5 resulted in a reduction of MDR1 mRNA level in the drug resistant cells.
8. In both cell lines HATi II treatment caused a decrease of genome-wide acetylation on studied lysine residues.
9. HATi II treatment of MCF7 cells resulted in a mild reduction of MDR1 mRNA level and no change of levels of acetyl lysine residues in the studied chromatin region of MDR1 gene. Interestingly, in MCF7-KCR cells HATi treatment caused a further

increase in the expression of MDR1, which was accompanied with higher acetylation level of H3K4 at MDR1 specific regions.

PUBLICATIONS

Publication related to the thesis

Toth M., Boros I., Balint E. *Elevated level of lysine 9-acetylated histone H3 at the MDR1 promoter in multidrug-resistant cells.* Cancer Science, 2012 Apr; 103(4): 659-69.

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Toth M, Molnar J, Boros I, Balint E. *Histone H3 K9 acetylation is highly elevated in the first exon of multidrug resistance 1 (MDR1/ABCB1) gene in a drug resistant cell line.* Epigenetics World Congress, 2009, Berlin, Germany

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Further publications

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