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Antibiotics induce overexpression of alpha satellite DNA accompanied with epigenetic changes at alpha satellite arrays as well as genome-wide

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Abstract

The transcription of satellite DNA is highly sensitive to environmental factors and represents a source of genomic instability. Therefore, tight regulation of (peri)centromeric transcription is essential for genome maintenance. Antibiotics are routinely used for in vitro studies and for medical treatment, however, their effect on pericentromeric satellite DNA transcription was not investigated. Here we show that antibiotics geneticin and hygromycin B, conveniently used in cell culture, as well as rifampicin (along with five other antibiotics), used to treat bacterial infections, increase transcription of a major human pericentromeric alpha satellite DNA in cell lines at standard concentrations. However, response differs among cell lines - maximal increase in A-1235 cells is obtained by rifampicin while in HeLa cells and fibroblasts by geneticin. There is also a positive correlation between antibiotic concentration and the level of alpha satellite transcription. The increase of transcription is accompanied with either H3K9me3 decrease or H3K18ac increase at tandemly arranged alpha satellite arrays while H3K4me2 remains unchanged. Our results suggest that induced alpha satellite DNA transcription upon antibiotic stress could be linked to epigenetic changes - histone modifications H3K9me3 and H3K18ac, which are associated with transcription of heterochromatin.

Keywords Satellite DNA, Heterochromatin, Transcription, Antibiotic, Histone modifications

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Introduction

Antibiotics are routinely used for in vitro studies while culturing cells in order to avoid bacterial contamination and for selection purposes. However, different studies have shown that use of antibiotics can affect gene expression and could modify the results of studies focused on drug response, cell cycle regulation and cell differentiation [1–3]. Antibiotic rifampicin (10 μM for 24 h) was shown to induce genome-wide drug dependent changes in gene regulation and expression in human hepatocytes, some of them linked to changes in histone marks H3K4me1 and H3K27ac [1]. Cells cultured with standard 1% Penicillin-Streptomycin (PenStrep) supplemented media showed significantly altered gene expression and regulation, as observed in a common liver cell line such as HepG2. Drug-associated genes were differentially expressed following PenStrep treatment and differential enrichment of active promoter and enhancer regions marked by H3K27ac was reported [2]. The human peripheral blood mononuclear cells (PBMCs) expressed DNA damage features such as activation of a serine/threonine kinase ATM and p53, as well as epigenetic changes - phosphorylation of H2AX and H3K4me2/3 modifications at some promoter sites after the in vitro exposure to antibiotic oxytetracycline (OTC, 2 μg/ml or 4,3 μM) [4]. Since OTC is largely employed in veterinary practices, this reveals a potential influence of OTC on animal and human health. While the effect of antibiotic treatment on gene expression was previously at least partially characterized, the influence of antibiotics on non-coding regions of genome, in particular on (peri)centromeric satellite DNAs which are related to genome stability, is poorly investigated. Therefore, the molecular consequences of growing human cell lines with antibiotics at standard cell culture concentrations as well as of antibiotics use in veterinary and medical practice have yet to be thoroughly investigated.

Satellite DNAs are tandemly repeated sequences preferentially clustered in (peri)centromeric regions of eukaryotic chromosomes [5]. Recent studies reveal that their expression is highly sensitive to environmental factors such as heat stress, DNA damaging agents, genotoxic and hyperosmotic stress [6–11]. Satellite DNA expression is also significantly increased under different pathological conditions, such as in diverse types of cancer [5, 12, 13]. Transcription of satellite DNA may represent a source of genomic instability through collision between replication and transcription forks, formation of secondary structures and cytotoxic DNA–RNA hybrids known as R-loops [14]. Therefore, tight regulation of centromeric and pericentromeric transcription is essential for the maintenance of genome stability [15] and cells with aberrant satellite DNA expression can feature substantial mitotic defects and large-scale genetic aberrations, including chromosomal instability and aneuploidy [16]. Satellite DNAs located within heterochromatin seem to be at least partially under epigenetic control and their arrays in cancer cells are characterized by lower level of repressive heterochromatic histone modification H3K9me3 as well as by global DNA hypomethylation relative to normal cells [17, 18]. On the other hand, heat stress induces the increase of silent histone mark H3K9me3 at (peri)centromeric satellite repeats as well as at the satellite repeats dispersed within euchromatin [9, 19], resulting in downregulation of expression of nearby genes [19].

Here, we analyzed whether antibiotics such as geneticin and hygromycin B, which are conveniently used in cell culture [20], as well as rifampicin which is used to treat several types of bacterial infections [21], affect expression of a major, most abundant human alpha satellite DNA clustered in (peri)centromeric regions of all human chromosomes [22]. We also studied if potential changes in satellite DNA expression under antibiotic stress were accompanied by epigenetic changes such as histone marks at heterochromatic satellite arrays and at the satellite repeats dispersed within euchromatin, as well as genome-wide. Using different cell types, we show that all three antibiotics induce overexpression of alpha satellite DNA at concentrations routinely used for in vitro studies and for medical treatment. In addition, overexpression is accompanied by changes in epigenetic modifications on histone marks at alpha satellite arrays located in heterochromatin as well as genome-wide. We proposed that epigenetic changes in heterochromatin, such as decrease of silent histone modification H3K9me3 whose loss affects satellite DNA expression [23], and increase of H3K18ac, which is characteristic for transcriptional activation of heterochromatin [16], could be linked to induced alpha satellite DNA expression upon antibiotic stress.

Results

Alpha satellite DNA transcription after antibiotic treatment

To investigate whether antibiotics affect the transcription of alpha satellite DNA we followed its transcription dynamics in human cell lines by RT-qPCR under standard conditions and after antibiotic treatment. Primers used for transcriptional analysis were able to amplify only tandemly arranged repeats (Figure S1) and since in human pericentromeric heterochromatin alpha satellite DNA is organized in tandemly arranged monomers [22], it is expected that the primers preferentially recognize transcripts deriving from pericentromeric regions. Cells were incubated for 48 h at 37 °C in complete medium with concentrations of antibiotics used for routine treatment, selection and maintenance of eukaryotic cells: geneticin 300–600 μg/ml, hygromycin B 50–100 μg/ml as

well as with rifampicin 8.2–82 μg/ml (10–100 μM). The transcription of alpha satellite DNA was checked immediately after antibiotic treatment and compared with a control. The transcription of alpha satellite DNA was monitored in immortalized fibroblasts (MJ90hTERT), glioblastoma cell line A-1235 and cervix carcinoma HeLa cells.

Treatment of A1235 cells with hygromycin B (50 μg/ml), geneticin (400 μg/ml) and rifampicin (82 μg/ml) revealed the increase of transcription by 1.6x ($P = 0.01$), 1.7x ($P = 0.008$), and 3.0x ($P = 0.02$), respectively (Fig. 1b). To test if transcription responds to antibiotic concentration, A-1235 cells were treated with three different concentrations of rifampicin (8.2, 41, and 82 μg/ml). The results showed no effect after 8.2 μg/ml treatment while 41 μg/ml and 82 μg/ml induced increase of 1.8x ($P = 0.009$) and 3.0x ($P = 0.02$), respectively (Fig. 1c), suggesting positive correlation between transcription and antibiotic concentrations.

Hela cells treated with hygromycin B (50 μg/ml), geneticin (400 μg/ml) and rifampicin (82 μg/ml) showed the increase of transcription by 3.1x ($P = 0.02$), 4.9x ($P = 0.01$), and 1.5x ($P = 0.01$), respectively (Fig. 1a), while decreased concentration of geneticin (300 μg/ml) showed no effect on alpha satellite transcription (Fig. 1a).

Immortalized fibroblast MJ90hTERT cell line showed a modest increase of transcription of 1.5x ($P = 0.01$) after geneticin (400 μg/ml) while treatment with hygromycin

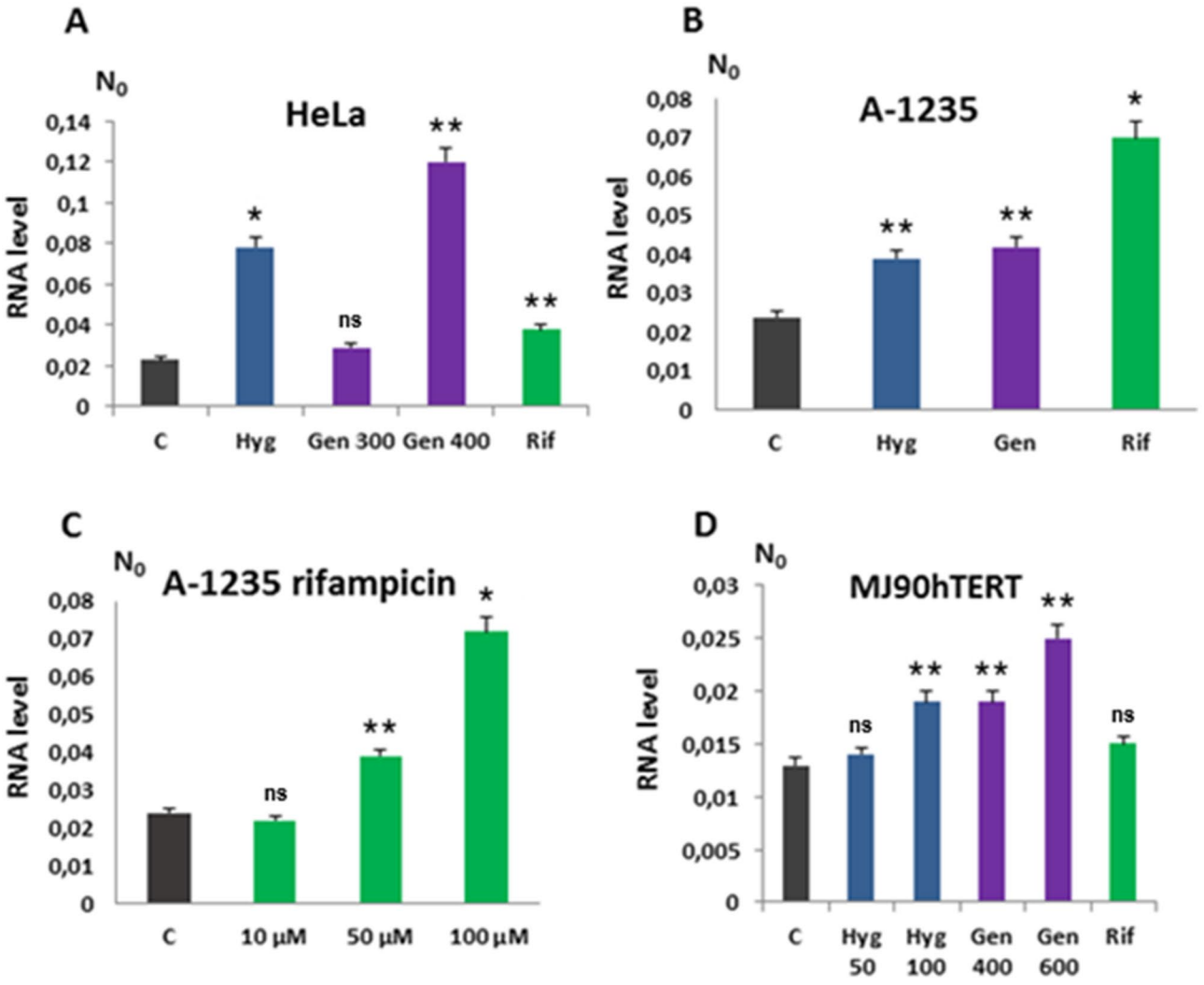

Fig. 1 Transcription of alpha satellite DNA in different cell lines after antibiotic treatment for 48 h: (A) HeLa cells treated with hygromycin B (50 μg/ml), geneticin (300 μg/ml and 400 μg/ml), rifampicin (82 μg/ml); (B) A-1235 cells treated with hygromycin B (50 μg/ml), geneticin (400 μg/ml), rifampicin (82 μg/ml); (C) A-1235 cells treated with rifampicin 8.2 μg/ml, 41 μg/ml, and 82 μg/ml; (D) MJ90hTERT cells treated with hygromycin B (50 μg/ml and 100 μg/ml), geneticin (400 μg/ml and 600 μg/ml), rifampicin (82 μg/ml). Two independent experiments were performed on each cell line. N_0 represents normalized average N_0 value and C denotes control. Columns show averages of two different RT-qPCR experiments performed in triplicates and error bars represent standard deviations. Statistical significance between controls and treated samples was calculated using the t-test and is indicated by stars (** $p < 10^{-2}$, * $p < 0.05$, ns - no significant difference)

B (50 μg/ml) and rifampicin (82 μg/ml) did not show a significant change of transcription. Higher concentration of hygromycin B (100 μg/ml) showed 1.5x ($P = 0.01$) increase of alpha satellite, however, with high percentage of dead cells. On the other hand, higher concentration of geneticin (600 μg/ml) induced 1.9x ($P = 0.008$) increase of alpha satellite transcription (Fig. 1d), while preserving the number of cells and their morphology.

The results revealed a general increase of alpha satellite DNA transcription in cell lines after treatment with different antibiotics at standard concentrations. However, the response differs among cell lines - maximal increase in A-1235 cells was obtained by rifampicin (82 μg/ml) while other two antibiotics showed a modest change (Fig. 1b). On the contrary, in HeLa cells the maximal effect on alpha satellite DNA transcription was obtained by geneticin (400 μg/ml; Fig. 1a), while in MJ90hTERT only higher concentration of geneticin (600 μg/ml) induced a significant change in transcription (Fig. 1d). The results also revealed a positive correlation between antibiotic concentration and the level of alpha satellite transcription.

Additionally, we tested influence on alpha satellite transcription of generally used prokaryotic antibiotics at standard concentrations routinely used in cell culture: amoxicillin (80 μg/ml), ampicillin (50 μg/ml), cefepime (131 μg/ml), cefuroxime (50 μg/ml) and streptomycin (50 μg/ml). The results, after 48 h treatment of MJ90hTERT cells, showed consistent overexpression of alpha satellite DNA in all samples relative to controls, aligning with our previous findings for geneticin, hygromycin B, and rifampicin (Figure S2).

H3K9me3, H3K18ac and H3K4me2 levels at alpha satellite repeats after antibiotic treatment

We analysed the distribution of silent histone mark H3K9me3 characteristic for heterochromatin, H3K18ac mark which is characteristic for transcriptional activation of heterochromatin [16] and H3K4me2, typical for open euchromatin, at tandemly arranged alpha satellite repeats as well as at those dispersed within euchromatin, under standard conditions and after antibiotic treatment. We performed chromatin immunoprecipitation (ChIP) coupled with quantitative real-time PCR, using specific primers for tandemly arranged satellite repeats as well as for six alpha repeats dispersed within introns of genes: AR 1, 10, 21, 25, 29 and 31 [9], (Table S1). Sequences flanking dispersed alpha repeats were used to construct single locus-specific primers. ChIP assay was performed on chromatin isolated from A-1235, HeLa and MJ90hTERT cells subjected to antibiotic treatment of 48 h at 37 °C. The level of tested histone modifications was measured immediately after antibiotic treatment and was compared to the level of control using the unpaired t-test. In addition, we followed the dynamics of IgG binding to dispersed alpha satellite repeats and tandemly repeated satellite arrays and the amount of bound IgG was very low, resulting in a signal below the qPCR threshold.

HeLa cells were treated with geneticin 400 μg/ml which exhibited the strongest effect on alpha satellite transcription (Fig. 1a) and decrease of H3K9me3 level of 2.1x ($P = 0.011$) at tandemly arranged heterochromatic alpha repeats was observed, while no significant change at six euchromatic repeats located within introns was found (Fig. 2a).

Decrease of geneticin concentration to 300 μg/ml resulted in no significant change of H3K9me3 level at tandemly arranged satellite arrays (Figure S3a) corresponding to no significant change of alpha satellite transcription at this concentration (Fig. 1a). The levels of H3K18ac and H3K4me2 were not significantly changed at tandemly arranged alpha satellite repeats as well as at alpha repeats dispersed within euchromatin after treatment with geneticin 400 μg/ml (Figure S5).

The treatment of A-1235 cells with 82 μg/ml rifampicin revealed 2.0x ($P = 0.02$) decrease of H3K9me3 level at tandemly arranged alpha satellite DNA repeats and no significant change at dispersed alpha satellite repeats (Fig. 2b). No significant change in H3K18ac or H3K4me2 level was detected, either at tandem or dispersed alpha repeats (Figure S4a). Lower rifampicin concentration of 41 μg/ml resulted in a slight but not statistically significant decrease of H3K9me3 level at tandem alpha arrays (Figure S3a), while an increase of alpha transcription of 1.8x was detected at this concentration (Fig. 1c).

The treatment of MJ90hTERT cells with geneticin 600 μg/ml, which showed the strongest effect on alpha satellite transcription (Fig. 1d), revealed a significant increase of 2.3x ($P = 0.01$) of H3K18ac level at tandemly arranged alpha satellite arrays but not on dispersed alpha repeats (Fig. 2d). Additionally, histone mark H3K9me3 was significantly decreased by 2.1x ($P = 0.02$) (Fig. 2c). On the other hand, no significant change in H3K4me2 level was found at tandemly arranged or dispersed alpha repeats (Figure S4b).

Results from HeLa and MJ90hTERT cells treated with geneticin and A-1235 cells with rifampicin revealed a decrease of H3K9me3 at heterochromatic alpha repeats which corresponds to increased transcription of alpha satellite DNA. In MJ90hTERT cells however, alongside H3K9me3 decrease after geneticin treatment, H3K18ac was significantly increased, which also corresponds to increased transcription of alpha satellite. On alpha repeats dispersed within euchromatin we did not detect changes in tested histone modifications after any antibiotic treatment. Although at 41 μg/ml rifampicin treatment an increase of alpha satellite transcription

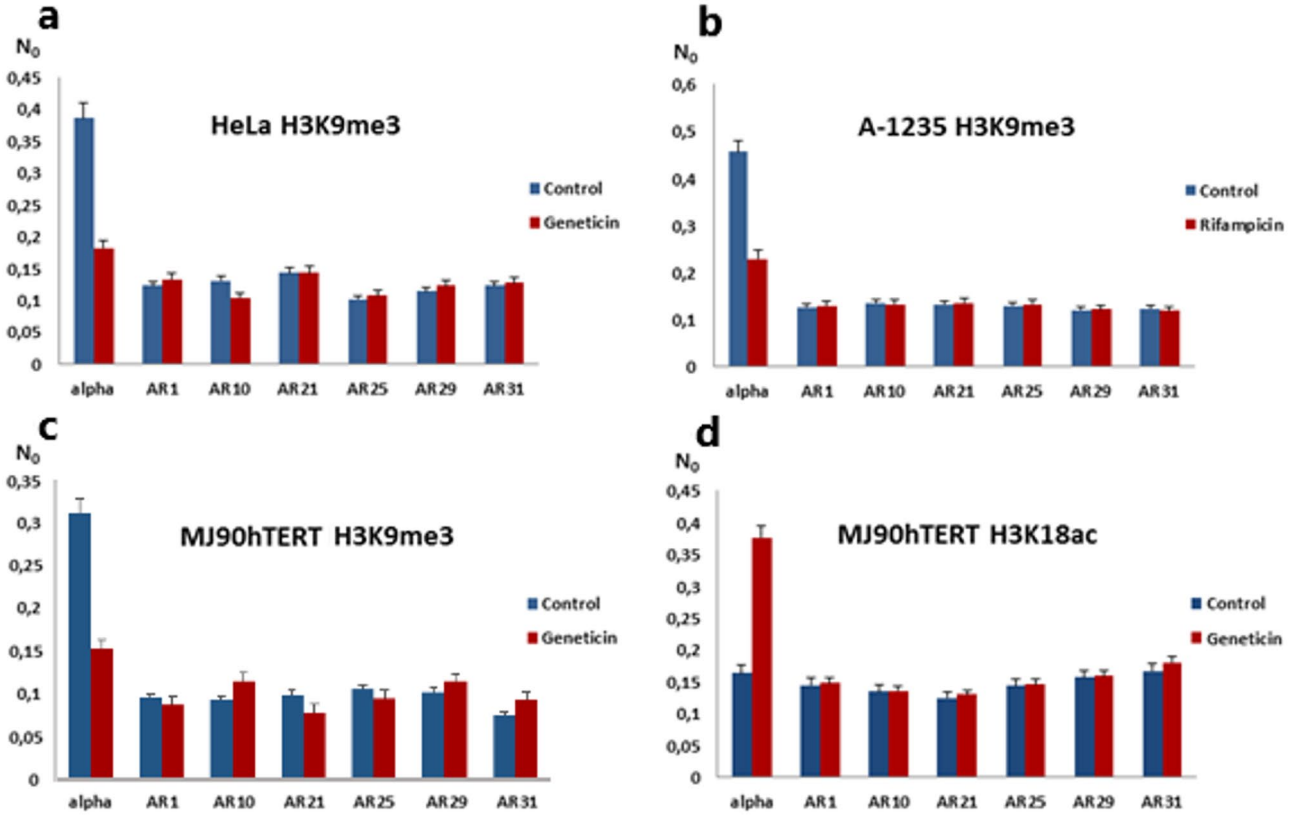

Fig. 2 Levels of histone modifications at tandemly arranged alpha satellite repeats characteristic for heterochromatin and at alpha repeats (ARs) dispersed within euchromatin after antibiotic treatment for 48 h: (A) H3K9me3 level in HeLa cells after treatment with geneticin (400 µg/ml). Significant decrease of H3K9me3 level of 2.1x ($P=0.011$) at tandemly arranged heterochromatic alpha repeats was observed; (B) H3K9me3 level in A-1235 cells after treatment with rifampicin (100 µM). 2.0x ($P=0.02$) decrease of H3K9me3 level at tandemly arranged alpha satellite DNA repeats was detected; (C) H3K9me3 level in MJ90hTERT cells after treatment with geneticin (600 µg/ml). Histone mark H3K9me3 was significantly decreased by 2.1x ($P=0.02$) at tandem alpha repeats. (D) H3K18ac level in MJ90hTERT cells after treatment with geneticin (600 µg/ml). A significant increase of 2.3x ($P=0.01$) was detected at tandemly arranged alpha satellite arrays. Levels of histone modifications were measured by ChIP coupled with quantitative real-time PCR at standard conditions (control) and after antibiotic treatment. N_0 values were normalized using N_0 values of input fractions and represent the levels of histone modifications. Columns show averages of two independent experiments and error bars indicate standard deviations

was observed in A-1235 cells despite a slight change in H3K9me3 level, this could be explained by low sensitivity of ChIP experiments. Similar to that, we observed no statistically significant change in H3K9me3 level at tandem alpha repeats in A-1235 cells as well as in H3K9me3 and H3K18ac levels in MJ90hTERT cells after geneticin 400 µg/ml treatment (Figure S3a, b) despite the slight increase in alpha transcription level of 1.7x and 1.5x respectively (Fig. 1b, d).

H3K9me3, H3K18ac and H3K4me2 levels genome-wide after antibiotic treatment

To see if antibiotic treatment affects epigenetic changes genome-wide we performed immunofluorescence on HeLa, A-1235 and MJ90hTERT cells using primary antibodies against histone marks H3K9me3, H3K18ac and H3K4me2, followed by secondary antibody marked with Alexa Fluor® 488.

After treatment of HeLa cells with geneticin 400 µg/ml for 48 h H3K9me3 level was increased genome-wide by 2.03x ($P<10^{-3}$), H3K18ac showed slight increase of 1.19x ($P<10^{-3}$), while H3K4me2 was decreased by 1.45x ($P<10^{-3}$) (Fig. 3; Table 1). Decrease of geneticin concentration to 300 µg/ml resulted in a slight increase of H3K9me3 of 1.32x ($P<10^{-3}$) while H3K18ac and H3K4me2 levels were downregulated 1.81x and 1.5x ($P<10^{-3}$), respectively (Table 1).

In MJ90hTERT cells after treatment with 600 µg/ml geneticin H3K9me3 and H3K18ac levels were increased 1.86x and 1.27x ($P<10^{-3}$), respectively, while H3K4me2 was slightly increased, 1.1x, but not statistically significant ($P>0.05$) (Fig. 4; Table 1). Decrease of geneticin concentration to 400 µg/ml induced the increase of H3K9me3 level of 2.28x ($P<10^{-3}$) while H3K18ac and H3K4me2 were not significantly changed (Table 1).

Different profile of histone changes was detected after treatment of A-1235 cells with 82 µg/ml rifampicin.

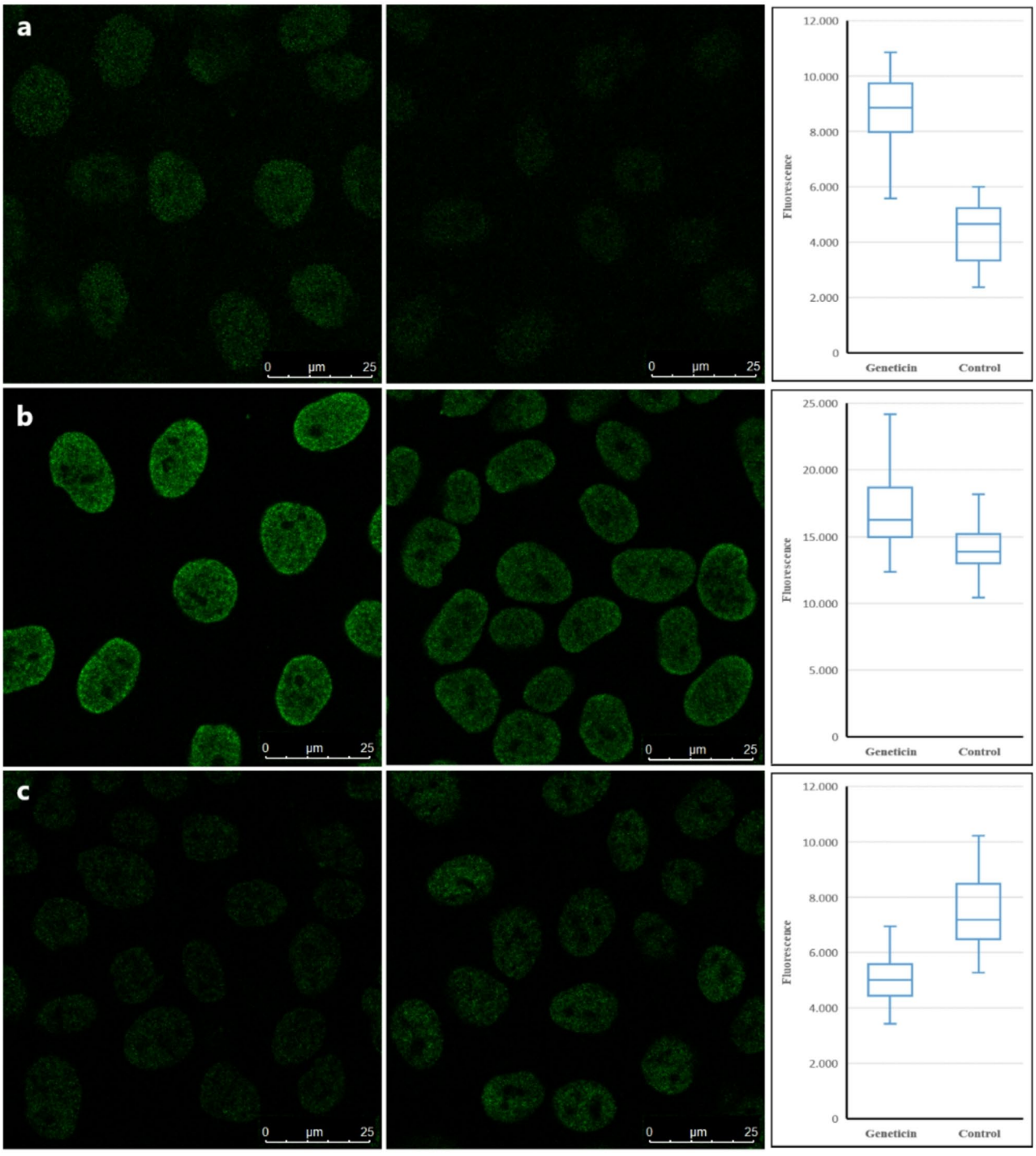

Fig. 3 Genome-wide analysis of H3K9me3 (a), H3K18ac (b) and H3K4me2 (c) levels in HeLa cells after geneticin treatment (400 µg/ml, left panels) and in controls (right panels). H3K9me3 level was increased genome-wide by 2.03x ($P<10^{-3}$), H3K18ac showed slight increase of 1.19x ($P<10^{-3}$), while H3K4me2 was decreased by 1.45x ($P<10^{-3}$). The fluorescence intensity is shown by box plots. Median values are indicated and error bars represent standard deviations

Namely, the level of H3K9me3 was slightly changed (1.08x, $P<10^{-3}$), while H3K18ac level was decreased by 2.38x and H3K4me2 increased 1.33x ($P<10^{-3}$) (Fig. 5; Table 1). Decrease of rifampicin concentration to 41 µg/ml resulted in H3K9me3 and H3K18ac decrease of 2.27x and 2.28x ($P<10^{-3}$), respectively, while H3K4me2 was upregulated by 1.4x ($P<10^{-3}$) (Table 1). In the same cell line, geneticin 400 µg/ml induced the decrease of

Table 1 The genome-wide fold changes of epigenetic modifications H3K9me3, H3K18ac and H3K4me2 in different cell lines after treatment with varying concentrations of antibiotics geneticin (Gen) and rifampicin (Rif) (up-upregulation; dw-downregulation; n.s.c.-no significant change)

Antibiotic	Cell line	H3K9me3 (X)	H3K18ac (X)	H3K4me2 (X)
Gen 400 µg/ml	HeLa	2.03 up	1.19 up	1.45 dw
Gen 300 µg/ml	**HeLa**	**1.32 up**	**1.81 dw**	**1.5 dw**
Gen 600 µg/ml	MJ90hTERT	1.86 up	1.27 up	n.s.c.
Gen 400 µg/ml	**MJ90hTERT**	**2.28 up**	**n.s.c.**	**n.s.c.**
Gen 400 µg/ml	A-1235	2.86 dw	2.58 up	1.35 up
Rif 82 µg/ml	**A-1235**	**1.08 up**	**2.38 dw**	**1.33 up**
Rif 41 µg/ml	A-1235	2.27 dw	2.78 dw	1.40 up

H3K9me3 level by 2.86x and the increase of H3K18ac and H3K4me2 levels by 2.58x and 1.35x ($P<10^{-3}$), respectively (Table 1). Changes of histone modifications genome-wide in three cell lines are summarized in Table 1.

The results reveal genome-wide increase of H3K9me3 levels in HeLa and MJ90hTERT cells induced by geneticin (300–600 µg/ml), while H3K4me2 levels were either downregulated or not significantly changed (Table 1). On the other hand, the level of H3K18ac was significantly downregulated in HeLa cells treated with 300 µg/ml geneticin, while higher concentrations of geneticin only slightly changed H3K18ac levels in HeLa and MJ90hTERT cells, indicating that the effect depends on the concentration of antibiotic but is not positively correlated with it. In A-1235 cells however, geneticin in the concentration of 400 µg/ml induced genome-wide downregulation of H3K9me3 and upregulation of H3K18ac, indicating that response to the antibiotic differs among cell lines. Also, different antibiotics affect differently epigenetic marks in the same cell line as shown by rifampicin which in A-1235 cells stimulates H3K18ac decrease while geneticin induces H3K18ac upregulation (Table 1).

Although different antibiotics induce overexpression of pericentromeric alpha satellite DNA, their effect on heterochromatin differs among cells, characterized either by decrease of H3K9me3 or increase of H3K18ac (Fig. 2). In a similar way, the effect of antibiotics genome-wide also differs among cells. Namely, while the effect of geneticin on histone marks H3K9me3 and H3K4me2 in HeLa and MJ90hTERT cells is similar, it differs from the one observed in A-1235 cells, as well as from the effect of rifampicin on the same marks in A-1235 cells. The results suggest that the heterochromatin, as well as the rest of chromatin, respond to antibiotics in diverse ways, depending on the cell line, type of antibiotic and antibiotic concentration.

Discussion

It is well known that antibiotics influence human microbiome and change its composition which can have a negative impact on host health including reduced microbial diversity and selection of antibiotic-resistant strains, making hosts more susceptible to infection [24]. However, besides targeting bacterial cells, antibiotics affect metabolism of eukaryotic cells as revealed by studies in vitro, on human cell lines [3, 4]. Some in vivo studies, such as those performed on male pseudoscorpions treated with the antibiotic tetracycline, showed significantly reduced sperm viability, which was passed to the next generation and suggests that a similar effect could occur in other species [25]. It was shown that clinically relevant doses of bactericidal antibiotics quinolones, aminoglycosides and β-lactams cause mitochondrial dysfunction and ROS overproduction in mammalian cells, and mice treated with these antibiotics exhibited elevated oxidative stress markers in the blood as well as oxidative tissue damage [26]. It is also known that treatment of some diseases requires high doses of antibiotic, e.g. for tuberculosis 35 mg/kg rifampicin per day is used (which corresponds to approx. 43 µM conc.) [21], and it is therefore interesting to know how similar doses affect metabolism of mammalian cells. Rifampicin is usually well-tolerated and rarely causes serious toxicity in eukaryotic cells. In extreme doses, however, rifampicin is known to produce hepatic, renal and hematological

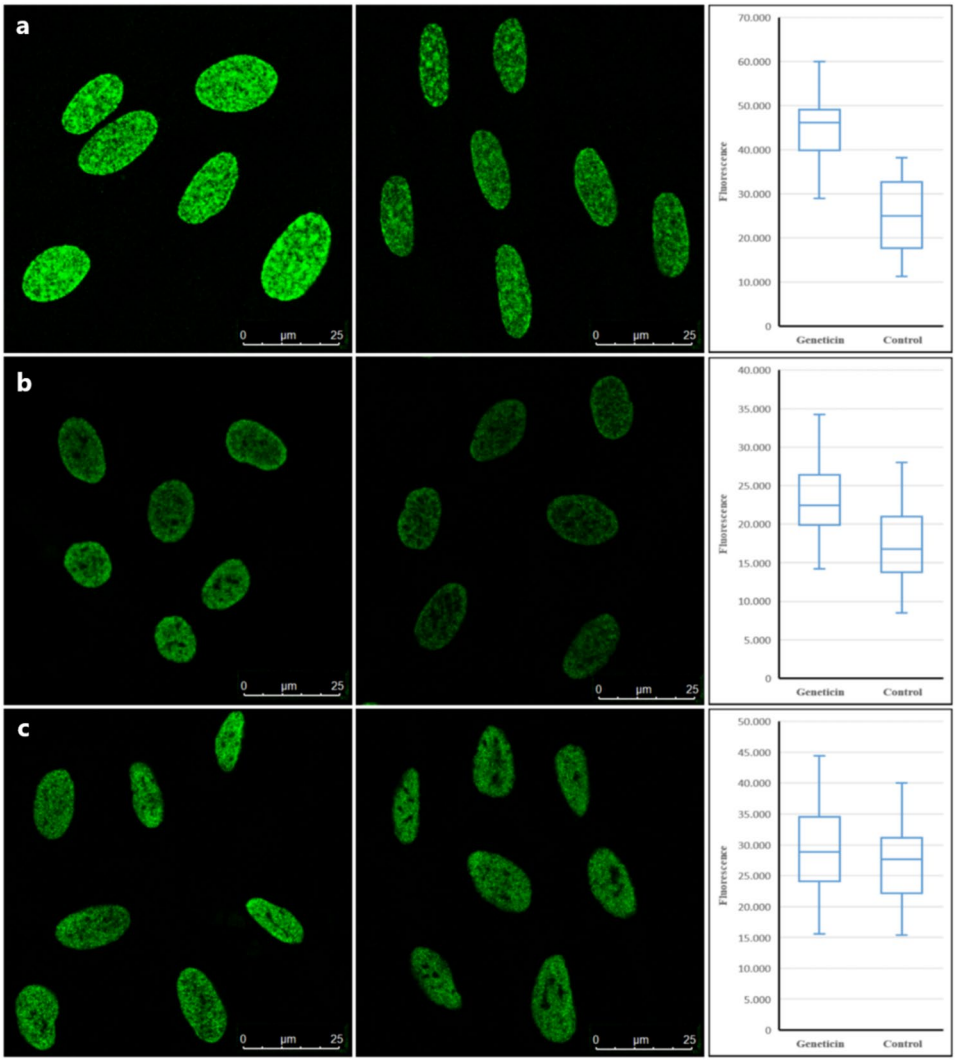

Fig. 4 Genome-wide analysis of H3K9me3 (a), H3K18ac (b) and H3K4me2 (c) levels in MJ90hTERT cells after treatment with geneticin 600 µg/ml (left panels) and in controls (right panels). H3K9me3 and H3K18ac levels were increased 1.86x and 1.27x ($P < 10^{-3}$), respectively, while H3K4me2 was slightly increased, 1.1x, but not statistically significant ($P > 0.05$). The fluorescence intensity is shown by box plots. Median values are indicated and error bars represent standard deviations

disorders and metabolic acidosis [27]. Its toxicity is predominantly hepatic and immuno-allergic in character. Rifampicin induces a dose-dependent hepatotoxicity in HHL-17 cells (IC50; 600 µM), and increases the serum levels of liver injury markers, e.g., alanine transaminase (ALT) and aspartate transaminase (AST) [28]. Also, it was found that rifampicin at exorbitant concentration exerts adverse effects on the proliferation of MSCs in human bone marrow and the differentiation of osteoblasts [29].

Tandemly arranged satellite DNA repeats represent a challenge for the maintenance of genomic stability, during normal cellular functions such as replication and transcription [15]. The increased pericentric satellite DNA transcription has negative effects on cellular physiology, leading to defects typically associated with tumorigenesis and ageing. Overexpressed transcripts of pericentromeric major satellite DNA in mice sequester BRCA1-associated network, cause accumulation of RNA loops, DNA damage and induce breast cancer [30]. Satellite DNAs are sensitive to different exogenous and endogenous stress conditions and here we investigated if the antibiotics commonly used in cell culture studies affect the expression of non-coding major human alpha satellite DNA. We used the aminoglycoside antibiotics geneticin G418 and hygromycin B which are effective against both eukaryotic and prokaryotic cells and are used to select for cells that express antibiotic resistance. Both antibiotics affect protein synthesis. We also tested rifampicin, an ansamycin antibiotic used to treat some bacterial infections, including tuberculosis. Rifampicin blocks bacterial DNA transcription by inhibiting bacterial RNA

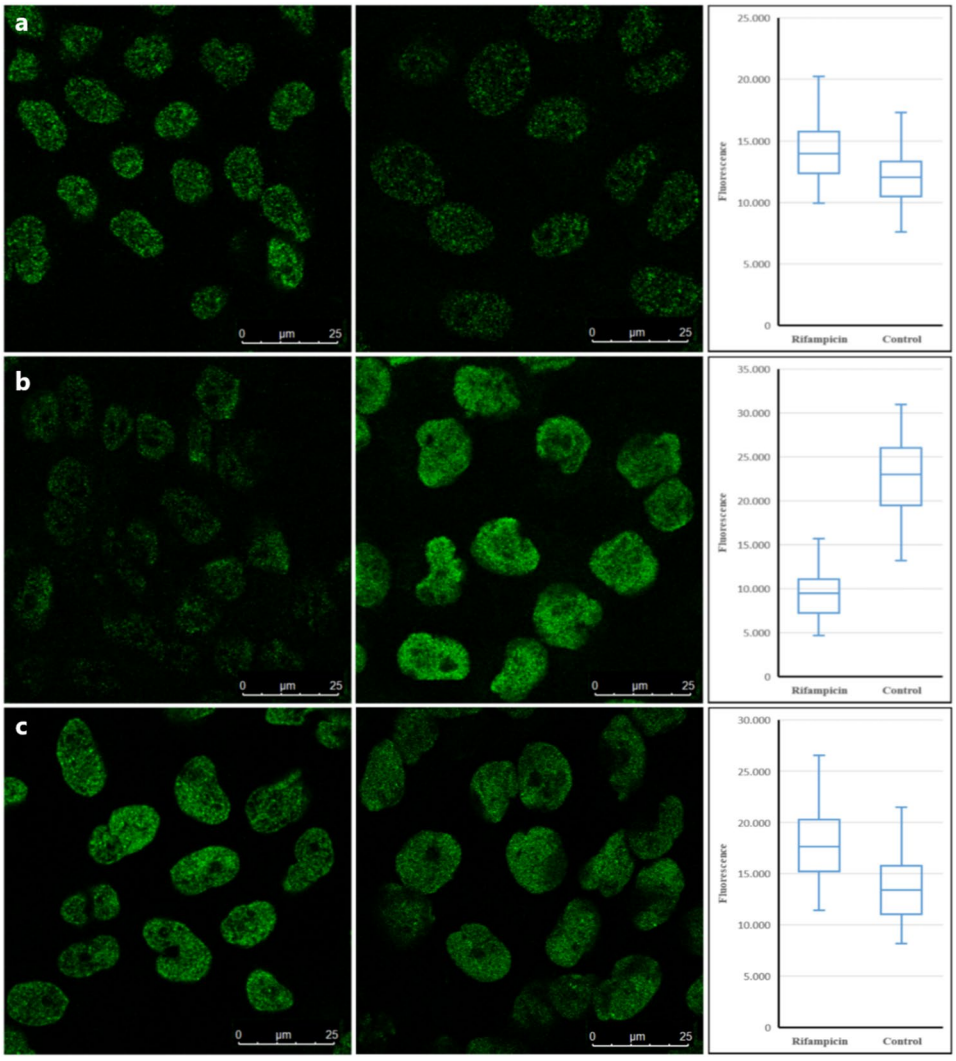

Fig. 5 Genome-wide analysis of H3K9me3 (a) H3K18ac (b) and H3K4me2 (c) levels in A-1235 cells after treatment with rifampicin 100 µM (left panels) and in controls (right panels). The level of H3K9me3 was slightly changed (1.08x, $P < 10^{-3}$), while H3K18ac level was decreased by 2.38x and H3K4me2 increased 1.33x ($P < 10^{-3}$).The fluorescence intensity is shown by box plots. Median values are indicated and error bars represent standard deviations

polymerase, whereas in our study it had an opposite effect on human heterochromatic DNA transcription, i.e. it stimulated it. The present study revealed that alpha satellite DNA is highly susceptible to antibiotic stress. Namely, using three cell lines: glioblastoma A-1235, HeLa and MJ90hTERT, we observed increased transcription of alpha satellite DNA using all three antibiotics at standard concentrations, although the response differed among cell lines. Maximal increase in A-1235 cells was obtained by rifampicin while in MJ90hTERT and HeLa cells geneticin induced the most significant increase of transcription under standard concentrations. The results also reveal a positive correlation between antibiotic concentration and the level of alpha satellite transcription.

Several other antibiotics that specifically target prokaryotes, such as amoxicillin and ampicillin (both penicillin derivatives that inhibit bacterial cell wall synthesis), cefepime and cefuroxime (both β-lactam antibiotics that also inhibit cell wall synthesis), and streptomycin (which binds to bacterial ribosomes and inhibits protein synthesis), also induced increased transcription of alpha satellite DNA. This suggests that their effects may reflect the activation of a general cellular stress response. This raises the question of how these antibiotics affect human cells, independent of their known actions on prokaryotic physiology. Further research is clearly needed to better understand the physiological consequences of applying these commonly used antibiotics.

The cell lines used as models in our research were selected for two specific reasons. First, in our previous experiments they demonstrated robustness and high viability (> 80%; using sublethal, physiological doses) during treatments and manipulations, providing results that were both highly reproducible and significant. Second,

while the effect of antibiotics on satellite expression was observed in all cases, as is clearly shown in Fig. 1., the most significant combinations were chosen for further epigenetic testing. It should also be noted that MJ90 are fibroblasts, immortalized with hTERT, therefore considered non-transformed cells, both morphologically and physiologically.

The increase of alpha satellite expression upon treatment with antibiotics is associated with significant decrease of silent histone mark H3K9me3 at heterochromatic alpha satellite repeats in HeLa and MJ90hTERT cells treated with geneticin and A-1235 cells with rifampicin, respectively, suggesting possible influence of this epigenetic change on alpha satellite DNA transcription. At concentrations of antibiotics which did not significantly affect satellite transcription, no change of H3K9me3 level in HeLa and A-1235 cells was observed, also supporting a possible relation between satellite transcription and H3K9me3 levels at heterochromatin. In MJ90hTERT cells, however, H3K18ac level was increased upon treatment with geneticin alongside the decrease of H3K9me3 level. Since it is known that H3K18 hyperacetylation leads to aberrant accumulation of pericentric transcripts [16], we propose that increased level of H3K18ac might also be responsible for overexpression of alpha satellite DNA in MJ90hTERT cells after geneticin treatment. Previous studies have shown that alpha satellite transcription is not simply a byproduct of deregulation of other genetic elements under normal or stress conditions. While both transposons and satellite DNAs are expressed during the cell cycle, there is no evidence that transposon activity influences alpha satellite transcription. Instead, the alpha satellite transcription seems to be controlled by the presence of centromere–nucleolar contacts [31] and by CENP-B protein which promotes the binding of the zinc-finger transcriptional regulator (ZFAT) responsible for activation of RNA Pol II transcription [32]. It was shown that Topoisomerase I (TopI) promotes the transcription of α-satellite DNAs which is also stimulated in response to DNA double-stranded breaks (DSBs) [33]. However, the results presented here, as well as the increased transcription of alpha satellite DNA in cancer which is associated with decreased H3K9me3 level at satellite repeats [17], suggest a regulation of alpha satellite transcription by epigenetic changes, in particular by histone marks H3K9me3 and H3K18ac. Loss of epigenetic heterochromatic marks was shown to be responsible for overexpression of satellite DNA in ageing [34] and neurodegenerative diseases [23]. It is also important to mention that although present at lower levels than transposon transcripts, alpha satellite RNAs are essential for centromere assembly, kinetochore formation, heterochromatin organization, and may modulate expression of associated genes. These roles highlight the importance of studying their activation and function under stress conditions, alongside other repetitive elements.

Apart from H3K9me3 and H3K18ac we did not observe a change in histone mark H3K4me2, characteristic for open but inactive euchromatin [35], at tandemly arranged heterochromatic alpha satellite repeats. Also, no change in all three epigenetic modifications was detected on alpha satellite repeats dispersed within euchromatin upon antibiotics treatment. More diverse changes, including not only H3K9me3 and H3K18ac but also H3K4me2, were detected genome-wide using immunofluorescence and these changes depended on cell type, antibiotic and antibiotic concentration.

We acknowledge that gene expression and epigenetic responses vary depending on cell type. In our study, we observed distinct regulatory patterns of alpha satellite expression across the tested cell lines, likely influenced by differences in chromatin organization, baseline transcriptional activity, and cellular metabolism. These variations may reflect intrinsic differences between cancerous and normal cells in their response to external stimuli, including antibiotics. Further studies are needed to dissect the molecular mechanisms driving these cell-type-specific effects. Different signaling pathways, overactivated in different cell lines, possibly confer resistance to certain antibiotics as well as types of stress or damage that they induce. It is likely that the level of certain type of stress the cells can resist varies between different cell lines and that their coping mechanisms against different types of stress are also diverse as a general consequence of their specific genetic background.

Our findings suggest that antibiotics may influence satellite DNA transcription by modulating specific histone marks. While the histone modification pathways are well known and highly conserved among all eukaryotes, the underlying mechanisms by which antibiotics potentially interact with said pathways are currently unknown. Ubiquitous effector enzymatic complexes and their functions; such as activating demethylases and acetyl-transferases; suppressing methyl-transferases and deacetylases, as well as chromatin remodeling factors such as SWI/SNF are well understood. Integrated stress response could also play a role by interacting with above-mentioned pathways. Many types of stress, including antibiotic, activate integrated stress response cellular machinery resulting in overproduction of specific transcription factors (such as ATF4), stimulating downstream chromatin remodeling of specific loci and neighboring promoter activation, consequently explaining their overexpression.

It should be noted that transcription of alpha satellite DNA upon antibiotic treatment in some cases exceeded normal transcription rate almost 5-fold (e.g. HeLa cells treated with geneticin 400 μg/ml), possibly affecting cell physiology as well as genome stability in the process.

This paper shows that commonly used antibiotics not only affect bacterial cells but also induce significant transcriptional and epigenetic changes in eukaryotic cells, particularly at alpha satellite DNA regions. The observed epigenetic changes, such as the reduction in H3K9me3 and increase in H3K18ac, provide a potential mechanistic explanation for the transcriptional upregulation of alpha satellite DNA. These findings suggest that researchers using antibiotics in cell culture studies should consider the results described in this paper when such experiments are performed.

Our research highlights the following key points:

1. (1) Routine antibiotic use in cell cultures affects metabolism and genomic stability, warranting caution.
2. (2) Antibiotic treatment in humans may have systemic effects beyond bacterial targeting, varying across tissues.
3. (3) Differential antibiotic effects on cancer vs. healthy tissues could inform potential anticancer strategies.
4. (4) Antibiotics may influence gene expression beyond alpha satellite DNA, potentially affecting other genomic regions and regulatory elements.
5. (5) Understanding individual variability in antibiotic response could improve drug safety and personalized treatment strategies.
6. (6) Chronic antibiotic exposure may have cumulative effects on genomic stability and epigenetic regulation.

These findings underscore broader implications for gene regulation, drug safety, and long-term antibiotic effects, highlighting the need for further research.

Materials and methods

Human cell lines

The following human cell lines were used in experiments: MJ90hTERT (immortalized human skin fibroblasts), HeLa (human cervical cancer) and A-1235 (human glioblastoma). Human diploid fibroblast strain MJ90hTERT (HCA2hTERT) was kindly provided by Dr Olivia M. Pereira-Smith (University of Texas, Health Science Center, San Antonio, TX, USA) [36–38]. HeLa and A-1235 cells were were provided by Thermo Fisher Scientific. Cells were cultured in appropriate medium (DMEM) supplemented with 10% FBS and 5% CO2 at 37 °C. Cells were incubated for 48 h at 37 °C with antibiotics geneticin, rifampicin and hygromycin B (Carl Roth) in complete medium. The concentration range of the antibiotics were: geneticin 300–600 µg/ml, hygromycin B 50–100 µg/ml, as well as with rifampicin 8.2–82 µg/ml (10–100 µM). Data regarding cell viability testing are shown in Figures S7 and S8. Trypan blue assay demonstrated approx. 5–10% of cell death across all antibiotic treatment combinations as well as in untreated controls.

RNA isolation and reverse transcription

For RNA isolation from cell cultures lysis buffer was added directly after the PBS washing step, avoiding trypsin treatment. RNA was quantified with the Quant-IT RNA assay kit using a Qubit fluorometer (Invitrogen). Integrity of RNA was checked by gel electrophoresis. Approximately 1 µg of RNA was reverse transcribed using the PrimeScript RT reagent Kit with gDNA Eraser (perfect Real Time, Takara) in 20 µl reaction using specifically modified primer for alpha satellite rev AATGCAC ATATCACTATGTAC, designed to produce cDNA molecules that differ from genomic DNA in order to avoid DNA contamination [39]. For all samples, negative controls without reverse transcriptase were used.

Quantitative real-time PCR (qPCR) analysis

qPCR analysis was performed according to the previously published protocol (Feliciello et al. 2015). Primers used for transcriptional analysis of alpha satellite DNA were constructed based on consensus sequence derived from cloned alpha satellite monomers of wide-ranging chromosomal origins [40] and the same modified primer used previously in reverse transcription was used in qPCR amplification along with the second primer fw C ACTCTTTTTGTAGAATCTGC. In this way, amplification was unaffected by any potential DNA contamination [34]. Glucuronidase beta (GUSB) [41] was used as an endogenous control for normalization in human samples. GUSB gene (Gene ID: 2990) was stably expressed without any variation among samples after antibiotic treatment. Three additional reference genes (GAPDH, TOP3A and DEK) were also tested and they showed stable expression in all samples, not affected by geneticin treatment (Figure S6). The primers for these genes are listed in Table S2. The following thermal cycling conditions were used: 50 °C 2 min, 95 °C 7 min, 95 °C 15 s, 60 °C 1 min for 40 cycles followed by dissociation stage: 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s and 60 °C for 15 s. Amplification specificity was confirmed by dissociation curve analysis and specificity of amplified products was tested on agarose gel. Control without template (NTC) was included in each run. Post-run data were analysed using LinRegPCR software v.11.1 [42, 43]. which enables calculation of the starting concentration of amplicon ("N_0 value"). N_0 value is expressed in arbitrary fluorescence units and is calculated by taking into account PCR efficiency and baseline fluorescence. N_0 value determined for each technical replicate was averaged and the averaged N_0 values were divided by the N_0 values of the endogenous control. Statistical analysis of qPCR data was done using GraphPad v.6.01 and normalized N_0 values were compared

using the unpaired t-test which compares the means of two unmatched groups.

Chromatin immunoprecipitation

MJ90hTERT, A-1235 and HeLa cells were grown to subconfluence, washed in PBS, scraped in Nuclear Isolation buffer (10 mM MOPS; 5 mM KCl; 10 mM EDTA; 0.6% Triton X-100) with protease inhibitor cocktail CompleteMini (Roche) and chromatin immunoprecipitation was performed according to the published protocol (Feliciello et al. 2015, 2020), with the exception of sonication step which was performed 30 times for 30 s on ice, high sonicator amplitude. The antibodies used were: Anti-Histone H3 (tri methyl K9, Abcam, ab8898), Anti-Histone H3 (di methyl K4, tri methyl K4, Abcam, ab6000), Anti-Histone H3 (acetyl K18, Abcam, ab1191), Anti-Histone H3 (di methyl K4, Abcam, ab7766) and IgG (Santa Cruz Biotechnology, sc2027). Binding of precipitated target was monitored by qPCR using the SYBR Green PCR Master mix (Bio-Rad). Primers used for H3K9me3, H3K18ac and H3K4me2 level analyses at heterochromatic alpha regions as well as at dispersed alpha repeats are listed in Table S1. The N_0 values were normalized using N_0 values of input fractions.

Immunofluorescence

Cells were grown on cover slips up to 70% confluence, washed with PBS and fixed for 5 min in cold methanol. Permeabilization was done by 0.5% triton X-100 for 5 min and blocking with DAKO Protein Block Serum-free ready to use reagent for 1 h at RT. Primary antibodies anti-H3K9me3 (Abcam, ab8898), anti-H3K18ac (Abcam, ab1191) and anti-H3K4me2 (Abcam, ab7766) were diluted in DAKO Antibody Diluent according to the instructions of Abcam, and incubation was performed overnight at 4 °C. After washing in PBS, goat polyclonal secondary antibody to rabbit IgG (ab150081) was diluted 1/1000 in DAKO Antibody Diluent and incubation was performed for 1 h at RT in the dark. Cells were stained with 1 µg/ml DAPI and a drop of DAKO Anti-Fade Fluorescence Mounting Medium was added. Cell slides were sealed with nail polish and analysed by confocal microscopy (Laser Scanning Confocal Microscope Leica SP8 X FLIM). For each sample slide (control and treated), five images were taken and the mean fluorescence values of all structurally and morphologically intact nuclei were quantified using „ImageJ" software [44]. The Shapiro–Wilk test was used to test data normality. Mean fluorescence values of treated samples and controls were tested for statistical significance using the parametric 2-tailed Welch's t-test if the data had normal distribution and non-parametric Mann–Whitney test when it did not. P-values less than 0.05 were considered statistically significant.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13072-025-00628-z.

Supplementary Material 1 :The following supporting information can be downloaded at: https://www.mdpi.com/xxx/s1. Figure S1: Consensus sequence of 171 bp alpha satellite monomer; Figure S2: Transcription of alpha satellite DNA in MJ90hTERT cell line after treatment with prokaryote-specific antibiotics. Figure S3a: H3K9me3 level at tandemly arranged alpha satellite arrays in HeLa cells; Figure S3b: H3K18ac level at tandemly arranged alpha satellite arrays in MJ90hTERT cells; Figure S4a: H3K18ac and H3K4me2 levels at tandemly arranged alpha satellite repeats and dispersed alpha repeats; Figure S4b: H3K4me2 levels of MJ90hTERT cells treated with geneticin; Figure S5: H3K18ac and H3K4me2 levels in HeLa cells after treatment with geneticin; Figure S6: Expression profiles of four housekeeping genes in MJ90hTERT cell line after 48 h treatment with geneticin. Figure S7: Images of tested cell lines treated with most significant antibiotic concentrations. Figure S8: Cell counts after viability assays. Table S1: List of primers used in ChIP-qPCR experiments; Table S2: List of housekeeping genes used as endogenous controls and their primers.

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Author contributions

Conceptualization, I.F.; methodology, M.M., S.L.; formal analysis, S.L., M.M., D.Đ., M.C.F., A.P., I.F.; investigation, S.L., M.M., I.F.; writing original draft preparation, I.F. and Đ.U.; writing—review and editing, Đ.U., I.F., D.Đ.; supervision, I.F; All authors have read and agreed to the published version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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