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The interactions between SATB1 and F-actin are important for mechanisms of active cell death

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Abstract

Introduction. The direct involvement of nuclear actin filaments in gene transcription and remodeling of chromatin is still debatable. However, nuclear localization of F-actin and its interactions with other nuclear matrix proteins have been reported. The aim of the study was to estimate the interactions between nuclear F-actin and one of the matrix proteins, special AT-rich sequence-binding protein 1 (SATB1), during active cell death induced *in vitro* by geldanamycin (GA).

Material and methods. The expression of SATB1 was modified by the transfection of non-aggressive breast cancer MCF-7 cells with siRNA against SATB1 or expression plasmid with cloned cDNA of SATB1. The amount and localization of F-actin were altered by changes of cofilin-1 (CFL1) expression in MCF-7 cells. The association between SATB1 and F-actin during GA-induced cell death was analyzed using confocal and transmission electron microscopy.

Results. Our studies revealed the colocalization between nuclear F-actin and SATB1 protein, during GA-induced death of breast cancer MCF-7 cells. The colocalization was enhanced in cells with overexpressed SATB1 and cofilin-1. At the ultrastructural level the SATB1 and F-actin complexes were seen at the border of condensed and decondensed chromatin. The presence of SATB1/F-actin molecular complexes was confirmed by magnetic separation of F-actin and interacting proteins.

Conclusion. We suggest that the molecular interactions between SATB1 and F-actin are necessary for active cell death to occur. *(Folia Histochemica et Cytobiologica 2015, Vol. 53, No. 2, 152–161)*

Key words: SATB1; F-actin; cofilin-1; geldanamycin; apoptosis; MCF-7 cells; protein interactions; confocal microscopy; electron microscopy

Introduction

The presence of nuclear actin is well established, however, in contrast to its cytoplasmic functions, the role of actin in the nucleus is not well characterized [1]. Nuclear actin exists as a dynamic equilibrium of monomeric (globular or G-actin) and polymeric (filamentous or F-actin) forms [2–4]. The finding that nuclear actin cannot be detected by phalloidin staining [5] casted doubts on this localization; however, development of new methods allowed the detection of nuclear F-actin. Functional studies imply that actin must be present in a polymerized form [6], *e.g.* McDonald et al., using FRAP technique, showed that about 20% of the total nuclear actin pool is in the polymeric state [2]. Nuclear actin has been

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implicated in various processes, such as chromatin remodeling, transcriptional regulation, RNA processing, transcription of RNA polymerases, and nuclear export [3, 4, 6, 7]; however, the molecular details of nuclear actin's action are not well understood. Numerous actin-binding and actin-related proteins were detected in the nucleus [4], but it still remains unclear whether these proteins control nuclear processes on their own or in associations with actin.

One of the most important nuclear proteins involved in chromatin organization and gene expression is special AT-rich sequence-binding protein 1 (SATB1). It binds specifically to AT-rich sequences of DNA by recognizing the base unpairing regions (BURs) and recruits chromatin remodeling complexes to the anchored sites [8, 9]. SATB1, as a potent epigenetic regulator, may affect the transcription of numerous genes [10]. The results of earlier studies suggested the involvement of SATB1 also in the processes of cell death. Sun et al. demonstrated that SATB1 formed special three-dimensional network during early apoptosis. Changes of SATB1 distribution were associated with its cleavage and accompanied by collapse of the nuclear architecture [11]. Galande et al. suggested that mechanisms of nuclear degradation in early apoptotic T cells involved efficient removal of SATB1 by inhibiting its dimerization and cleavage of genomic DNA into loop domains which resulted in rapid and efficient disassembly of higher-order chromatin structures [12].

Altered expression of SATB1 protein was observed in several malignancies such as breast, laryngeal, gastric, colorectal, liver and ovarian cancers [13–18]. Our previous studies indicated the presence of F-actin in nuclei of different cell lines after induction of the active cell death process due to cells' treatment with anti-cancer drugs [19–23]. Moreover, we observed the colocalization of SATB1 and F-actin in the transcriptional active regions of the cell nucleus during active cell death of the Chinese hamster ovary cell line (CHO AA8 cell line) following treatment with doxorubicin [24]. Results of our recent study revealed that targeted regulation of SATB1 and cofilin-1 (CFL1), a regulator of actin dynamics responsible for its translocation to the nucleus [25, 26], changed the apoptotic response of breast cancer cells (MCF-7 cell line) to geldanamycin (GA) [27]. The aim of the present study was to estimate the nuclear localization and interactions between F-actin and SATB1 during active cell death of MCF-7 cells treated *in vitro* by GA in conditions of the controlled expression of these proteins.

Material and methods

Cell culture and treatment. Human breast adenocarcinoma MCF-7 cell line was purchased from American Type Culture Collection (ATCC; HTB-22). The cells were cultured and treated with 2 μM GA (Sigma-Aldrich, St. Louis, MO, USA) for 24 h, as described previously [27].

Cell transfection by nucleofection. For the nucleofection of MCF-7 cells, the cells were grown in cell culture flasks (BD Biosciences, Franklin Lakes, NJ, USA) up to 80–90% confluence in Minimum Essential Medium Eagle (MEM, Sigma-Aldrich, St. Louis, MO, USA) with the addition of 10% fetal bovine serum (FBS, Gibco/Life Technologies, Carlsbad, CA, USA) and 50 μg/mL gentamycin (Sigma-Aldrich). After trypsinization, a total of 2×10^6 cells were transfected using SE Cell Line 4D-Nucleofector X Kit (Lonza, Verviers, Belgium) according to the manufacturer's instructions and as described previously [27]. Briefly, the cells were suspended in 100 μL of the mixture containing SE Nucleofector Solution, together with 3 pmol siRNA against human SATB1 (Hs_SATB1_3; Qiagen, Hilden, Germany), 3 pmol siRNA against human cofilin-1 (Hs_CFL1_3), 2 μg human cDNA of SATB1 cloned into pCMV6-XL5 expression plasmid vector (NM_002971; OriGene, Rockville, MD, USA) or 2 μg human cDNA of cofilin-1 cloned into pCMV6-XL5 expression plasmid vector (NM_005507; OriGene, Maryland, USA), respectively. Then, the mixture was transferred into electroporation cuvettes and the transfection was done using 4D-Nucleofector device (Lonza) under the program EN-130. As a negative control, the commercially designed AllStars negative control siRNA (Qiagen) or pCMV6-XL5 control plasmid vector (OriGene) were used. After electroporation, the cells were grown in medium without antibiotics for 72 h and then used for further experiments.

Immunofluorescent staining of SATB1 and F-actin. After transfection and incubation with GA, the MCF-7 cells were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 20 min and washed with PBS (3 × 5 min). After permeabilization with 0.1% (v/v) Triton X-100 (Sigma-Aldrich) in PBS for 5 min and washing with PBS, the non-specific binding was blocked by 1% (w/v) bovine serum albumin in PBS (BSA/PBS) for 15 min. Subsequently, SATB1 protein was labeled by incubating of the cells with rabbit monoclonal anti-SATB1 antibody (Abcam, Cambridge, MA, USA), diluted 1:50 in 1% BSA/PBS for 1 h at room temperature (RT). Then, the cells were washed with PBS (3 × 5 min) and incubated in the dark with Alexa Fluor 488 goat anti-rabbit IgG (H + L; Invitrogen/Life Technologies) for 1 h at RT. For the visualization of F-actin, the cells were incubated with phalloidin-TRITC conjugate (Sigma-Aldrich) diluted 1:5 in 1% BSA/PBS for 20 min at RT, in the dark. After washing with PBS, the cell nuclei

were stained with 4',6'-diamidino-2-phenylindole dihydrochloride (DAPI, Sigma-Aldrich) for 10 min at RT, in the dark. The slides were mounted in Aqua-Poly/Mount (Polysciences, Warrington, PA, USA) and examined using C1 laser-scanning confocal microscope (Nikon, Tokyo, Japan) with ×100 oil immersion objective (Nikon). Images were captured using Nikon EZ-C1 software (Ver. 3.80; Nikon) using the same laser power, pixel dwell and gain settings.

Analysis of SATB1 and F-actin colocalization. The analysis of the degree of overlap between SATB1 and F-actin fluorescence was performed using ImageJ software (Ver. 1.47i) and Colocalization Threshold plugin. The data were presented as colocalization pixel map and a 2D intensity histogram with indicated linear regression.

Ultrastructural localization of SATB1 and F-actin. The localization of SATB1 and F-actin at the ultrastructural level was performed using combination of pre- and post-embedding labeling methods. After transfection and incubation with GA, the MCF-7 cells were fixed with 4% paraformaldehyde in PBS for 20 min and washed with PBS (3 × 5 min). After permeabilization with 0.1% Triton X-100 (Sigma-Aldrich) in PBS for 5 min and washing with PBS, the non-specific protein interactions were blocked by 6% BSA in PBS for 1 h. For F-actin localization, the cells were first incubated with biotinylated phalloidin (Sigma-Aldrich) diluted in blocking solution (1:85) for 20 min at RT. Next, after washing with PBS (3 × 5 min), the cells were post-fixed in 1% OsO_4 (Serva Electrophoresis GmbH, Heidelberg, Germany), dehydrated and embedded in LR White (Sigma-Aldrich). After polymerization of resin, the samples were cut into the ultrathin sections and placed on nickel grids (Sigma-Aldrich). Biotinylated phalloidin was detected using Qdot 525 streptavidin conjugate (Invitrogen/ /Life Technologies,) diluted 1:100 in blocking solution for 1 h at RT. For the ultrastructural localization of SATB1, a post-embedding immunogold method with rabbit monoclonal anti-SATB1 antibody (Abcam) in blocking solution and goat anti-rabbit IgG 20 nm Gold Conjugate (SPI Supplies, West Chester, PA, USA) was used. After staining with uranyl acetate, the preparations were examined under a JEM 100 CX electron microscope (Jeol, Tokyo, Japan) operating at 80 kV.

FRET acceptor bleaching method. To study SATB1 interactions with F-actin, the FRET acceptor bleaching (AB) method was used. The images were collected using a C1 laser-scanning confocal microscope (Nikon) and captured using Nikon EZ-C1 software (Ver. 3.80; Nikon). The preparations for FRET analysis were carried out analogically as for the immunofluorescence experiments; however, the counterstaining step was omitted. Briefly, for labeling of SATB1 and F-actin, Alexa Fluor 488 goat anti-rabbit IgG (H + L; Invitrogen/Life Technologies) and phalloidin-TRITC conjugate (Sigma-Aldrich) were used, respectively. Alexa Fluor 488 (acceptor) was induced using a diode laser 488 nm, while TRITC (donor) was induced using a HeNe laser 543 nm. FRET AB method was performed by comparing donor fluorescence intensity in the same sample before and after destroying the acceptor by photobleaching process. If FRET was initially present, a resultant increase in donor fluorescence would occur on photobleaching of the acceptor. The energy transfer efficiency was calculated using the following formula: $FRET_{eff} = (D_{post}–D_{pre})/D_{post}$; where D_{post} is the fluorescence intensity of the donor after acceptor photobleaching and D_{pre} the fluorescence intensity of the donor before acceptor photobleaching. The FRET efficiency ($FRET_{eff}$) was considered as positive when $D_{post} > D_{pre}$.

Magnetic separation of F-actin and interacting proteins. For the analysis of the molecular complexes between SATB1 and F-actin the method of magnetic separation of F-actin and interacting proteins was applied. The streptavidin-coated magnetic beads were used (Dynabeads M-280 Streptavidin; Invitrogen/Life Technologies) according to manufacturer's instructions with several modifications. Briefly, following transfection and incubation of the MCF-7 cells with GA, the actin cytoskeleton was stabilized by incubation for 20 min with biotinylated phalloidin diluted 1:85 in PBS at 37°C. Then, the cells were lysed in RIPA buffer in the presence of biotinylated phalloidin (1:85; both from Sigma-Aldrich) and clarified by centrifugation at 8,000 g. After normalization of protein concentration by BCA Protein Assay Kit (Thermo Scientific Pierce, Rockford, IL, USA) using spectrophotometer, 100 μg of total protein was incubated with 0.5 mg of streptavidin-coated magnetic beads. After five cycles of magnetic separation and washing in PBS containing 0.1% BSA (Sigma-Aldrich), the biotin-streptavidin bonds were broken by 10 min boiling in 0.1% SDS (Sigma-Aldrich). The presence of SATB1 in the molecular complex with F-actin was analyzed by Western blot method as described previously [27]. The immunoreactive bands were then visualized using ready-to-use solution of BCIP/NBT substrate for alkaline phosphatase. After scanning, the densitometry of the bands was quantified using the Quantity One Basic software (Ver. 3.6.5; Bio-Rad, Hercules, CA, USA).

Statistical analysis. The data were shown as mean ± SEM. Statistical comparisons between two groups of colocalization, $FRET_{eff}$ or densitometric analysis data were performed with Mann-Whitney U test. The differences between the groups were considered significant at $p \leq 0.05$. The GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA) was used for statistical analyses.

Results

Alterations of SATB1 and cofilin-1 expression in MCF-7 cells change SATB1/F-actin colocalization profile in response to geldanamycin

The analysis of the colocalization between SATB1 and F-actin was performed on micrographs captured by laser-scanning confocal microscope. It was found that the percentage of colocalized pixels increased statistically significantly together with the GA treatment after the transfection of MCF-7 cells with non-targeting siRNA or empty plasmid ($P = 0.0038$ and $P = 0.0383$, respectively) (Figure 1A: k; Figure 2A: k). These increases were seen both along F-actin stress fibers in cytoplasm and cell nucleus (Figure 1A: i; Figure 2A: i). In cells transfected with $siRNA_{SATB1}$, the GA-dependent changes in the colocalization of SATB1 and F-actin were not noticed (Figure 1B: k). In control cells, colocalization was observed mainly along thick stress fibers of F-actin and amounted to 1.33% (Figure 1B: d, e, k). Similar colocalization (1.70%) after the exposition to 2 μM geldanamycin was seen (Figure 1B: i, j, k).

In cells with the overexpressed SATB1, the colocalization of SATB1 and F-actin was observed mainly along stress fibers of F-actin and amounted to 9.86% (Figure 2B: d, e, k). Incubation of these cells with GA increased the colocalization to 32.13% ($P = 0.0083$) and the interactions SATB1 and F-actin were located mainly in the nuclear area (Figure 2B: i, j, k). After the down-regulation of CFL1, statistically significant changes in colocalization between SATB1 and F-actin were not observed (Figure 1C: k). On the other hand, in the cells transfected with plasmid vector with cloned cDNA of CFL1, the colocalization between SATB1 and F-actin was 6.17% and increased to 11.50% ($P = 0.0383$) after the treatment with 2 μM GA (Figure 2C: k). Similarly, as in the cells transfected with empty plasmid and in the cells with overexpression of CFL1, GA-induced nuclear localization of SATB1/F-actin complexes was also noticed (Figure 2C: i).

To confirm the results obtained at the level of confocal microscope, the localization of SATB1 and F-actin in the MCF-7 cells with overexpression of SATB1 and CFL1 was studied at the ultrastructural level. As shown in Figure 3, after the treatment of cells with 2 μM GA, SATB1 (labeled with 20 nm gold particles) and nuclear F-actin (labeled with Qdots 525) were colocalized at the border of electron-dense heterochromatin and electron-transparent euchromatin.

Overexpression of SATB1 and cofilin-1 in MCF-7 cells increases SATB1/F-actin interactions in the response to geldanamycin

The analysis of SATB1 and F-actin interactions by the FRET acceptor bleaching method revealed that labeled SATB1 and F-actin molecules were close enough to imply their structural interaction (Figure 4A). Moreover, statistically significant, GA-dependent increase of FRET efficiency was observed in the MCF-7 cells transfected with empty plasmid as well as in the cells transfected with plasmid vector with cloned cDNA of SATB1 or CFL1. More precisely, in the MCF-7 cells transfected with empty plasmid, GA induced 8.05% increase of FRET efficiency ($P = 0.0013$) and donor (SATB1) fluorescence increased mainly in the cell nucleus area (Figure 4B, C: a–b). After the exposure of cells with overexpression of SATB1 to 2 μM GA, the efficiency of FRET was statistically significantly increased by 17.65% ($P = 0.0048$) and the donor fluorescence increased both in the area of cell nucleus and along acceptor (F-actin) stress filaments (Figure 4D, E: a–b).

Similarly, the exposure of MCF-7 cells with overexpression of CFL1 to 2 μM GA increased donor fluorescence by 7.38% ($P = 0.0045$) both in the cell nucleus area and along F-actin stress filaments (Figure 4F, G: a–b).

Magnetic separation of F-actin and interacting proteins confirmed the presence of molecular complexes between SATB1 and F-actin. As shown in Figure 5, the treatment of MCF-7 cells with 2 μM GA increased the quantity of precipitated SATB1 by 5%, 22% and 8% for the cells transfected with empty plasmid, overexpression of SATB1 and CFL1, respectively.

Discussion

Despite some claims that nuclear actin exists only in a globular form, recent reports have revealed the presence of nuclear actin in multiple forms: monomeric, oligomeric and short-polymeric [2, 3, 7]. Also the results of our studies obtained by a modification of both confocal and transmission electron microscopy (TEM) and combination of pre- and post-embedding labeling techniques demonstrated the presence of actin filaments in cell nuclei of various cell lines [19–23]. In the present study we have also detected the presence of SATB1 protein in nuclei of MCF-7 cells, a human breast cancer cell line widely used to investigate SATB1 expression [13, 28, 29].

The main purpose of the present study was to evaluate the possible interactions between the nuclear

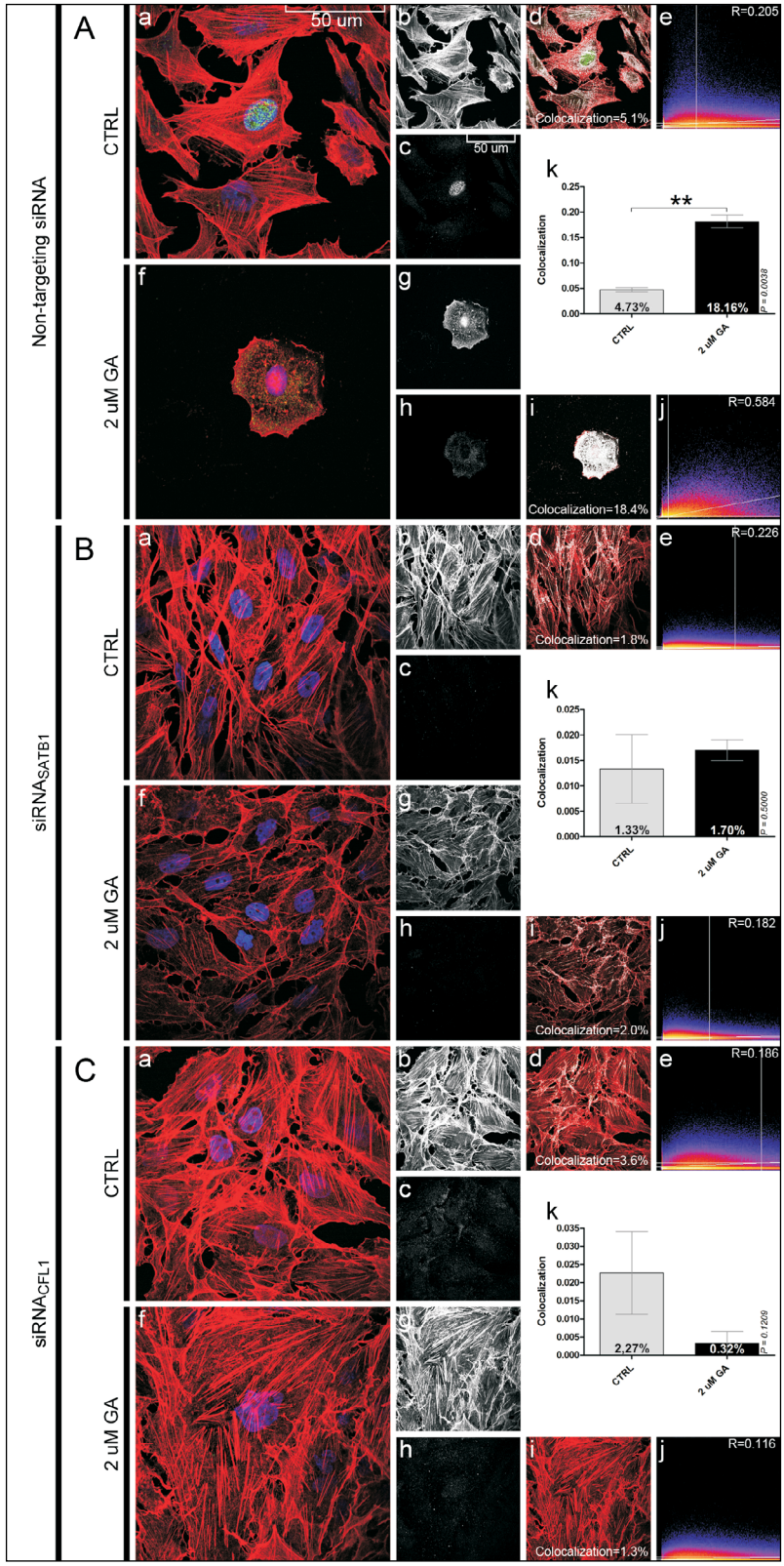

Figure 1. The influence of 2 μM geldanamycin (GA) on changes in the colocalization of SATB1 and F-actin in MCF-7 cells with down-regulated expression of SATB1 or cofilin-1 (CFL). **A.** Cells transfected with non-targeting siRNA; **B.** Cells transfected with siRNA against SATB1; **C.** Cells transfected with siRNA against CFL1. **a–e.** Control cells; **f–j.** Cells treated with 2 μM geldanamycin; **a, f.** Fluorescent localization of SATB1 and F-actin; **b, g.** Fluorescent localization of F-actin; **c, h.** Fluorescent localization of SATB1; **d, i.** Pixel map of interactions between SATB1 and F-actin; **e, j.** Correlation of pixel intensity; **k.** The influence of 2 μM geldanamycin on changes in SATB1 and F-actin colocalization. *Bars* represent mean ± SEM. **p < 0.01. CTRL — control

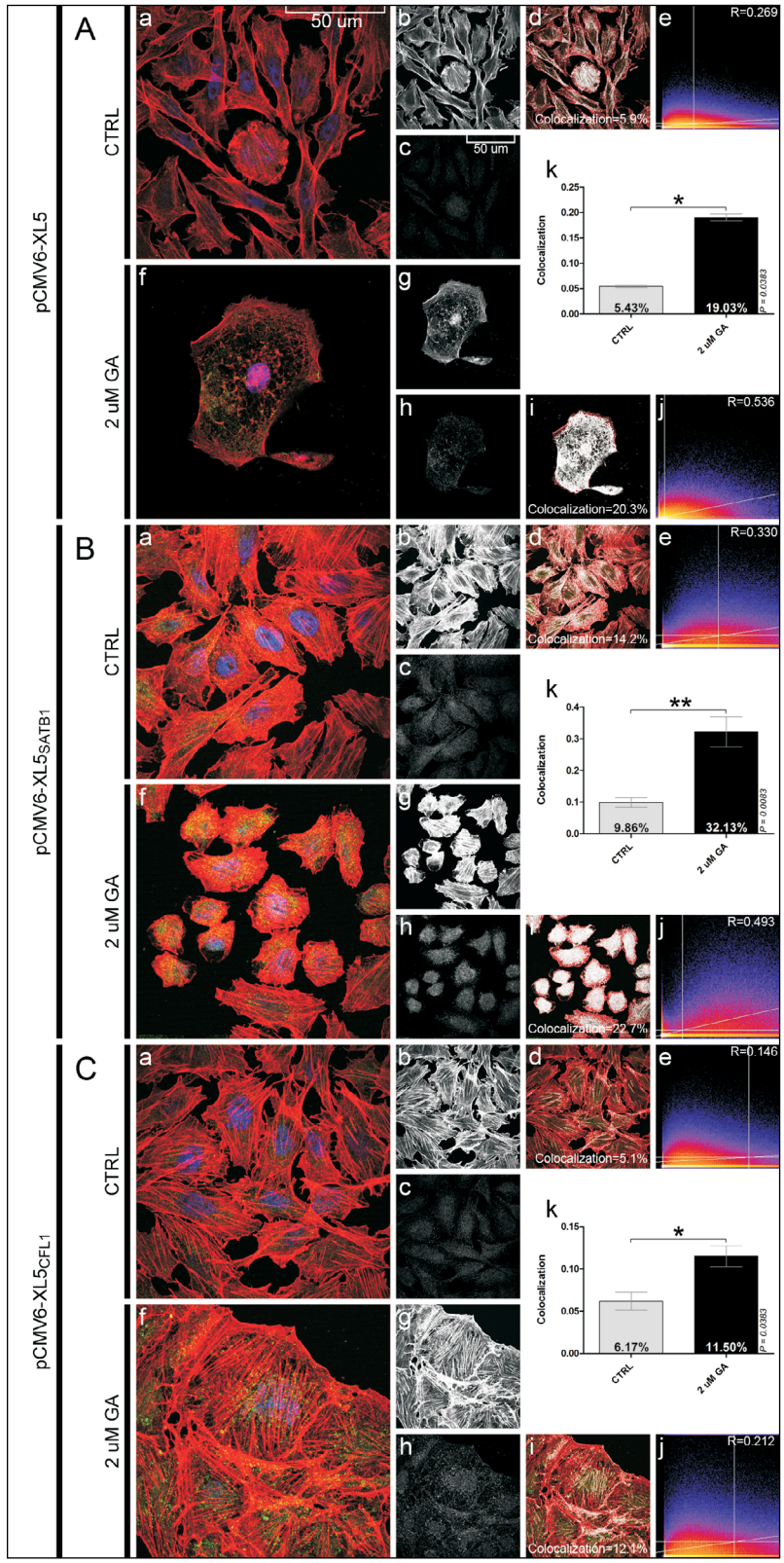

Figure 2. The influence of 2 μM geldanamycin (GA) on changes in the colocalization of SATB1 and F-actin in MCF-7 cells with overexpression of SATB1 or cofilin-1 (CFL1). **A.** Cells transfected with pCMV6-XL5 empty plasmid; **B.** Cells transfected with pCMV6-XL5 expression plasmid with cloned cDNA of SATB1; **C.** Cells transfected with pCMV6-XL5 expression plasmid with cloned cDNA of CFL1. **a–e.** Control cells; **f–j.** Cells treated with 2 μM geldanamycin; **a, f.** Fluorescent localization of SATB1 and F-actin; **b, g.** Fluorescent localization of F-actin; **c, h.** Fluorescent localization of SATB1; **d, i.** Pixel map of interactions between SATB1 and F-actin; **e, j.** Correlation of pixel intensity; **k.** The influence of 2 μM geldanamycin on changes in SATB1 and F-actin colocalization. *Bars* represent mean ± SEM. *p < 0.05, **p < 0.01. CTRL — control

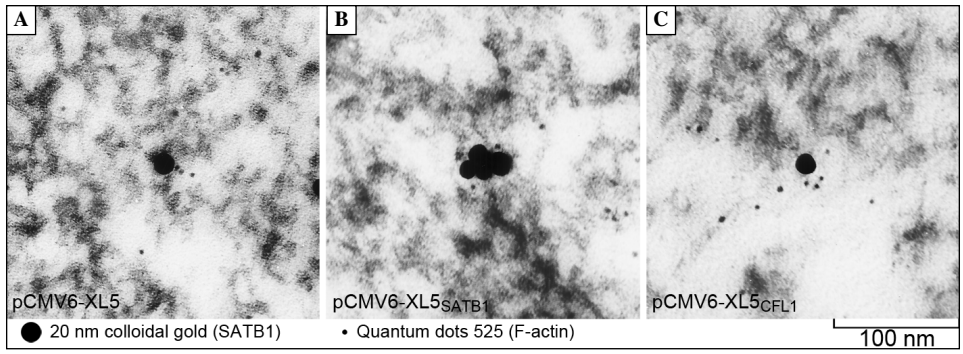

Figure 3. Ultrastructural colocalization of SATB1 and F-actin in the MCF-7 cells with overexpression of SATB1 or cofilin-1 (CFL1) after their treatment with 2 μM geldanamycin (GA). **A.** The nucleus of cells transfected with pCMV6-XL5 empty plasmid; **B.** The nucleus of cells transfected with pCMV6-XL5 expression plasmid; **C.** The nucleus of cells transfected with pCMV6-XL5 expression plasmid with cloned cDNA of CFL1

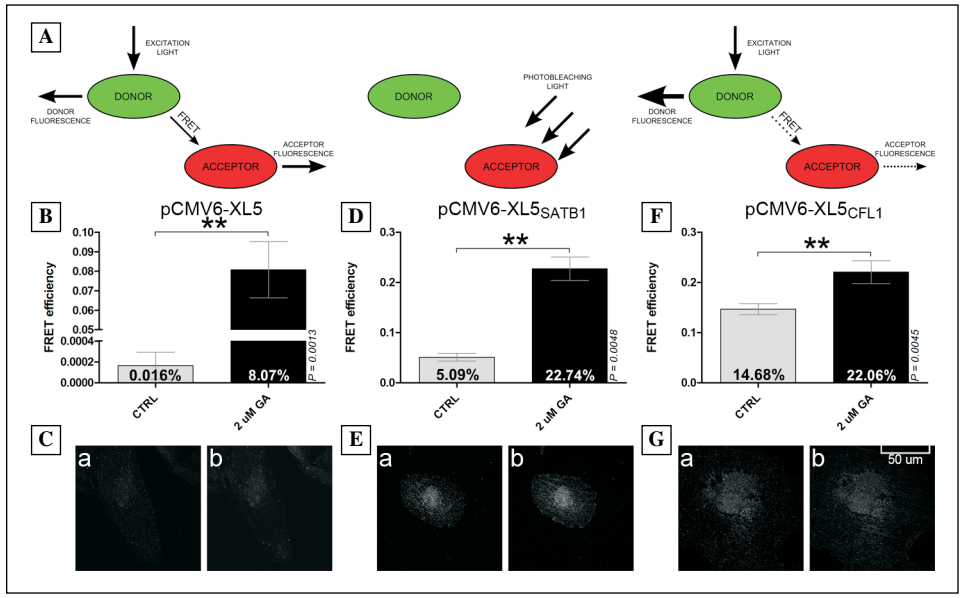

Figure 4. The influence of 2 μM geldanamycin (GA) on FRET efficiency in the MCF-7 cells with overexpression of SATB1 or cofilin-1 (CFL1). **A.** Schematic diagram of the FRET acceptor bleaching experiment; **B, D, F.** FRET efficiency in the MCF-7 cells transfected with **(B)** pCMV6-XL5 empty plasmid, **(D)** pCMV6-XL5 expression plasmid with cloned cDNA of SATB1, **(F)** pCMV6-XL5 expression plasmid with cloned cDNA of CFL1. *Bars* represent mean ± SEM. **p < 0.01. CTRL — control; **C,E,G.** Donor fluorescence signal before **(a)** and after **(b)** acceptor photobleaching in the MCF-7 cells transfected with **(C)** pCMV6-XL5 empty plasmid, **(E)** pCMV6-XL5 expression plasmid with cloned cDNA of SATB1, **(G)** pCMV6-XL5 expression plasmid with cloned cDNA of CFL1

pool of F-actin and SATB1 during active cell death of MCF-7 cells treated by geldanamycin in conditions of the controlled expression of both mentioned proteins. GA is an ansamycin-derivative benzoquinone compound that inhibits the function of Hsp90 (Heat Shock Protein 90) causing deregulation of the such processes as cell cycle, cell growth, and cell survival [30]. The effects of the Hsp90 inhibition are complex and depend on the biochemical and molecular features of treated cells [31]. Our previous studies indicated the increased incidence of apoptosis and the decreased frequency of polyploidy cells with the features of mitotic catastrophe after GA treatment of MCF-7 cells [27]. In the present study, we found that the induction of the MCF-7 cells death by GA increased the colocalization and interactions of SATB1 protein and F-actin. Moreover, using phalloidin-based magnetic separation of F-actin and interacting proteins, we confirmed the presence of molecular complexes between SATB1 and F-actin. These results suggest

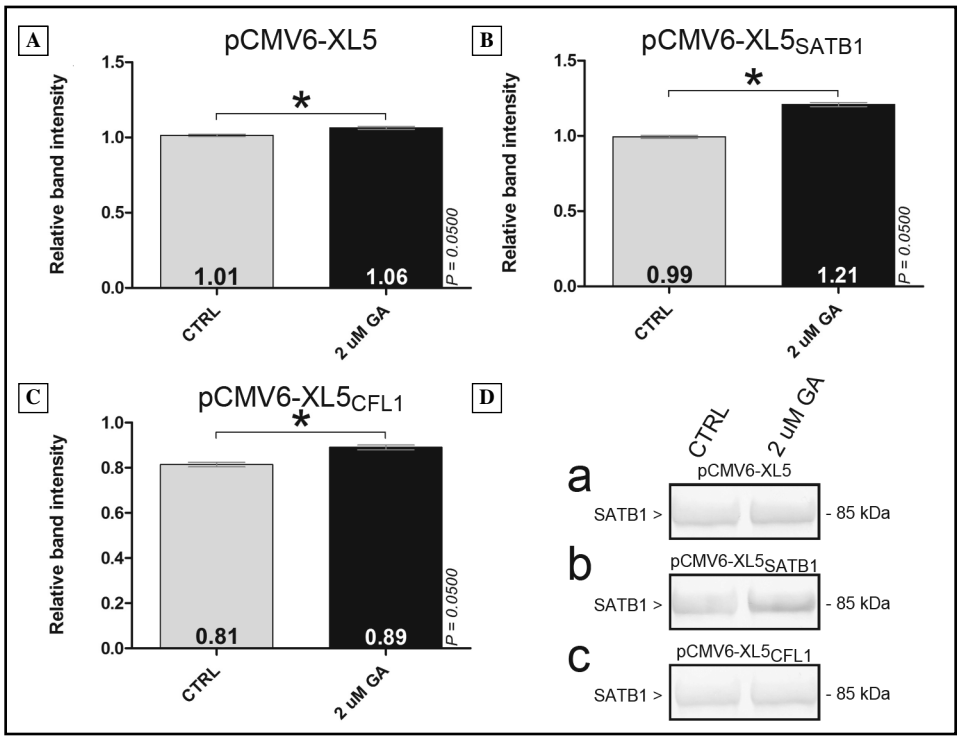

Figure 5. The influence of 2 μM geldanamycin (GA) on changes of SATB1/F-actin biological interactions at the molecular level in the MCF-7 cells with overexpression of SATB1 or cofilin-1 (CFL1). **A–C.** The amount of SATB1 in molecular complex with F-actin in the MCF-7 cells transfected with **(A)** pCMV6-XL5 empty plasmid, **(B)** pCMV6-XL5 expression plasmid with cloned cDNA of SATB1, **(C)** pCMV6-XL5 expression plasmid with cloned cDNA of CFL1 detected by Western blot method. CTRL — control. *Bars* represent mean ± SEM; **D.** Representative blots of studied cells transfected with **(a)** pCMV6-XL5 empty plasmid, **(b)** pCMV6-XL5 expression plasmid with cloned cDNA of SATB1, and **(c)** pCMV6-XL5 expression plasmid with cloned cDNA of CFL-1

that both proteins may jointly act to promote changes in chromatin and nuclear architecture during active cell death. This assumption was confirmed by finding that after the induction of cell death, the colocalization of SATB1 and F-actin was observed mainly in the nuclear area and, especially, at the border of condensed and decondensed chromatin as observed at the ultrastructural level. These observations are in agreement with the results of our previous investigation in which we found the colocalization of SATB1 and F-actin in the transcriptionally active regions of the nuclei of CHO AA8 cells undergoing active cell death [24].

Many authors showed that actin may translocate into the cell nucleus to function as a transcriptional modulator of gene expression [1, 3, 32–36]. We have previously demonstrated not only the translocation of F-actin into the nucleus, but also its involvement in chromatin remodeling during cell death [19–23]. Our current findings together with results of the previous report on CHO AA8 cells [24] collectively suggest that SATB1 may not only provide the structural background for chromatin organization but also it may play a significant role in the reorganization of chromatin during cell death. Findings presented in this and previous study [24] suggest that SATB1/F-actin complexes are present in cancer cells undergoing active cell death, and that chromatin remodeling by F-actin associated with SATB1 during cell death occurs independently on the cell type.

Moreover, in the MCF-7 cells overexpressing SATB1 and CFL1, GA-induced colocalization and interactions of SATB1 and F-actin were enhanced. Our previous investigations indicated that the alterations in SATB1 and CFL1 expression affected apoptotic response of the MCF-7 cells to GA. Moreover, the overexpression of these proteins potentiated GA-induced arrest of the cells in the G1 phase of cell cycle and increased the population of the hypodiploid cells [27]. Chua et al. reported that CFL1 plays an important role during the initiation phase of apoptosis [37]. Similarly, our previous observations suggested that the expression of CFL1 is necessary for the activation of apoptotic cell death [22]. CFL1 is a key regulator of the actin dynamics, by catalyzing actin polymerization and depolymerization [25, 26]. It binds actin and can translocate into the nucleus during times of cell stress [38]. CFL1 may also transport DNase I into

the nucleus by forming a tight cofilin-actin-DNase I ternary complex [39]. Our finding that the induction of CFL1 expression increased nuclear F-actin labeling could be explained by the involvement of CFL1 in the transport of actin monomers to the cell nucleus and their reassembly into short polymers [6].

The significance of SATB1 expression during active cell death is controversial and requires further investigations. Downregulation of SATB1 in Jurkat cells caused their resistance to induced cell death [40]. In line with these observations, Wang et al. reported that deficiency in SATB1 expression in Sézary cells caused resistance to apoptosis [41]. However, Chu et al. found that downregulation of SATB1 expression was responsible for the initiation of active cell death [42].

In summary, this is the first report evaluating the interactions and colocalization of SATB1 and nuclear F-actin during active cell death of the MCF-7 breast cancer cells. By using various methods we showed that the induction of cell death by GA increases nuclear colocalization of SATB1 and F-actin. Based on the results of both the current and previous our research, we may conclude that the SATB1/F-actin complexes are present in the nuclei of dying cells and interactions of SATB1 and F-actin seem not to be dependent on the cell type.

Acknowledgments

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