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The Relationships Between Snail1 and Estrogen Receptor Signaling in Breast Cancer Cells

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ABSTRACT

The loss of hormonal dependency of breast tumor cells is often accompanied with the appearance of epithelial-mesenchymal transition (EMT) features and increase in cell metastasis and invasiveness. The central role in the EMT belongs to transcription factors Snail responded for the decrease in E-cadherin expression and cell contacts, stimulation of cell mobility and invasiveness. Aim was to study the relationships between estrogen receptor machinery and Snail1 signaling, and mechanism of Snail1 regulation in hormone-resistant breast cancer cells. The experiments were performed on the estrogen-dependent MCF-7 breast cancer cells, estrogen-hyposensitive MCF-7/LS subline generated through long-term cultivation of the parental cells in steroid-free medium, and ER-negative estrogen-resistant HBL-100 cells. Snail1, estrogen receptor, p65 NF-κB, E-cadherin levels were analyzed by Western blot. We found that decrease in the estrogen dependency is correlated with increase in Snail1 expression and activity, we demonstrated the Snail1 involvement in the negative regulation of ER, and showed that Snail1 inhibition partially restores the sensitivity of the estrogen-hyposensitive cells to antiestrogen tamoxifen. Furthermore, NF-κB was found to serve as a positive regulator of Snail1 in breast cancer cells, and simultaneous inhibition of NF-κB and Snail1 resulted in additional increase in cell response to tamoxifen. In general, the results obtained demonstrate the phenomenon of Snail1 activation in the hormone-resistant breast cancer cells, and show that Snail1 and NF-κB may serve as an important targets in the treatment of breast cancer, both estrogen-dependent and estrogen-independent tumors. J. Cell. Biochem. 113: 2147–2155, 2012.

KEY WORDS: EPITHELIAL-MESENCHYMAL TRANSITION; SNAIL1; ESTROGEN RECEPTOR; BREAST CANCER; NF-κB; HORMONE RESISTANCE

The efficiency of endocrine therapy of tumors, including breast cancer, is limited by development of hormone-independent tumors which are resistant to antiestrogens initially or acquire resistance under prolonged therapy with antiestrogens (tamoxifen, raloxifene) [Alvarez, 2010; Orlando et al., 2010]. The most frequent mechanism of hormonal resistance is based on the activation of hormone-independent mitogenic signaling, including receptor tyrosine kinases, cell cycle-regulating proteins, PI3K/Akt cascade, etc. [Gnatyshak and Dryzhak, 1991; Dowsett, 2001; Weinberg et al., 2005; Scherbakov et al., 2006; Steelman et al., 2011].

Recently it was shown that the loss of hormonal dependency of breast cancers is often accompanied with increase in cell metastasis, invasiveness and appearance of epithelial–mesenchymal transition (EMT) features. The central role in the EMT belongs to transcription factors Snail1 responded for the decrease in E-cadherin expression and cell contacts, stimulation of cell mobility and invasiveness. Several studies documented the possible estrogen involvement in Snail1 down-regulation [Fujita et al., 2004; Park et al., 2008], demonstrated the correlation between development of hormonal resistance and increase in Snail1 expression in the breast tumors [Kim et al., 2009; Lundgren et al., 2009]. However, the mechanisms of relationship between EMT and estrogen signaling, as well as the role of Snail1 signaling in the regulation of estrogen receptor (ER) machinery still remains unclear.

Abbreviations: TPA, 12-*O*-tetradecanoylphorbol-13-acetate; ER, estrogen receptor; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; siRNA, small interfering RNA; E2, 17beta-estradiol.

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In the present article, using in vitro cultured breast cancer cell lines we found that acquisition of hormonal resistance correlates with increase in Snail1 expression and activity. We show Snail1 involvement in the negative regulation of ER, and demonstrate that Snail1 inhibition partially restores the sensitivity of the resistant breast cancer cells to antiestrogen tamoxifen.

MATERIALS AND METHODS

CELL CULTURE

The human breast cancer cell line MCF-7 and human breast epithelial cell line HBL100 (immortalized by SV40 large T antigen) were cultured in standard DMEM medium supplemented with 7% fetal calf serum (FCS) (HyClone) at 37°C and 5% CO_2. The subline MCF-7/LS was developed by long-term (60 days) cultivation of the parental cell line MCF-7 in phenol red-free DMEM medium supplemented with 7% steroid-free fetal serum. The steroid-free serum was prepared by treatment of fetal serum with dextran-coated charcoal (Sigma–Aldrich), according to the routine method described by Provost et al. [2000]. The cell growth was evaluated by the MTT-test based on the accumulation by living cells of an MTT reagent (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) [Sobottka and Berger, 1992].

TRANSIENT TRANSFECTION AND MEASUREMENT OF REPORTER GENE ACTIVITY

The plasmids containing the full-length wSnail1 and ERalpha were kindly provided by Dr. Antonio García de Herreros (Universitat Pompeu Fabra, Barcelona) and Dr. Craig Jordan (Georgetown University Medical Center). The pMIG plasmid containing IκBα repressor was kindly given by Dr. Alexander Gasparian (Cbiolabs).

To determine the transcriptional activity of Snail1 and ER, the cells were transfected with the plasmids containing luciferase reporter gene controlled by the promoter with Snail1 responsive or estrogen responsive elements, respectively. The reporter plasmids used in this work were kindly provided by Dr. Antonio García de Herreros [Vincent et al., 2009] and Dr. George Reid [Reid et al.,

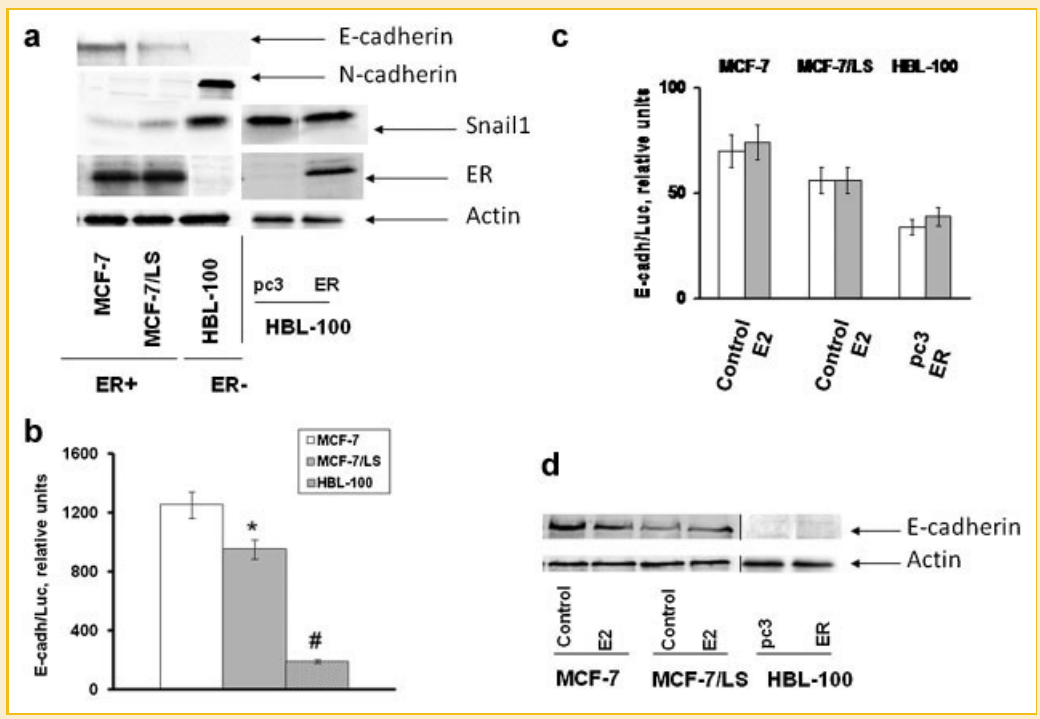

Fig. 1. Expression and activity of Snail1 in breast cancer cell lines. Western blot of E-cadherin, N-cadherin, Snail1, and ER (a) and Snail1 activity (b) in MCF-7, MCF-7/LS, and HBL-100 cells. Protein loading was controlled by membrane hybridization with anti-actin Abs. The blot represents the results of one of the three similar experiments. The HBL-100 cells were transfected with the full-length ERalpha or control PC3 plasmids. To analyze Snail1 activity the MCF-7, MCF-7/LS, and HBL-100 cells were transfected with the luciferase reporter gene construct containing wild-type E-cadherin promoter sequences. The transfection efficiency was controlled by co-transfection of the cells with plasmid containing the β-galactosidase gene. Twenty-four hours after transfection the luciferase and β-galactosidase activities were determined as described in "Materials and Methods" Section, and relative luciferase activity was calculated in arbitrary units as the ratio of the luciferase to the galactosidase activity. Data represent mean value ± SD of at least three independent experiments. $P < 0.05$ * versus MCF-7 and HBL-100 cells, # versus MCF-7 and MCF-7/LS cells. Estradiol influence on Snail1 activity (c) and E-cadherin level (d) in MCF-7, MCF-7/LS, and HBL-100 cells. The cells were transfected with the luciferase reporter gene construct under E-cadherin promoter. The transfection efficiency was controlled by co-transfection with β-galactosidase plasmid. Three hours after transfection MCF-7 and MCF-7/LS cells were treated with or without 10^{-9} M E2 for 24 h. HBL-100 cells were co-transfected with pc3 (empty vector) or ERalpha for 24 h, and reporter luciferase activity was determined. Data represent mean value ± SD of at least three independent experiments. The cells were treated with 10^{-9} M E2 for 24 h, and subjected to Western blot analysis for E-cadherin expression. The blot represents the results of one of the three similar experiments, protein loading was controlled by anti-actin hybridization.

2003]. The transfection was carried out for 4 h at 37°C using Metafectene (Biontex Laboratories GmbH). To control the efficiency and potential toxicity of the transfection, the cells were transfected with the β-galactosidase plasmid. All subsequent experiments were performed during 48 h after the transfection. The luciferase activity was measured according to a standard protocol (Promega) using a Turner BioSystems 20/20n luminometer. The luciferase activity was calculated in arbitrary units evaluated as the ratio of the luciferase activity to the galactosidase activity.

SMALL INTERFERING RNA OLIGONUCLEOTIDES

Scrambled nonspecific small interfering RNA (siRNA; sense 5′-CAGUCGCGUUUGCGACUGGdTdT-3′), Snail1 specific siRNA (sense 5′-AGGCCUUCAACUGCAAAUAdTdT-3′), and NF-κB specific siRNA (5′-GCCCUAUCCCUUUACGUCAdTdT-3′) along with their corresponding antisense RNA oligonucleotides were purchased from Syntol (Russia). These RNAs were dissolved in annealing buffer [10 mM Tris–HCl (pH 7.5), 50 mM NaCl, 1 mM EDTA] as 10 μM solutions and annealed at room temperature following heating to 95°C.

Transfection of the RNA oligonucleotides was performed using Lipofectamine Reagent to result in a final RNA concentration of 50 nM.

WESTERN BLOT ANALYSIS

The cells were removed from the dishes with 1.2 ml of phosphate buffer, washed twice, and incubated for 10 min on ice in the modified lysis buffer containing 50 mM Tris–HCl, pH 7.4, 1% SDS, 1% Igepal CA-630, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF; 1 μg/ml each aprotinin, leupeptin, pepstatin; 1 mM Na-orthovanadate, and 1 mM NaF. Samples were sonicated four times for 5 s each at 30% output and centrifuged for 5 min at 15,000*g*, and supernatants were then used as total cell extracts. Protein content was determined by the Bradford method. Soluble and cytoskeletal fractions were prepared as described by Gout et al. [2004]. Nuclear fraction was prepared according Dr. Jong In Yook's lab protocol [Ko et al., 2007].

Cell lysates (60 μg protein) were separated in 12% SDS–PAGE under reducing conditions, transferred to a nitrocellulose membrane (Hybond-C extra, GE Healthcare) and processed according to the standard protocol. To prevent nonspecific absorption, the filters were treated with 5% nonfat milk (Applichem) solution in TBST buffer (20 mM Tris, 500 mM NaCl, pH 7.5, 0.1% Tween-20) and then incubated with primary antibodies overnight at +4°C.

Primary antibodies to Snail1 (Cell Signaling Technology), ER (Sigma–Aldrich), E-cadherin (Cell Signaling Technology), N-cadherin (BD Transduction Laboratories), and p65 NF-κB (Cell Signaling Technology) were used; antibody against β-actin (Cell

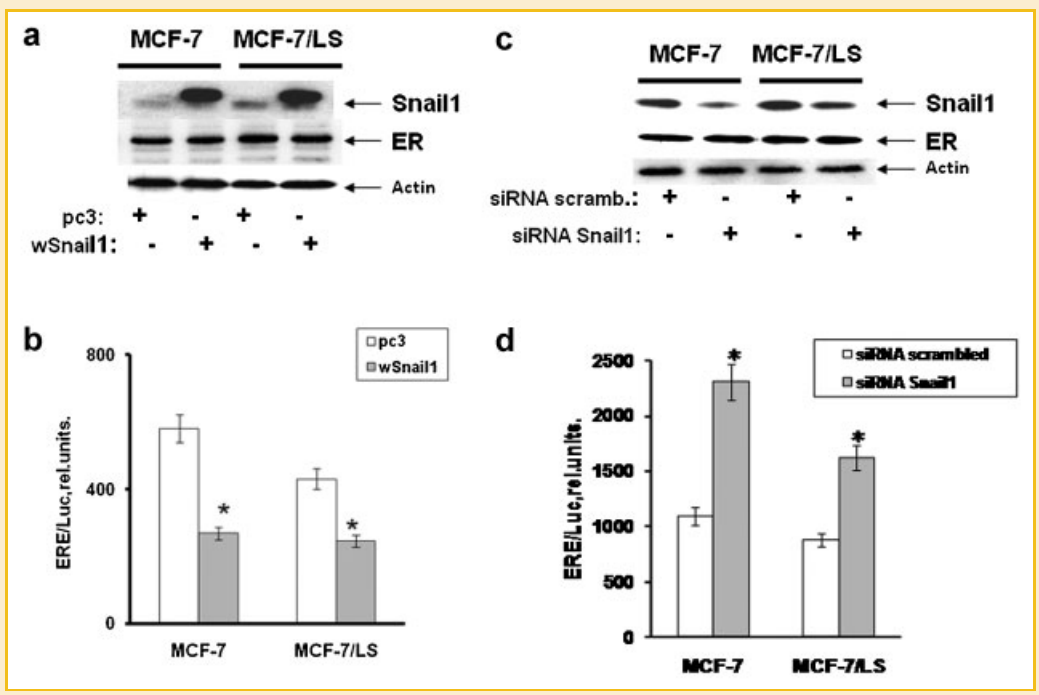

Fig. 2. Snail1 signaling and ER activity. Western blot of Snail1 and ER (a) and ER reporter assay (b) after transfection with wSnail1 plasmid. MCF-7 cells were transfected with the plasmid containing wild-type of Snail1 and subjected to Western blot analysis for Snail1 and ER expression. The blot represents the results of one of the three similar experiments. MCF-7 cells were transfected with empty vector or Snail1 plasmid together with the ERE-TK-LUC plasmid containing the luciferase reporter gene under the estrogen responsive element (ERE), and β-galactosidase plasmid. Twenty-four hours after transfection the luciferase and β-galactosidase activities were determined as described above. $P < 0.05$ * versus pc3-treated cells. Western blot of Snail1 and ER (c) and ER activity (d) after Snail1 knockdown. The MCF-7 cells were transfected with the scrambled siRNA or siRNA to Snail1, after 24 h the level of Snail1 and ER was determined by Western blot analysis. The MCF-7 cells were transfected with the scrambled siRNA or siRNA to Snail1 with subsequent ER reporter assay. Data represent mean value ± SD of at least three independent experiments. $P < 0.05$ * versus scrambled RNA-treated cells.

Signaling Technology) and Lamin B1 (Invitrogen) were used to standardize loading. Appropriate IgG's (Jackson ImmunoResearch and Cell Signaling Technology) conjugated to horseradish peroxidase were used as secondary antibodies. Signals were detected using ECL reagent (GE Healthcare) and ImageQuant LAS4000 system (GE Healthcare).

ASSESSMENT OF APOPTOSIS

The apoptosis was determined by flow cytometry using staining with propidium iodide (PI; Sigma–Aldrich). Cells were fixed in 70% cold ethanol, centrifuged, and resuspended in 1 ml of solution containing PI (5 μg/ml), 0.1% sodium citrate, 0.1% Triton X-100, and then incubated for 15 min in the dark. Then the samples were analyzed in the FACScanto II flow cytometer (Becton Dickinson). The further processing of the data was performed with the WinMDI 2.9 software program (Joseph Trotter, La Jolla). The percent of apoptotic cells was determined as a pre-G1 peak in the DNA histogram.

STATISTICAL ANALYSIS

Statistical analysis was performed using the STATISTICA program. The criterion for statistical significance was $P < 0.05$.

RESULTS

THE EXPRESSION AND ACTIVITY OF SNAIL1 IN DIFFERENT BREAST CANCER CELLS

The cell lines were used as follows: (1) ER-positive (high ER level) estrogen-dependent MCF-7 cells, (2) ER-positive estrogen-hyposensitive MCF-7/LS subline generated through 2-month cultivation of the parental cells in steroid-free medium [Lobanova et al., 2009], (3) ER-negative estrogen-independent HBL-100 cells.

The data of Western blotting analysis showed the inverse relationship between ER and Snail1 content: the highest Snail1 level was revealed in ER-negative HBL-100 cells, the lower Snail1 content in ER-positive MCF-7 and MCF-7/LS cells (Fig. 1a). The comparison between ER-positive lines: estrogen-dependent MCF-7 and estrogen-hyposensitive MCF-7/LS cells showed the relative increase in Snail1 content in MCF-7/LS cells (Fig. 1a).

The similar tendency was demonstrated by luciferase reporter assay with the plasmid, containing Snail-binding element of E-cadherin promoter (the plasmid was described in Vincent et al., 2009) the highest trans-repressor Snail1 activity was in ER-negative HBL-100 cells, and the low activity was in ER-positive cells, coming down to minimum value in MCF-7 cells (Fig. 1b). The comparative analysis of E-cadherin and N-cadherin expression revealed the "cadherin switch"—loss of E-cadherin

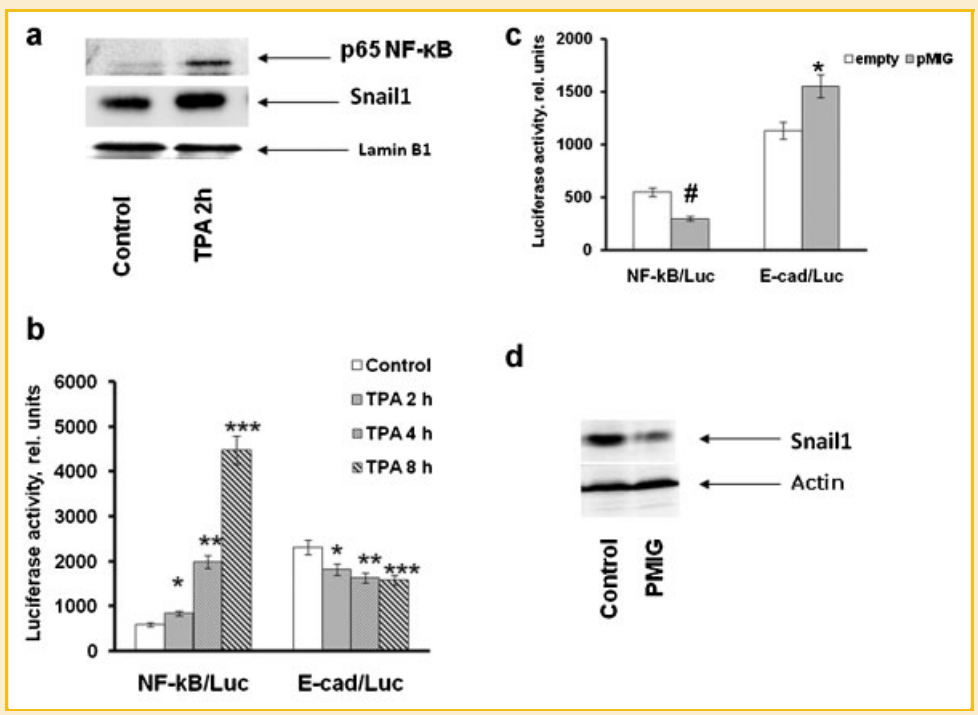

Fig. 3. Snail1 signaling and NF-κB. Western blot of NF-κB and Snail1 (a) and E-cadherin reporter assay (b) in MCF-7 cells after TPA treatment. MCF-7 cells were treated with TPA in a final concentration 200 ng/ml for 24 h and subjected to Western blot analysis of nuclear fraction. Protein loading was controlled by anti-Lamin B1 hybridization. The blot represents the results of one of the four similar experiments. The cells were transfected with the luciferase reporter construct under E-cadherin promoter, and β-galactosidase plasmid. Three hours after transfection the cells were treated with or without 200 ng/ml TPA for 2, 4, and 8 h with subsequent determination of the reporter luciferase activity. Data represent mean value ± SD of at least three independent experiments. $P < 0.05$ *, **, *** versus control cells. Influence of NF-κB inhibition by pMIG plasmid on Snail1 level (d) and activity (c). The cells were transfected with the pMIG plasmid containing IkBα repressor. Data represent mean value ± SD of at least three independent experiments. Protein loading was controlled by anti-Actin hybridization. The blot represents the results of one of the four similar experiments. $P < 0.05$ *, # versus empty plasmid-treated cells.

and accumulation of N-cadherin—in the cells with increased Snail1 level (Fig. 1a).

THE NEGATIVE FEEDBACK BETWEEN SNAIL1 AND ER SIGNALING

We proposed that such inverse relationship between ER and Snail1 in different cell lines may be caused, at least in part, by the existence of the negative feedback between Snail1 and ER. The analysis of estrogen action on Snail1 activity in the ER-positive MCF-7 and MCF-7/LS cells showed no changes in the transcriptional Snail1 activity and E-cadherin expression (Fig. 1c,d). Similarly, ER transfection into HBL-100 cells neither altered the trans-repressor Snail1 activity and expression nor restored the E-cadherin level (Fig. 1c,d).

To further investigate the links between Snail1 and ER, we transfected the wild-type Snail1 into MCF-7 and MCF-7/LS cells with subsequent determination of ER expression and activity. As revealed, Snail1 transfection caused no changes in ER content (Fig. 2a), but significantly decreased ER transcriptional activity determined by reporter assay with ERE-TK-LUC plasmid (Fig. 2b). The transfection of siRNA Snail1 into MCF-7 and MCF-7/LS cells caused the opposite effect stimulation of ER activity, totally supporting the hypothesis about Snail1 involvement in ER down-regulation (Fig. 2c,d). Accordingly, Snail1 transfection into ER-negative HBL-100 cells was not accompanied with the alterations in the ER reporter activity (data not shown).

THE ROLE OF NF-κB IN THE SNAIL1 REGULATION

NF-κB signaling plays an important role in the Snail1 regulation in cancer cells being involved in the both direct and indirect activation of Snail1 molecules [Wu and Zhou, 2010]. We have found that NF-κB activation by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) correlated with the increase in Snail1 expression and activity in MCF-7 cells (Fig. 3a,b). NF-κB inhibition by pMIG plasmid containing IkBα repressor decreased Snail1 expression and activity (Fig. 3c,d). Similarly, NF-κB knockdown by siRNA led to suppression of Snail1 activity showing that NF-κB may be considered as an important positive regulator of Snail1 signaling in breast cancer cells (Fig. 4a,b). Furthermore, similar to Snail1, inhibition of NF-κB resulted in the increase in ER transcriptional activity in both MCF-7 and MCF-7/LS cells demonstrating the possible involvement of NF-κB in the regulation of hormonal sensitivity (Fig. 4c).

THE INVOLVEMENT OF SNAIL1 IN THE REGULATION OF DRUG SENSITIVITY

To further elaborate the role of Snail1 signaling in the hormonal resistance, the Snail1 influence on the breast cancer cell sensitivity to tamoxifen was studied. We found that Snail1 knockdown by siRNA potentiated the tamoxifen inhibitory effect on ER transcriptional activity in MCF-7 and MCF-7/LS cells (Fig. 5a). The subsequent analysis of cell growth revealed the amplification of the response to antiproliferative tamoxifen action in the cells treated with siRNA Snail1 (Fig. 5b).

The study of cell sensitivity to doxorubicin, one of the widespread anti-tumor drugs, showed that knockdown of Snail1 led to enhance in apoptotic response to doxorubicin in both parental MCF-7 cells and estrogen-hyposensitive MCF-7/LS subline (Fig. 6). The same tendency was revealed in ER-negative HBL-100 cells demonstrating the important role of Snail1 in the regulation of antiapoptotic pathways independently from ER status (Fig. 6).

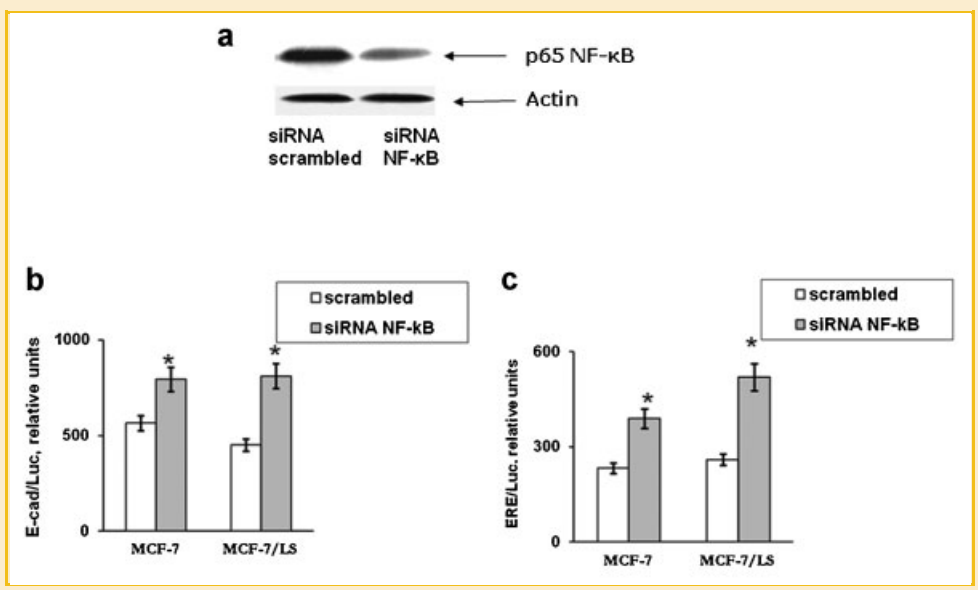

Fig. 4. NF-κB knockdown and activity of Snail1 and ER. Western blot of NF-κB in total cell extracts after NF-κB knockdown (a). The MCF-7 cells were transfected with the scrambled siRNA or siRNA to NF-κB, after 24 h the level of NF-κB was determined by Western blot analysis. b: E-cadherin reporter assay. The cells were transfected with siRNAs together with E-cadherin reporter construct, 24 h after transfection the relative luciferase activity was determined. c: ER reporter assay. Similar to (b), only ERE-TK-LUC reporter construct was used. b,c: $P < 0.05$ * versus scrambled RNA-treated cells.

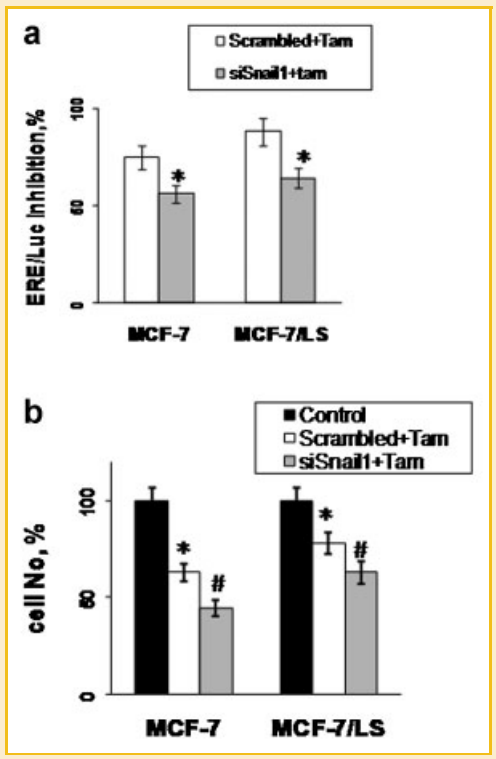

Fig. 5. Snail1 and cell sensitivity to tamoxifen. ER reporter assay (a). MCF-7 and MCF-7/LS cells were transfected with the scrambled siRNA or siRNA to Snail1 together with ERE-TK-LUC plasmid. Three hours after transfection the cells were treated with or without 10^{-6} M tamoxifen for 24 h with subsequent determination of the reporter luciferase activity. The data are presented as percentage ratio of luciferase activity in tamoxifen-treated cells to respective untreated cells. $P < 0.05$ * versus control cells. Growth response to tamoxifen (b). Cells were transfected with siRNAs, and treated with control vehicle or 10^{-6} M tamoxifen for 2 days. Cell growth was evaluated using MTT-test. The data are presented as a number of viable tamoxifen-treated cells in percent to respective control. $P < 0.05$ * versus control and siSnail1-Tamoxifen-treated cells, # versus control and scrambled RNA-Tamoxifen-treated cells.

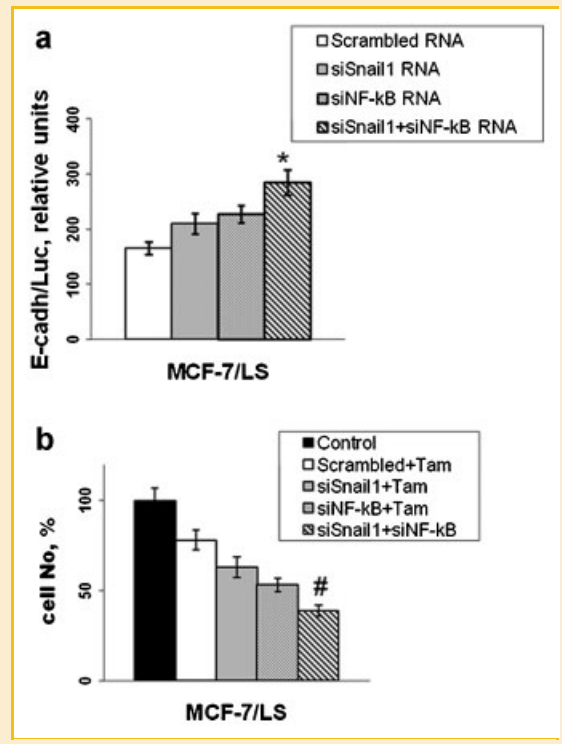

Fig. 7. The combined effect of Snail1 and NF-κB RNA interference. E-cadherin reporter assay (a). MCF-7/LS cells were transfected with scrambled siRNA, siRNA to Snail1, NF-κB or their combination, together with E-cadherin reporter construct, 24 h after transfection the relative luciferase activity was determined. $P < 0.05$ * versus other combinations and control. Growth response to tamoxifen (b). MCF-7/LS cells were transfected with siRNAs, and treated with or without tamoxifen for 2 days. The data are calculated as a number of tamoxifen-treated cells in percents to respective control. Data represent mean value ± SD of at least three independent experiments. $P < 0.05$ # versus other combinations and control.

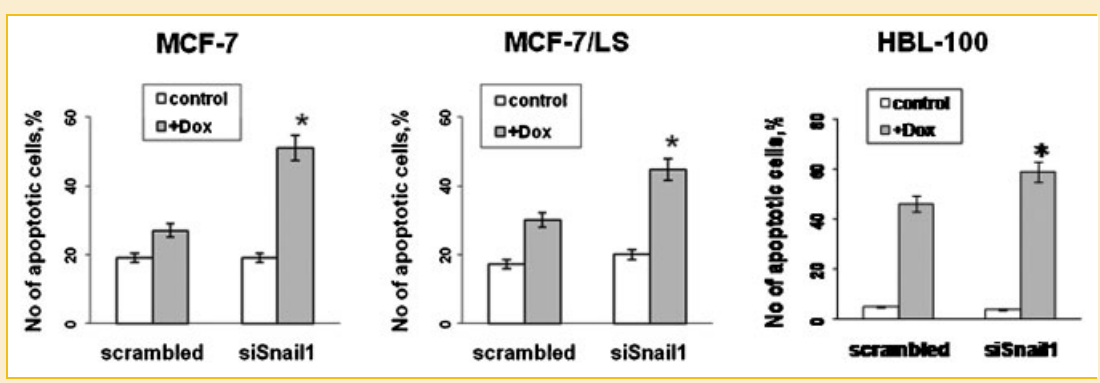

Fig. 6. Effect of Snail1 knockdown on cell apoptosis. MCF-7, MCF-7/LS, and HBL-100 cells were transfected with the scrambled siRNA or siRNA to Snail1, and treated with or without 10^{-6} M doxorubicin for 2 days. The apoptosis level was assessed by propidium iodide staining using flow cytometry analysis, the percentage of apoptotic cells is indicated. Data represent mean value ± SD of at least three independent experiments. $P < 0.05$ * versus Dox-treated cells after scrambled RNA transfection.

Totally, the results presented show that progression of hormonal resistance may be accompanied with the increase in Snail1 expression which in turn inhibits ER activity and diminishes cell response to both (anti)hormonal and proapoptotic drugs.

THE COMBINED EFFECT OF SNAIL1 AND NF-κB RNA INTERFERENCE ON THE HORMONAL SENSITIVITY

Taking into account the NF-κB ability to up-regulate Snail1, we proposed that simultaneous inhibition of both pathways, NF-κB and Snail1, might result in strong inhibition of Snail1 activity and parallel activation of hormonal response. To test this hypothesis, we transfected estrogen-hyposensitive MCF-7/LS cells with siRNA to NF-κB, Snail1 and their combination with subsequent analysis of Snail1 activity and cell sensitivity to tamoxifen. We have found that simultaneous suppression of NF-κB and Snail1 led to a maximum inhibition of transcriptional activity of Snail1 (Fig. 7a) and a marked increase in cell response to tamoxifen (Fig. 7b), demonstrating that suppression of both pathways effectively enhances the hormonal sensitivity of estrogen-hyposensitive cells.

DISCUSSION

In many cases progression of breast cancer tumors to estrogen-independent phenotype is not associated with the ER alterations but caused by the changes in the intracellular signaling pathways, including the activation of receptor tyrosine kinases, disorders in the regulation of cell cycle proteins, etc. [Arpino et al., 2009; Musgrove and Sutherland, 2009; Haagenson and Wu, 2010]. Moreover, estrogen-independent tumors may acquire several mesenchymal features pointing out the possible involvement of EMT-associated pathways in the regulation of hormonal sensitivity [Lundgren et al., 2009; Micalizzi et al., 2010].

Here we show that one of the main EMT-associated proteins Snail1 is involved in the negative regulation of ER machinery in the breast cancer cells. We have found that progression of MCF-7 cells to estrogen-independent stage is accompanied with Snail1 activation and decrease in E-cadherin content. Snail1 suppression or hyperexpression lead to stimulation or inhibition of ER transcriptional activity, respectively. Our data support both experimental and clinical observations demonstrated the inverse relationship between Snail1 and ER in breast cancers [Dhasarathy et al., 2007; Yu et al., 2009]. However contrary to some reports [Fujita et al., 2004; Park et al., 2008], we did not find any changes in Snail1 activity after 17beta-estradiol (E2) treatment. Probably, it may reflects the simultaneous ER influence on different Snail1-related pathways in breast cancer cells—both Snail1-stimulating pathways (PI3K, Akt/PKB) and Snail1-inhibiting ones (MTA3) [Planas-Silva and Waltz, 2007]. Ye et al. [2010] have shown that ERalpha signaling decreased Slug (SNAI2) expression by two different mechanisms: directly, by repression of Slug transcription by the formation of a corepressor complex of ligand-activated ERalpha, HDAC inhibitor (HDAC1), and nuclear receptor corepressor (N-CoR) that bound the Slug promoter; indirectly by phosphorylation and inactivation of GSK-3beta through phosphoinositide 3-kinase/Akt. Certainly, the further studies are required to delineate the mechanism of ER influence on EMT signaling.

We have found that Snail1 knockdown results in the increase in the cell apoptotic response, including the ER-negative HBL-100 cells, thereby showing the Snail1 ability to up-regulate proapoptotic pathways in a parallel and independently from its influence on ER machinery. Testing the cell response to antiestrogen tamoxifen

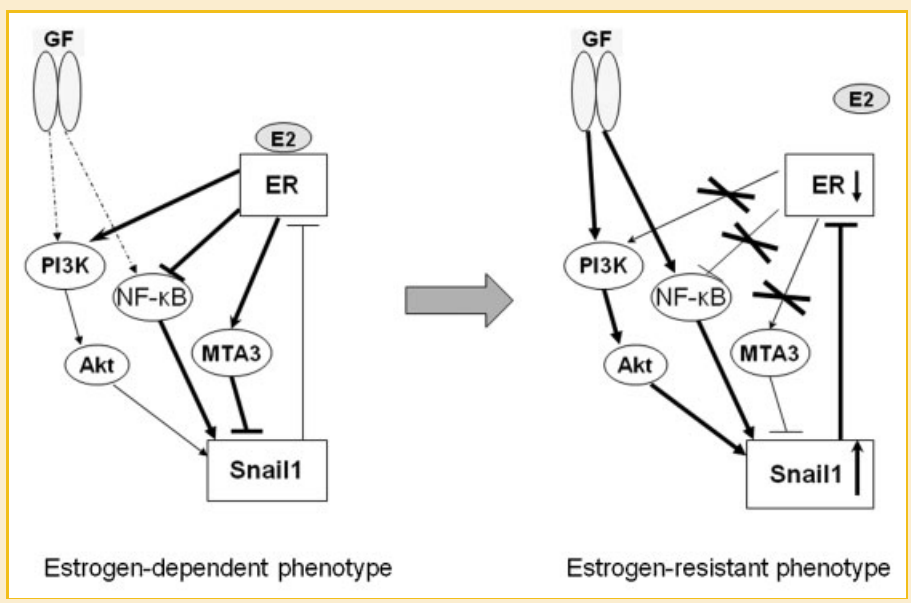

Fig. 8. Snail1 regulation in estrogen-dependent and resistant breast cancer cells. The cell progression to estrogen-independent stage is accompanied with the alterations in ER signaling, namely blockage of ER-mediated Snail1-inhibiting pathways and stimulation of growth factor-dependent Snail1-activating pathways. Activated Snail1 in turn diminishes the ER activity maintaining the cell resistance to estrogens.

revealed the marked increase in cell sensitivity to anti-proliferative tamoxifen action by Snail1 suppression. In agreement with other observations [Kim et al., 2009; Storci et al., 2010; Wu and Zhou, 2010], NF-κB was found to serve as a positive regulator of Snail1 in breast cancer cells, and inhibition of NF-κB resulted in additional increase in cell response to tamoxifen. Zhang's et al. [2011] results showed that Snail1 activation and consequent repression of E-cadherin may depend on NF-κB activation, and NF-κB promotes migration and invasion by upregulating Snail1 and consequent repression of E-cadherin in cholangiocarcinoma cell.

One of the important paradigms of the anti-tumor target therapy is that drugs must block at least two intracellular targets (usually growth/apoptosis-related signaling proteins), in other case cancer cells will compensate the deficiency of one targeted protein by the activation of parallel signaling [Azim and Piccart, 2010; Davies and Hiscox, 2010; Uray and Brown, 2011]. Here we demonstrate that simultaneous inhibition of Snail1 and NF-κB lead to maximum suppression of Snail1 activity and cell sensibilization to antiestrogens. Figure 8 illustrates the possible interrelations between ER, Snail1 and NF-κB signaling and their evolution in estrogen-resistant tumors. We propose that Snail1 may be considered as one of the negative regulators of ER in breast tumors. High level of Snail1 diminishes the ER activity resulting in the decrease of estrogen dependency in the cells. Direct suppression of Snail1 and inhibition of main Snail1 activator—NF-κB may partially restore ER activity and in parallel—increase apoptotic potency of tumor cells. Totally, we suggest that Snail1 and NF-κB may serve as an important targets in the treatment of breast cancer, both estrogen-dependent and (that is more valuable) estrogen-independent tumors.

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