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# *N*-Acetylcysteine Increases Susceptibility of HeLa Cells to Bacterial Invasion

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## ABSTRACT

*Serratia grimesii* are non-pathogenic bacteria capable, however, to invade eukaryotic cells provided that they synthesize intracellular metalloprotease grimelysin (Bozhokina et al. [2011] Cell. Biol. Int. 35: 111–118). To elucidate how invasion of grimelysin containing bacteria depends on physiological state of host cells, we studied the effect of *N*-acetylcysteine (NAC) on susceptibility of HeLa cells to invasion by the wild-type *S. grimesii* and recombinant *E. coli* expressing grimelysin gene. Incubation of HeLa cells with 10 mM NAC resulted in changes of cell morphology and disassembly of actin cytoskeleton that were reversed when NAC was removed from the culture medium. Both in the presence of NAC and upon its removal, the entry of grimelysin producing bacteria increased by a factor of 1.5–2 and 3–3.5 for wild-type *S. grimesii* and recombinant *E. coli*, respectively. This effect does not correlate with cytoskeleton rearrangements but may be due to the NAC-induced up-regulation of cell surface receptors playing a role in cell adhesion and cell–cell junctions. A twofold difference in the efficiency of *S. grimesii* and recombinant *E. coli* to enter the NAC-treated cells suggests that the entry of the wild-type and recombinant bacteria occurs via different receptors which activity is differently affected by NAC. J. Cell. Biochem. 114: 1568–1574, 2013. 

**KEY WORDS:** ANTIOXIDANTS; BACTERIAL INVASION; CYTOSKELETON; SERRATIA; GRIMELYSIN

Bacterial invasion is a process of interaction of bacteria with eukaryotic cells. To penetrate non-phagocytic host cells invasive bacteria have to initiate signal transduction pathway of the host by either expressing proteins on their surfaces that interact with cellular receptors or injecting protein effectors that activate signaling cascades or modify cytoskeleton assembly by a direct interaction with actin and actin-binding proteins [Bhavsar et al., 2007]. Bacterial effectors often mimic natural activators of small GTPases Rho, Rac, and Cdc42 or directly stimulate the host signaling pathways by a functional mimicry to host GTPases [Stebbins and Galán, 2001; Huang et al., 2009]. On the other side, efficiency of bacterial invasion depends on physiological state of host cells. Susceptibility of cells to bacterial invasion is promoted by immortalization [Velge et al., 1994; Efremova et al., 2001], cell proliferation [Velge et al., 1997], the loss of contact inhibition and anchorage-dependent growth [Velge et al., 1995], that is, by the processes associated with changes in distribution of cell surface receptors and cytoskeleton rearrangements.

We have previously found that spontaneously isolated non-pathogenic bacteria *Serratia grimesii* invade eukaryotic cells, provided that they synthesize thermolysin-like metalloprotease ECP32/grimelysin characterized by high specificity towards actin [Efremova et al., 2001; Bozhokina et al., 2011]. Furthermore, transformation of non-invasive *E. coli* with plasmid encoding grimelysin confers to the bacteria invasive capacity [Bozhokina et al., 2011]. To reveal mechanisms of this invasion, in the preceding work [Efremova et al., 2004, Gamaley et al., 2006], we have treated cultured cells with *N*-acetylcysteine (NAC) that can modulate redox status of cells [Atkuri et al., 2007] and modify multiple signaling pathways, including regulation of gene expression [Zafarullah et al., 2003; Gustafsson et al., 2005; Parasassi et al., 2010]. Incubation of normal 3T3 and transformed 3T3-SV40 fibroblasts with 10–20 mM NAC resulted in a reversible increase in the intracellular level of reduced glutathione and disorganization of actin cytoskeleton [Efremova et al., 2004; Gamaley et al., 2006]. Thereby neither changes in glutathione levels, nor cytoskeleton disassembly/assembly abolished resistance of 3T3 cells to invasion by the bacteria. At the same time, removal of NAC from the culture medium led to reversion of 3T3-SV40 cells to well-adhered "pseudo-normal" fibroblasts that displayed pronounced stress fibers and resistance to bacterial invasion. These results suggested that the NAC-induced assembly of actin filaments in 3T3-SV40 cells and sensitivity of

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these cells to bacterial invasion depend on some molecules whose activities are irreversibly changed in the presence of NAC, rather than on intracellular glutathione levels [Gamaley et al., 2006]. However, visual analysis did not allow us to evaluate a direct effect of NAC on susceptibility of 3T3-SV40 fibroblasts to bacteria. Moreover, 3T3-SV40 fibroblasts were produced in vitro using transformation by oncogenic virus SV40 [Torado et al., 1967], and their properties may differ from the properties of spontaneously transformed cells. Therefore the present work was aimed to evaluate, using quantitative assays, the effect of NAC on susceptibility of spontaneously transformed HeLa cells to the wild-type and recombinant grimelysin producing bacteria. As small GTPases of the Rho family are recruited to the site of pathogen entry, in some experiments, the Rho-transfected HeLa cells were studied along with regular HeLa cells.

Our results show that treatment of HeLa cells with NAC increases entry of grimelysin producing bacteria by a factor of 1.5–2 and 3–3.5 for wild-type *S. grimesii* and recombinant *E. coli*, respectively. This effect does not correlate with cytoskeleton disassembly-assembly but correlates with an increased expression of E-cadherin gene, indicating that the NAC increased internalization of the bacteria involves modulation of the E-cadherin-β-catenin pathway. While *Serratia* are facultative pathogens, common in hospital infection, these data may be important in the NAC clinical use.

## MATERIALS AND METHODS

### REAGENTS

Culture media αMEM and DMEM were obtained from Biolot LLC (Russia). Fetal bovine serum (FBS) was from Thermo Scientific (Thermo Fisher Scientific Inc.). Peptone and yeast extract were obtained from Difco (Franklin Lakes, NJ), nonessential amino acids (NEAA), NAC, PBS, sodium deoxycholate, Triton X-100, reagents for electrophoresis, reagents for PCR, FITC, rhodamine–phalloidin, DAPI and Mounting medium were purchased from Sigma Chemical Co. (St. Louis, MO).

### BACTERIAL STRAINS, CELL CULTURES, AND GROWTH CONDITIONS

Human cervical carcinoma HeLa M cells were obtained from the Russian Cell Culture Collection (Institute of Cytology, St. Petersburg, Russia). RhoA- and RhoC-expressing HeLa cell lines were kindly provided by Dr. T. Adam (Charite, Berlin, Germany). Cells were grown in the antibiotic-free αMEM supplemented with 10% FBS and 1% NEAA at 37°C under 5% $CO_2$ atmosphere, for the time required to form a monolayer.

*S. grimesii* strain 30063 was from the German Collection of Microorganisms and Cell Cultures (DSMZ). The recombinant *E. coli* SCS1, expressing grimelysin gene was obtained as described previously [Bozhokina et al., 2008]. Bacteria were grown in Luria broth (LB medium) containing 1% peptone, 0.5% yeast extract, and 1% NaCl at pH 7.0 at 37°C with aeration.

### INCUBATION WITH *N*-ACETYLCYSTEINE (NAC)

NAC was freshly dissolved in the culture medium, and pH was adjusted to a neutral one with 0.5 mM NaOH. Cells were grown on glass coverslips in culture dishes for 24 h, and NAC was added to the medium to the final concentration of 2.5–20 mM for 22–24 h. After the medium was replaced by the fresh one without NAC, the cells were fixed with paraformaldehyde either immediately or after growing for 24 h more.

### FLUORESCENCE MICROSCOPY

The control and infected cells in PBS were fixed with 3.7% paraformaldehyde in PBS (10 min at room temperature, washed three times with PBS), permeabilized for 5 min with 0.1% Triton X-100 in PBS and washed three times with PBS. To visualize F-actin cells were then stained with rhodamine–phalloidin for 15 min and washed with PBS. Cell number was determined by counting the number of cell nuclei stained with DAPI (diluted 1:2,000). Samples were analyzed using a fluorescence Leica SP5 TCS inverted microscope. Cell number was counted using ImageJ software (version 1.42l).

### GENTAMYCIN INVASION ASSAY

Efficiency of invasion was evaluated by gentamycin invasion assay [Prouty and Gunn, 2000]. Bacteria were grown in LB medium at 37°C with aeration until actinase activity on their extracts could be determined [Bozhokina et al., 2011]. Bacteria were pelleted by centrifugation at 5,000 rpm for 10 min, resuspended in αMEM, and the bacteria suspension was added to HeLa cells grown overnight in 6-well plates containing nearly confluent monolayers of $\sim 2 \times 10^5$ cells per well, to a multiplicity of infection (MOI) of approximately 100 bacteria per cell. Then the plates were centrifuged at 2,000 rpm for 5 min to get bacteria to attach the cells. The plates were incubated for 2 h at 37°C, and αMEM with bacteria was removed. After washing three times with 1 ml of PBS per well, the infected cells were trypsinized and incubated in PBS containing gentamicin (50 mkg/ml) with shaking at 37°C for 2 h, to get rid of extracellular bacteria. At the concentration used, gentamycin is impermeable to mammalian cells; therefore it kills only extracellular rather than internalized bacteria. The cells were lysed with 1.5% sodium deoxycholate in water, pipetted to disperse bacterial aggregates, quickly serial diluted and plated out on LB agar to determine the number of colony forming units (CFU). The results for each experiment are the average of an assay performed in triplicate and independently repeated three times.

### LIMITED ACTIN PROTEOLYSIS

For proteolytic digestion, bacteria grown in LB medium were harvested by centrifugation at 5,000 rpm for 10 min. The pellets were resuspended in αMEM, and HeLa cells were infected with bacteria for 2 h as described above. Then the infected cells were trypsinised, incubated in PBS containing gentamicin (50 mkg/ml) with shaking at 37°C for 2 h, washed three times with PBS, and lysed by five to six cycles of freezing and thawing. The lysates were clarified by centrifugation at 5,000 rpm for 10 min. Actin solution of 0.5 mg/ml was mixed with the clarified lysates at the actin/lysate ratio of 1:3 and incubated overnight at 4°C. Reaction was stopped by addition of SDS buffer; the products were analyzed by SDS–PAGE [Laemmli, 1970] using 12% polyacrylamide gel.

### PCR ANALYSIS

To reveal intracellular bacteria in the infected HeLa cells, the HeLa M cells were infected with bacteria and treated as described above for limited proteolysis. Total DNA of the cells and internalized bacteria was extracted using the QIAamp DNA Mini Kit (Qiagen), according to the manufacturer's instructions. For the polymerase chain reaction (PCR), the following grimelysin specific oligonucleotides were used:

Gri-Forward: 5′-ATTCTCGGATCCATGCCGACCCTAACAGCACGA-3′
Gri-Reverse: 5′-ATTCTCGCGGCCGCTTATGCCACCCCCACCTGATGCCA-3′

DNA was PCR amplified under standardized conditions as follows: denaturation at 94°C for 2 min, followed by 35 cycles of 30 s each at 94°C; 1 min at 58°C, 1 min at 72°C, and a final elongation step at 72°C for 15 min. Negative control without DNA was present in all amplifications. PCR products were separated by electrophoresis in 1.5% agarose gel.

### SEMI-QUANTITATIVE RT-PCR

Expression analyses of E-cadherin gene was conducted by semi-quantitative RT-PCR. Total RNA was extracted from cell lysates using Qiagene RNA extraction Kit according to the manufacturer's instructions. Reverse transcription was performed with the MMLV Reverse Transcriptase (Promega) and the resulting cDNA was diluted to 20 ng/μl. Gene-specific primer pairs were designed using BLAST-primer software and were as followed:

β-Actin forward: 5′-CTGGGACGACATGGAGAAAA-3′
β-Actin reverse: 5′-AAGGAAGGCTGGAAGAGTGC-3′
E-cadherin forward: 5′-CCCTGGCTTTGACGCCGAGA-3′
E-cadherin reverse: 5′-CTCGGTCCAGCCCAGTGGTG-3′

PCR reactions were optimized to 94°C for 3 min, 33 amplification cycles at 94°C for 1 min, the appropriate annealing temperature (60°C for β-actin and 64°C for E-cadherin) for 1 min, 72°C for 1 min, and a final extension of 10 min at 72°C. Amplified products were resolved on 1.5% agarose gels and visualized by ethidium bromide staining.

### STATISTICAL ANALYSIS

Data were analyzed statistically using one-way analysis of variance (ANOVA) with Excel Data Analysis Pack. A difference was considered significant at the $P < 0.05$ level.

## RESULTS

### EFFECT OF NAC ON CELL GROWTH

Quantitative analysis of bacterial invasion under different conditions requires that similar amounts of the cultured cells were infected by bacteria. However, incubation of cultured cells with NAC can lead to the reduction in cell number due to the cell cycle arrest [Ovesen et al., 1993; Gamaley et al., 2003] or apoptosis [Gamaley et al., 2003; Guan et al., 2010]. Specifically, NAC has been shown to induce apoptosis in multiple types of human cancer cells, including HeLa cells [Guan et al., 2010 and references therein]. Therefore we first elucidated growth characteristics of HeLa cells in the presence of NAC.

HeLa M cells grown for 24 h on coverslips to confluecy of about 80% were incubated with 2.5–20 mM NAC for the next 24 h. Figure 1A depicts the effect of different NAC concentration on cell density. Incubation during 24 h with 2.5, 10, and 20 mM NAC decreased the cell density by about 13%, 40%, and 45%, respectively. Similar results were obtained upon incubation with 2.5 and 10 mM NAC for 48 h (data not shown).

Control HeLa M cells demonstrated epithelium-like cell morphology, cells were polygonal in shape with regular dimensions, and grow attached to a substrate in discrete patches (Fig. 1C). Incubation of HeLa cells with 2.5 mM NAC did not result in any appreciable changes of cell morphology. Within incubation with 10 mM NAC the cells became less spread and more round (Fig. 1D). Thereby in approximently 10% of the cells a membrane blebbing was observed (marked with arrow in Fig. 1D). Though the blebbing may be one of

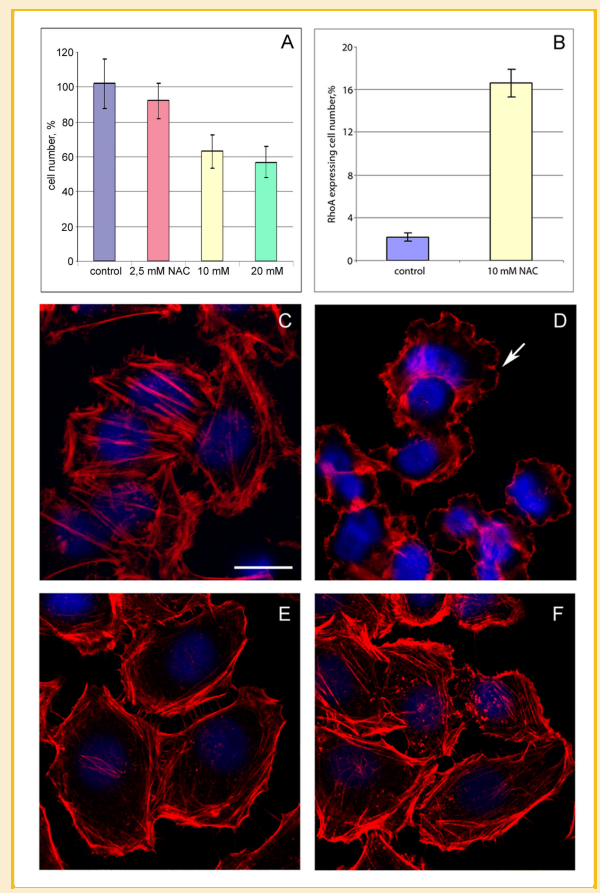


Fig. 1. Growth characteristics and actin cytoskeleton of NAC-treated HeLa-M cells. A: the number of HeLa M cells incubated with 2.5, 10, and 20 mM NAC for 24 h. B: the number of HeLa-RhoA cells after incubation with 10 mM NAC for 24 h. C–F: reorganization of actin cytoskeleton in HeLa M cells incubated with 10 mM NAC for 24 h (D), and in 24 h after NAC was removed from the culture medium (E,F) The scans in (E) and (F) are taken from two different experiments. C: control HeLa M cells. White arrow points to a cell with membrane blebbing. Red-cytoskeleton, Blue-nuclei. Bar, 20 mkm. Values are expressed as mean ± S.D. (*error bars*). A difference was considered significant at the $P < 0.05$ level.

the markers of apoptosis [Lane et al., 2005] we did not observe any nuclear fragmentation or other signs of apoptosis or cell death. Our data are also consistent with the results of Guan et al. [2010] showing that an induction of apoptosis followed treatment of HeLa cells with 10 mM NAC was observed only after 48 h of incubation.

The cells incubated with 20 mM NAC were similar to those incubated with 10 mM NAC (data not shown). Therefore in further experiments 10 mM NAC was used.

In contrast to its effect on the cell density in HeLa M culture, incubation with NAC promoted growth of Rho-GFP-transfected HeLa cells. In the initial cell culture, the RhoA-transfected cells comprised about 3% of the cells. The cells treatment with 10 mM NAC for 24 h increased this amount to 25% (Fig. 1B) indicating that NAC induces proliferation of Rho-transfected cell, which confirms the literature data [Gustafsson et al., 2005].

## EFFECTS OF NAC ON CYTOSKELETON

Maintenance of normal cell shape and adhesion requires the presence of intact cytoskeleton and specific cell–matrix interactions. The change in epithelium-like cell morphology, as well as decrease in cell adhesion, prompted us to examine possible alterations of cytoskeletal organization.

As demonstrated in Figure 1, the NAC-produced cytoskeleton rearrangements paralleled the changes in cell morphology. Within incubation with 10 mM NAC while the cells were rounded off and decreased in square, the well-structured stress fibers characteristic of untrearted HeLa M cells disassembled, and amorphous actin aggregates appeared. Instead, the NAC-treated cells exhibited strong accumulation of actin under the cell membrane (Fig. 1D). After removal of NAC from the incubation medium followed by the 24 h of growth the cells became more spread and a partial cytoskeleton restoration was observed (Fig. 1E,F).

## VISUALIZATION OF INTRACELLULAR BACTERIA IN THE NAC-TREATED HELA CELLS

To reveal effects of NAC on susceptibility of HeLa M cells to bacteria we used a reference strain *S. grimesii* 30063 producing actin-specific protease ECP32/grimelysin, and the recombinant *E. coli* expressing grimelysin gene [Bozhokina et al., 2008]. These bacteria can be internalized within the transformed eukaryotic cells, with about 10% of the cells being invaded either by the reference or recombinant bacteria expressing the protease [Bozhokina et al., 2011]. Bacteria stained with DAPI could be detected in the cytoplasm of HeLa M cells. In addition, the invasion was monitored by limited actin proteolysis and PCR analysis.

Figure 2 illustrates internalization of *S. grimesii* 30063 (Fig. 2A) and recombinant *E. coli* SCS1 (Fig. 2B) within NAC-treated HeLa M cells visualized by fluorescence miscroscopy. To reveal the bacteria in the cytoplasm fraction of the infected cells by limited proteolysis the lysates of NAC-pretreated infected cells were incubated with skeletal muscle actin as described in Materials and Methods Section. Elecrophoretic analysis of these samples revealed appearance of the 36 kDa actin fragment, characteristic of limited actin proteolysis with grimelysin [Bozhokina et al., 2008] (Fig. 2C). Using specific primers toward full-length grimelysin gene, we had also applied PCR analysis and confirmed the presence of bacteria in the lysates of the infected cells (Fig. 2D).

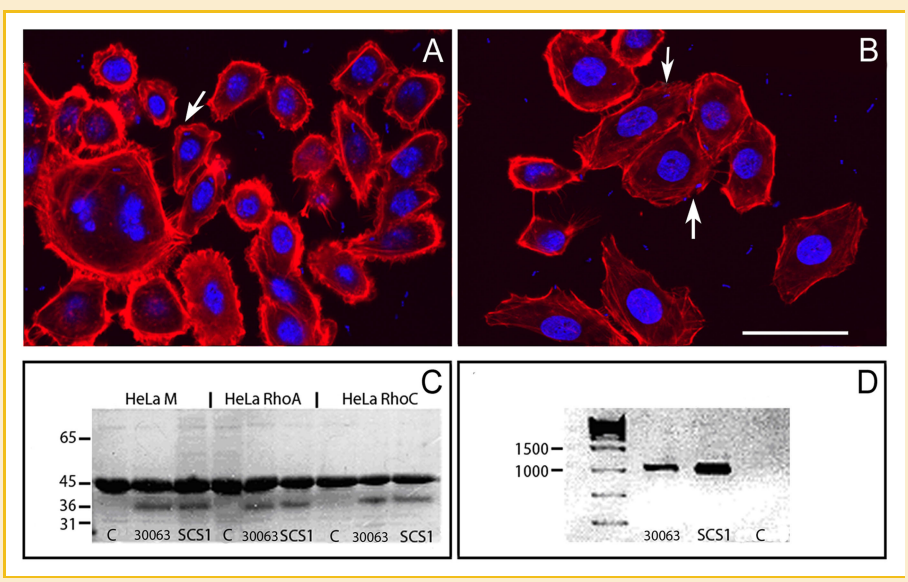


Fig. 2. Detection of intracellular grimelysin-producing bacteria in the NAC-treated HeLa M cells. A: fluorescent microscopy of HeLa M cells infected by *S. grimesii*. B: fluorescent microscopy of HeLa M cells infected by recombinant *E. coli* SCS1. Arrows point to the intracellular bacteria. Bar, 20 mkm. C: "Actinase" activity in the lysates of HeLa M, HeLa-RhoA, and HeLa-RhoC cells not incubated with bacteria (C), and infected with *S. grimesii* (30063) or recombinant *E. coli* (SCS1) as described in Materials and Methods Section. M, molecular mass markers. D, PCR analysis of HeLa M cells infected with *S. grimesii* (30063) and recombinant *E. coli* (SCS1) as described in Materials and Methods Section; (C), control uninfected cells. The size of PCR product ~1,200 bp.

### QUANTITATIVE EFFECTS OF NAC ON SUSCEPTIBILITY OF HELA CELLS TO BACTERIAL INVASION

To quantitate the amount of the intracellular bacteria, we used the gentamycin invasion assay [Prouty and Gunn, 2000]. In addition to the control HeLa M cells, HeLa cell lines containing a fraction of the cells constantly transfected with RhoA-GFP and RhoC-GFP were incubated with 10 mM NAC under the same conditions and infected with the bacteria. Figure 3 shows the number of intracellular *S. grimesii* 30063 in the HeLa cells pretreated with NAC for 24 h. The efficiency of invasion into HeLa M, HeLa-RhoA, and HeLa-RhoC cells was increased by a factor of 1.2, 1.5, and 1.3, respectively (Fig. 3A, striped boxes) indicating that the Rho-transfected HeLa cells are somewhat more sensitive to invasion than HeLa M cells. This difference may be due to the proliferative activity of the Rho-transfected cells rather than to a direct effect of Rho overexpression on the invasion. This point is to be further elucidated on the enriched HeLa-Rho cell cultures.

Removal of NAC from the culture medium (Fig. 3, black boxes) additionally increased efficiency of the invasion making it twice higher than it was in the control cells not treated with NAC (Fig. 3, white boxes).

The invasion capacity of recombinant *E.coli* SCS1 carrying grimelysin gene was much higher than that of *S. grimesii* 30063 (Fig. 3B). This effect was similar for all three cell lines used in the work. When the cells were infected with bacteria after removal of NAC from the culture medium, the efficiency of invasion was additionally increased (Fig. 3C).

### EFFECT OF NAC ON E-CADHERIN EXPRESSION

It has previously been shown that treatment with NAC up-regulates expression of several genes including E-cadherin and increases focal adhesion, thereby newly established adherence junctions with E-cadherin/β–catenin complexes were revealed [Parasassi et al., 2005]. Because E-cadherin, via β- and α-catenins, can trigger

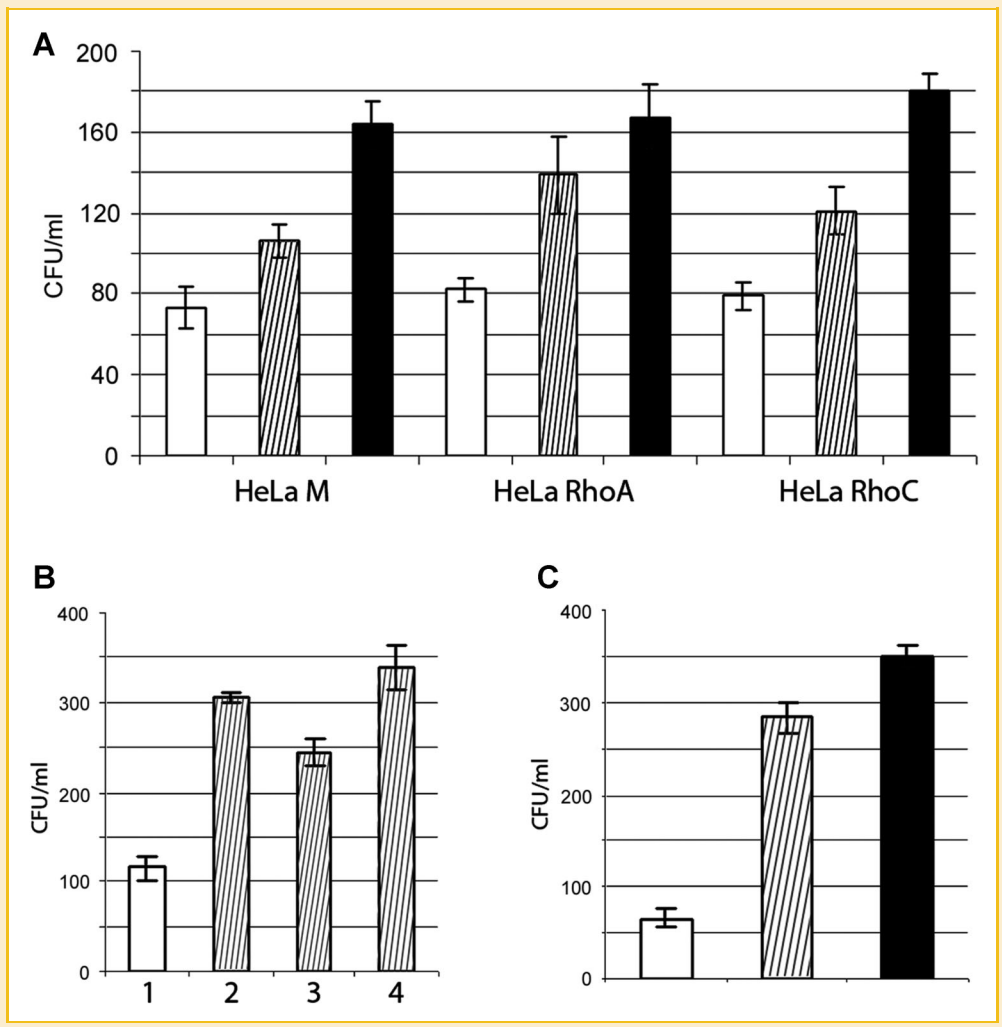


Fig. 3. Quantitative evaluation of the effect of NAC on susceptibility of HeLa cells to invasion by the wild-type and recombinant bacteria. HeLa M, HeLa RhoA, and HeLa-RhoC cells were infected by *S. grimesii* (A) and recombinant *E. coli* SCS1 (B,C) as described in Materials and Methods Section. *White boxes*, infected cells without NAC treatment, *striped boxes*, the cells incubated with 10 mM NAC before the infection, and *black boxes*, the cells preincubated with 10 mM NAC and washed out before the infection. Numbers 1–4 in (B) correspond to untreated cells, HeLa RhoA, HeLa M, and HeLa RhoC cells, respectively. In (C) HeLa-RhoC cells were infected. Values are expressed as mean ± S.D. (*error bars*). A difference was considered significant at the $P < 0.05$ level.

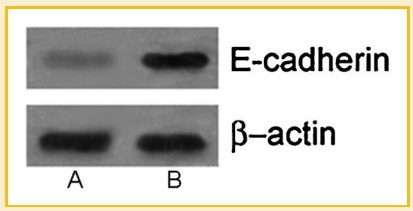


Fig. 4. Expression of E-cadherin determined by Semi-quantitative RT-PCR. A: untreated cells, control. B: cells treated with 10 mM NAC for 24 h. β-Actin was used as standard.

dynamic events of actin polymerization and membrane extensions culminating in bacterial uptake [Lecuit et al., 2000] we examined the expression of E-cadherin in NAC treated cells by semi-quantitative RT-PCR using β-actin housekeeping gene as internal control. Figure 4 shows that treatment of Hela M cells with 10 mM NAC resulted in an increased expression of E-cadherin gene.

## DISCUSSION

Studies at the cellular level have previously demonstrated that NAC promotes differentiation in normal primary human keratinocytes as well as a reversion of a colon carcinoma cell line from neoplastic proliferation to apical-basolateral differentiation [Parasassi et al., 2005]. Microarray data analysis of the NAC-treated cells identified up- and down-regulated genes whose joint activity correlated with inhibition of proliferation and stimulation of differentiation in both cell types [Gustafsson et al., 2005]. The NAC-induced shift from proliferation to appearance of well adhered pseudo-normal cells displayed pronounced stress fibers was also observed for 3T3-SV40 fibroblasts [Gamaley et al., 2006]. As susceptibility of cells to bacterial invasion increased with proliferation level [Velge et al., 1995], we could expect that NAC would decrease susceptibility of eukaryotic cells to bacteria. Our results showed, however, that exposure of HeLa cells to NAC restrained cell growth but considerably increased penetration of the grimelysin-producing bacteria. On the other hand, NAC promoted proliferation of a fraction of Rho-transfected cells of Hela-Rho cell lines, and this effect correlated with enhancement of the invasion. Together, these data corroborate our earlier suggestion [Gamaley et al., 2006] that the effect of NAC on susceptibility of eukaryotic cells to bacterial invasion, albeit dependent on the cell type and physiological state of host cells, is determined by the NAC-induced variations of cell surface proteins.

This hypothesis is supported by a number of data demonstrating the NAC-induced changing in matrix metalloproteinase activity, collagen synthesis, and extracellular matrix organization, which involves integrin-mediated adhesion and intracellular communications [Björklund and Koivunen, 2005; Fisher et al., 2009; Dooley et al., 2010]. Thus, although NAC and other thiol compounds modulate redox status of cells, they can be involved in cell signaling independently of antioxidative function [Hayakawa et al., 2003; Bogani et al., 2007; Parasassi et al., 2010]. For example, NAC has been shown to inhibit nuclear factor NF-κB activation by blocking tumor necrosis factor (TNF)-induced signaling by lowering the affinity of receptor to TNF [Hayakawa et al., 2003]. Other studies provide evidence for a molecular mechanism by which $H_2S$ or L-cysteine can improve glucose metabolism via activation of the PI3K/PIP3/phospho-AKT insulin signaling pathway and inhibition of the phosphatase and tensin homolog (PTEN) protein/NF-κB pathway [Manna and Jain, 2011]. Thiol compounds can also be involved in a direct regulation of gene expression.

Screening for the genes whose expression in normal and cancer cells is regulated by NAC revealed increased levels of products involved in cell–cell junctions and cell adhesion, including E-cadherin and β-catenin [Gustafsson et al., 2005]. This was in line with a remarkable increase in E-cadherin and β-catenin immunofluorescence labeling and redistribution of the oncogenic β-catenin from the nucleus to adherence junctions [Parasassi et al., 2005]. Our data confirmed the increased expression of E-cadherin gene in the NAC-treated HeLa cells. Both E-cadherin and β-catenin are known to participate in the initial contact of bacteria with eukaryotic cells [Lecuit et al., 2000]. We assume therefore that the NAC-induced stimulation of the E-cadherin-catenin pathway contributes to the NAC-increased internalization of the grimelysin producing bacteria within HeLa cells. It is interesting thereby that the recombinant bacteria enter the NAC-treated cells twice more efficiently than the wild-type ones. This difference suggests that the entry of the wild-type and recombinant bacteria occurs via different receptors which activity is differently affected by NAC.

The efficiency of the internalization of grimelysin-producing bacteria is usually rather low, with only about 10% of the cells being invaded either by the wild-type or recombinant bacteria [Bozhokina et al., 2011]. This implies that production of the enzyme does not make the bacteria pathogenic but rather provides them with an opportunity to render pathogenic under specific conditions. However, our present data show that treatment with NAC results in a two- to threefold increase in susceptibility of HeLa cells to invasion by these bacteria. While *Serratia* are facultative pathogens, common in hospital infection [Grimont and Grimont, 1978] these data should be taken into account in the NAC clinical use.

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