Data Article

*In vitro* effects of resistin on epithelial to mesenchymal transition (EMT) in MCF-7 and MDA-MB-231 breast cancer cells — qRT-PCR and Western blot analyses data

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**Abstract**

Resistin is an adipokine produced by the white adipocytes and adipose-derived macrophages, which mediates inflammation and insulin resistance Huang et al., 1997 and Renehan et al., 2008 Feb. Here, we provide data on the effect of resistin on epithelial to mesenchymal transition (EMT) in breast cancer cells *in vitro*. As model systems, we used human MCF-7 (low-metastatic) and MDA-MB-231 (high-metastatic) breast cancer cell lines. To optimize experimental conditions, we treated the cells with various concentrations of resistin (12.5, 25 and 50 ng/ml) for different time intervals (6 and 24 hours), and measured SOCS3 mRNA expression by using qRT-PCR analysis. Further, we used qRT-PCR and Western blot analyses to measure the expression of various epithelial (E-cadherin, claudin-1) and mesenchymal (SNAIL, SLUG, ZEB1, TWIST1, fibronectin, and vimentin) markers after resistin...
treatment. This data article is part of a study Avtanski et al., 2019 May, where detailed interpretation and discussion can be found. © 2019 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Data

1.1. Effect of resistin on suppressor of cytokine signaling 3 (SOCS3) mRNA expression

Resistin is a cytokine produced by the white adipose tissue (WAT) that is known to play a major role in mediating insulin sensitivity of the insulin target tissues [1,2]. Previous research by Steppan et al. [4] demonstrated that resistin treatment of 3T3-L1 adipocytes (at concentration of 20 ng/ml) markedly induced the gene expression of suppressor of cytokine signaling 3 (SOCS3). Here, we used SOCS3 as a control gene to optimize the treatment conditions of human MCF-7 and MDA-MB-231 breast cancer cells with resistin. Cells were treated with increasing concentrations of human resistin (8.3, 12.5, 25, and 50 ng/ml) for 6 and 24 hours, and resistin mRNA expression levels were measured by qRT-PCR analysis. Data were presented as fold-change of resistin treatment vs. non-treated (Control) cells at 6 and 24 hours. Results demonstrated that resistin statistically significant upregulated SOCS3 mRNA levels in both, MCF-7 and MDA-MB-231 cells, as maximal effect was reached at concentration of 12.5 ng/ml for 6 hours of treatment (Fig. 1 and Table 1).
1.2. Effect of resistin on the expression of epithelial to mesenchymal transition (EMT) markers in breast cancer cells in vitro

Using similar treatment conditions, we evaluated the effect of resistin on the expression of various mesenchymal and epithelial markers in MCF-7 and MDA-MB-231 cells. Results from qRT-PCR analysis showed that in MDA-MB-231 cells, resistin significantly upregulated mRNA levels of key transcription factors involved in EMT (SNAIL, SLUG, ZEB1, and TWIST1) as well as their target genes, fibronectin and vimentin (Fig. 2 and Table 2). Results from Western blot analyses using MCF-7 and MDA-MB-231 cells demonstrated that resistin significantly increased the protein expression levels of SNAIL and vimentin in both MCF-7 and MDA-MB-231 cells, as well as concomitantly suppressed the protein levels of E-cadherin and claudin-1 in MCF-7 cells ([3] and Table 3). In order to investigate EMT transcription factor nuclear translocation events, we further performed fractioned Western blot, analyzing ZEB1 and SNAIL protein expression in separate cytoplasmic and nuclear fractions from MCF-7 and MDA-MB-231 cells treated with resistin (12.5 ng/ml for 6 hours). The results from this experiment demonstrated that resistin time-dependently induced the nuclear translocation of these two transcription factors in both, MCF-7 and MDA-MB-231 cells ([3] and Table 4).

Table 1

| Treatment Time | Cell Line | MCF-7 | MDA-MB-231 |
|----------------|-----------|-------|-------------|
|                | Concentration (ng/ml) | mean ± SEM | p Value | mean ± SEM | p Value |
| 6h             | Control   | 1.047 ± 0.088 | 1.066 ± 0.107 |
|                | 8.3       | 1.542 ± 0.184 | 0.0248 | 1.706 ± 0.183 | 0.0053 |
|                | 12.5      | 1.829 ± 0.192 | 0.0002 | 2.187 ± 0.216 | 0.0001 |
|                | 25        | 1.277 ± 0.166 | 0.1864 | 1.430 ± 0.172 | 0.0752 |
|                | 50        | 1.016 ± 0.256 | 0.8854 | 1.182 ± 0.039 | 0.4322 |
| 24h            | Control   | 0.988 ± 0.112 | 1.017 ± 0.061 |
|                | 8.3       | 1.505 ± 0.194 | 0.0353 | 1.611 ± 0.136 | 0.0002 |
|                | 12.5      | 1.815 ± 0.231 | 0.0252 | 1.769 ± 0.185 | 0.0020 |
|                | 25        | 1.272 ± 0.162 | 0.2091 | 1.290 ± 0.124 | 0.0549 |
|                | 50        | 0.823 ± 0.139 | 0.4503 | 0.918 ± 0.104 | 0.4699 |

Fig. 1. Optimization of resistin treatment of MCF-7 and MDA-MB-231 cells. Concentration-response and time-dependent experiments. MCF-7 (A) and MDA-MB-231 (B) cells were treated with various concentrations of resistin (8.3, 12.5, 25, and 50 ng/ml) for 6 and 24 hours. SOCS3 mRNA levels were measured by using qRT-PCR analysis. GAPDH was used as a housekeeping gene. Relative mRNA levels and p values are listed in Table 1.
2. Experimental design, materials and methods

2.1. Reagents

Human recombinant resistin (Cat. # PF-138) was purchased from Calbiochem® (EMD Millipore Corp.). The lyophilized form was reconstituted with water to a concentration of 100 µg/ml and further to a series of dilutions of 8.3, 12.5, 25, and 50 µg/ml, which were used for cell treatment.

![Graphs showing effects of resistin on mesenchymal gene expression in MDA-MB-231 cells.](image)

**Fig. 2.** Effect of resistin on mesenchymal gene expression in MDA-MB-231 cells. Quantitative RT-PCR analyses data on the relative mRNA expression levels of SNAIL, SLUG, ZEB1, TWIST1, fibronectin and vimentin in MDA-MB-231 cells, untreated or treated with resistin (12.5, 25, and 50 ng/ml for 6 and 24 hours). All fold-change data and p values are listed in Table 2.
### Table 2

**Effect of resistin on mesenchymal gene expression in MDA-MB-231 cells.** Concentration-response and time-dependent experiment for resistin treatment of MDA-MB-231 cells (see Fig. 2). Cells were treated with various concentrations of resistin (12.5, 25 and 50 ng/ml) for 6 and 24 hours and relative mRNA levels of SNAIL, SLUG, ZEB1, TWIST1, fibronectin and vimentin were measured by using qRT-PCR analysis. *p* values < 0.05 are shown in bold.

| Gene      | Condition | Resistin (ng/ml) | Treatment Time (h) | mean ± SEM | p Value | mean ± SEM | p Value | mean ± SEM | p Value | mean ± SEM | p Value | mean ± SEM | p Value |
|-----------|-----------|------------------|--------------------|------------|---------|------------|---------|------------|---------|------------|---------|------------|---------|
| SNAIL     | Control   | 1.015 ± 0.048    | 6                  |            |         | 3.400 ± 0.549 | <0.0001 |            |         | 1.922 ± 0.434 | 0.0398 | 2.457 ± 0.621 | 0.0040 |
|           | Resistin  | 4.669 ± 0.721    |                    | **0.0006** | **0.0027** | 3.743 ± 0.823 |         | 0.980 ± 0.079 |         | 2.271 ± 0.452 | **0.0020** | 0.560 ± 0.1036 | 0.4247 |
| SLUG      | Control   | 1.025 ± 0.043    | 6                  |            |         | 1.553 ± 0.315 | **0.0267** |            |         | 1.909 ± 0.404 | **0.0263** | 0.797 ± 0.147 | 0.4247 |
|           | Resistin  | 1.921 ± 0.205    |                    | **0.0019** | <0.0001 | 2.282 ± 0.185 | <0.0001 |            |         | 1.036 ± 0.060 |         | 1.318 ± 0.193 | **0.0014** |
| ZEB1      | Control   | 1.002 ± 0.016    | 6                  |            |         | 1.096 ± 0.111 | 0.3641 |            |         | 1.223 ± 0.120 | 0.3614 | 0.748 ± 0.054 | **0.0014** |
|           | Resistin  | 1.526 ± 0.119    |                    | **0.0002** | <0.0001 | 1.357 ± 0.042 | <0.0001 |            |         | 1.238 ± 0.131 | 0.1813 | 0.748 ± 0.054 | **0.0014** |
| TWIST1    | Control   | 1.009 ± 0.035    | 6                  |            |         | 1.201 ± 0.243 | 0.4293 |            |         | 1.034 ± 0.058 |         | 1.427 ± 0.351 | 0.6319 |
|           | Resistin  | 3.335 ± 0.549    |                    | **0.0008** | **0.0108** | 1.500 ± 0.209 |         | 2.901 ± 0.684 |         | 1.088 ± 0.075 |         | 1.427 ± 0.301 | 0.6319 |
| Fibronectin| Control  | 1.001 ± 0.025    | 6                  |            |         | 0.947 ± 0.116 | 0.7563 |            |         | 1.420 ± 0.121 | **0.0362** | 1.191 ± 0.245 | 0.6319 |
|           | Resistin  | 1.742 ± 0.153    |                    | **0.0074** | **0.0093** | 1.573 ± 0.122 |         | 2.658 ± 0.732 | **0.0337** | 1.201 ± 0.189 | 0.5287 | 1.044 ± 0.122 | 0.9197 |
| Vimentin  | Control   | 1.125 ± 0.121    | 6                  |            |         | 1.607 ± 0.175 | 0.0289 |            |         | 2.658 ± 0.732 | **0.0337** | 1.201 ± 0.189 | 0.5287 |
|           | Resistin  | 3.241 ± 0.560    |                    | **0.0006** | **0.0014** | 2.050 ± 0.226 |         | 1.201 ± 0.189 | 0.5287 | 1.044 ± 0.122 | 0.9197 | 1.201 ± 0.189 | 0.5287 |
2.2. Cell lines and reagents

Human breast cancer MCF-7 and MDA-MB-231 cells were purchased from American Type Culture Collection (ATCC) (Cat. ## HTB-22 and HTB-26, ATCC). Cells were grown in Dulbecco’s Modified Eagle’s Medium (DMEM) (Cat. # 10-013-CF, Corning Inc.) supplemented with 10% FBS (Cat. # 1500-500, VWR International) and 1x Antibiotic/Antimycotic Solution (Cat. # 30-004-CI, Corning, Inc.).

2.3. Quantitative RT-PCR analyses

Total RNA was extracted by using TRIzol™ reagent (Ambion, Thermo Fisher Scientific) and chloroform, and precipitated with iso-propanol (VWR International). The precipitated RNA was washed

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### Table 3

Effect of resistin on epithelial and mesenchymal proteins expression in MCF-7 and MDA-MB-231 cells – densitometry analyses. MCF-7 and MDA-MB-231 cells were treated with resistin (12.5 ng/ml) for 6 and 24 hours. Densitometry analyses were performed using ImageJ software (NIH). Data are presented as % change of resistin treatment compared to control (no treatment) after normalizing to GAPDH levels. *p* values < 0.05 are shown in bold.

| Treatment Time | Protein | Condition | Protein Expression (% ± SEM) | p Value | Protein Expression (% ± SEM) | p Value |
|----------------|---------|-----------|-----------------------------|---------|-----------------------------|---------|
| 6h             | SNAIL   | Control   | 100.00 ± 0.660              |         | 236.10 ± 27.660             | 0.0002  |
|                | Resistin|           | 195.90 ± 20.090             | 0.0002  | 262.70 ± 15.400             | < 0.0001|
|                | Vimentin| Control   | 100.00 ± 1.801              |         | 82.59 ± 6.082               | 0.0201  |
|                | E-cadherin| Control | 100.00 ± 9.933              |         | 84.19 ± 9.331               | 0.0369  |
|                |         | Resistin  | 147.10 ± 12.320             | 0.0016  | 55.42 ± 6.600               |         |
|                | Claudin-1| Control  | 100.00 ± 12.460             |         | 262.70 ± 4.992              |         |
|                |         | Resistin  | 82.59 ± 6.082               | 0.2093  | 58.65 ± 4.992               |         |
| 24h            | MCF-7   | SNAIL     | 100.00 ± 21.420             |         | 235.80 ± 21.660             | 0.0004  |
|                | Vimentin| Control   | 249.80 ± 26.860             | 0.0005  | 201.00 ± 13.060             | 0.0122  |
|                |         | Resistin  | 201.00 ± 16.980             |         | 236.10 ± 15.400             |         |
|                | E-cadherin| Resistin | 147.10 ± 12.320             | 0.0016  | 55.42 ± 6.600               |         |
|                | Claudin-1| Resistin | 82.59 ± 6.082               | 0.2093  | 58.65 ± 4.992               |         |
|                | MDA-MB-231| SNAIL    | 100.00 ± 21.420             |         | 235.80 ± 21.660             | 0.0004  |
|                | Vimentin| Control   | 249.80 ± 26.860             | 0.0005  | 236.10 ± 27.660             | 0.0002  |
|                |         | Resistin  | 201.00 ± 13.060             | 0.0002  | 235.80 ± 21.660             | 0.0004  |

### Table 4

Effect of resistin on EMT-transcription factor nuclear translocation in MCF-7 and MDA-MB-231 cells – densitometry analyses. MCF-7 and MDA-MB-231 cells were subjected to resistin treatment (12.5 ng/ml) for 6 and 24 hours. Western blots were performed using separate (cytoplasmic and nuclear) protein fractions. β-Tubulin and lamin B1 were used as loading controls. Densitometry analyses were performed using ImageJ software (NIH). Data are presented as % change of resistin treatment compared to control (no treatment) after normalizing to the corresponding loading control. *p* values < 0.05 are shown in bold.

| Protein | Fraction | Condition | MCF-7 Protein Expression (% ± SEM) | p Value | MDA-MB-231 Protein Expression (% ± SEM) | p Value |
|---------|----------|-----------|-----------------------------------|---------|----------------------------------------|---------|
| SNAIL   | Cytoplasmic | Control | 100.00 ± 13.380                   | 0.0438  | 96.67 ± 9.349                          | 0.0465  |
|         |          | Resistin (12.5 ng/ml): 6h | 55.09 ± 7.723                   | 0.0398  | 58.63 ± 9.535                          | 0.0283  |
|         |          | Resistin (12.5 ng/ml): 24h | 40.23 ± 14.720                  | 0.0044  | 61.88 ± 4.449                          | 0.0159  |
|         | Nuclear  | Control | 100.00 ± 4.044                    | 0.0004  | 100.00 ± 8.182                         | 0.0100  |
|         |          | Resistin (12.5 ng/ml): 6h | 218.50 ± 20.000                 | 0.0004  | 158.10 ± 11.900                       | 0.0159  |
|         |          | Resistin (12.5 ng/ml): 24h | 208.10 ± 9.646                  | 0.0004  | 173.30 ± 13.670                       | 0.0100  |
| ZEB1    | Cytoplasmic | Control | 98.10 ± 7.932                    | 0.0213  | 93.33 ± 4.913                         | 0.0172  |
|         |          | Resistin (12.5 ng/ml): 6h | 54.61 ± 8.793                   | 0.0179  | 46.16 ± 10.970                        | 0.0283  |
|         |          | Resistin (12.5 ng/ml): 24h | 46.39 ± 10.730                  | 0.0001  | 37.42 ± 15.890                        | 0.0287  |
|         | Nuclear  | Control | 100.00 ± 4.884                   | 0.0179  | 168.60 ± 15.550                       | 0.0044  |
|         |          | Resistin (12.5 ng/ml): 6h | 219.80 ± 6.828                  | 0.0179  | 191.70 ± 8.551                        | 0.0044  |
|         |          | Resistin (12.5 ng/ml): 24h | 255.50 ± 7.281                  |         |                                        |         |
once with 70% ethanol, air-dried and resuspended in sterile, nuclease-free water, then the samples were incubated at 65 °C for 10 minutes and stored at −86 °C until analyzed. Total RNA concentration was determined using NanoDrop™ One Microvolume UV–Vis Spectrophotometer (Thermo Fisher Scientific), samples were normalized to 1 mg/ml RNA, and RT reaction was performed by using qScript cDNA Synthesis Kit (Quanta Bio) and SimpliAmp thermal cycler (Applied Biosystems, Thermo Fisher Scientific). Quantitative PCR analyses were performed by using PowerUp SYBR Green Master Mix and QuantStudio 3 Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific). The following primer sequences (5'-3') were used: SOCS3, (forward) CCTGCGCTCAAGACCTTC, (reverse) GTCATCCTCGCTCGTAAGA (PrimerBank ID 45439351c1); SNAIL, (forward) CTTCCTGGTGCAAGAAGACCA (reverse), GGGAATATCACTGTATGTGTG [6]; ZEB1, (forward) GCAACACAAATGCCAGAGA, (reverse) GCCTGGTCAGGAGAGATG [7]; TWIST1, (forward) GGTCCGTCAACCGTCCTC, (reverse) CTATGGGACGCAGACAT [8]; fibronectin, (forward) ATGATGAGGTCAGCTGTG, (reverse) CTCTGAATCCTGGCATTGGT [7]; vimentin, (forward) AGATGGCCCTTGACATTGAG, (reverse) TGGAAGAGGCAGAGAAATCC [7]; GAPDH, (forward) CCTGCCACAGCTTCTGGT [9]. The annealing temperature for all of the PCR analyses was 60 °C.

2.4. Western blot analyses

Whole cellular protein was extracted by using Pierce™ RIPA Lysis Buffer (Cat. # 89901, Thermo Fisher Scientific) supplemented with protease inhibitor cocktail (Mammalian Protease Arrest, Cat. # 786-331, G-Biosciences) and phosphatase inhibitors (Phosphatase Arrest™, Cat. # 786-450, G-Biosciences). Cytoplasmic and nuclear protein fractions were extracted by using Nuclear & Cytoplasmic Extraction Kit (G Biosciences®, Cat. # 786-182) following manufacturer’s protocol. Protein concentrations were quantified by using Pierce™ BCA Protein Assay Kit (Cat. # 23225, Thermo Fisher Scientific), BioTek® plate reader and Gen5™ data analysis software (BioTek®). Protein extracts were separated by polyacrylamide gel electrophoresis (SDS-PAGE), and transferred onto nitrocellulose membranes (Amersham, GE Healthcare). Membranes were blotted with SNAIL (C15D3) Rabbit mAb (Cat. # 3879), SLUG (C19G7) Rabbit mAb (Cat. # 9585), TCF8/LEB1 (D80D3) Rabbit mAb (Cat. # 3396), Vimentin (D21H3) XP Rabbit mAb (Cat. # 5741), E-cadherin (24E10) Rabbit mAb (Cat. # 3195), Claudin-1 (D5H1D) XP Rabbit mAb (Cat. # 13255), GAPDH (14C10) Rabbit mAb (Cat. # 2118) (Cell Signaling Technology), β Tubulin (D-10) mAb (Cat. # sc-5274), and Lamin B1 (B-10) mAb (Cat. # sc-374015) (Santa Cruz Biotechnology) primary antibodies and Pierce™ Goat Anti-Rabbit Horseradish Peroxidase Conjugated Secondary Antibody (Cat. # 31460, Thermo Fisher Scientific). Protein bands were visualized by using SuperSignal West Pico Chemiluminescent Substrate (Cat. # 34580, Cell Signaling Technology) and MyECL™ Imager (Thermo Fisher Scientific). Densitometry analyses were performed with ImageJ software (National Institutes of Health) and protein expression levels were presented as percentage difference compared to control (±SEM).

2.5. Statistical analyses

Quantitative RT-PCR analyses data were based on 4-6 separate experiments, as each experimental condition was set up in duplicates, and each sample was analyzed in duplicates. Densitometry analysis of the Western blot data was based on 3 different experiments. Statistical analysis were carried out using GraphPad Prism 7 software (GraphPad Software, La Jolla, CA). Data were analyzed using ANOVA. Results are expressed as mean ± SEM and are considered statistically significant if p < 0.05.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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