 doi:10.20944/preprints201907.0105.v1

Original research manuscript

Mechanisms underlying the altered proliferation, invasion and migration of endometrial carcinoma cells via small interfering RNA specific Snail-1 transcription factor in HEC-1A cells

Jun Jiang [1,*], Jianfen Wang [1], Yong Shen [1], Xuelu Jiang [1]

1 The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou (31006), China
* Correspondence: junjiang026@gmail.com

Abstract: Snail-1 is a transcription factor that play a role in the regulation of invasion, and metastasis of malignant cells via modulation of the epithelial-to-mesenchymal transition (EMT) process. Here in the current investigation, we knocked down the expression of Snail-1 through small interference RNA (siRNA) in Endometrial carcinoma (EC) associating cell line, namely HEC-1A, and then assessed the migration and proliferation of the transfected cells. We exerted Snail-1 specific siRNA to transfect the HEC-1A cells. Using quantitative Real-time PCR, the mRNA expression levels of Snail-1, MMP-9, Vimentin, and E-cadherin as well as expression level of miR-34a were measured. In addition, western blotting was carried out to determine the protein level of Snail-1. Using MTT and migration assay, the ability of transfected HEC-1A cells in proliferation and migration was evaluated. Snail-1 mRNA and protein expressions were decreased after transfection of HEC-1A cells. As well transfection caused decreased expression of MMP-9 and vimentin, while the expressions of E-cadherin and miR-34a were increased. Transfection of HEC-1A cells resulted in promoted apoptosis rate and reduced migration ability. EMT of EC tumor cells is partially under the impression of Snail-1, which alters the apoptosis and metastasis of these cells. As a consequence, silencing Snail-1 by siRNA may suggest a potentially promising tool in the EC therapy.

Keywords: Endometrial carcinoma; Snail-1; siRNA; metastasis; apoptosis

1. Introduction

Endometrial carcinoma (EC) is a most critical cause of malignancies in the female genital tract, that has recently been reported to confer an increasing incidence and death rates. A large number of affected subjects are detected when the disease is still found in the uterus, which is defined as stage I or II of the International Federation of Gynecology and Obstetrics (FIGO criteria. The 5-year survival rates in patients with EC is between 74% to 91%. That notwithstanding, a 5-year overall survival is 57–66% and 20–26% has been reported, in respective, for patients in the FIGO stage III and IV [1]. A number of treatment approaches, including hormonal therapy, surgery, chemotherapy, and radiation therapy has been prosperous in the therapy of EC patients when the disease is localized, in spite of a little treatment options for the patients when the disease shows metastasis [2]. As a consequence, it is paramount to reveal the precise molecular modulation that are involved in the progression of the disease from a localized phenotype toward metastatic and invasive phenotype.

The transforming from early-stage into advanced-stage tumor phenotype is related to a process called epithelial-mesenchymal transition (EMT), participating in the migration, metastasis and invasion of malignant cells [3, 4]. EMT can develop mesenchymal characteristics in the

 doi:10.20944/preprints201907.0105.v1

epithelial cells, that in turn, promotes the detachment of malignant cells from the origin source of tumor and allow their aftermath invasive mobilization, providing the metastatic ability to the cancer cells [5]. An association has been indicated between EMT and the erosion of E-cadherin-mediated cell-to-cell attachment in malignant cells [6]. EMT play a role in the triggering and activation of mesenchymal signatures, including vimentin, and a number of EMT-stimulating transcription factors, such as Snail.

Snail family transcriptional repressor 1 (SNAI1) gene is harbored by the chromosome 20, codifying a member of the Snail superfamily of zinc-finger transcription factors, namely Snail-1, which plays a role in the transcription inhibition. Snail superfamily of zinc-finger transcription factors are involved in the differentiation and survival cells that are expressed in them, charactering two curtail cancer developing mechanisms [7, 8]. By suppression of E-cadherin, Snail-1 takes part in the modulation of EMT, leading to controlling the mesenchymal features like migration and metastasis. EMT underlying Snail-1 dysregulation has been associated with E-cadherin and claudins underexpression as well as of fibronectin and vimentin overexpression [9]. Furthermore, Snail-1 has been shown to be involved in the upregulation of matrix metalloproteinase (MMP)-9, and therefore, regulates the migration and invasion and of tumor cells [9, 10]. A bulk of investigations has testified the role of Snail-1 in the modulation of molecules involved in the EMT, including E-cadherin, vimentin, and MMP-9, as well as microRNAs (miRNAs) like miR-34a, thereby, impressing the invasion, migration, and metastasis of cancer cells [11-15].

Herein, in this study, the expression of Snail-1 was silenced via siRNA in EC related HEC-1A cell line, and then evaluated the EMT process in the transfected cells through studying the migration and proliferation abilities of the HEC-1A cells.

2. Results

2.1. mRNA expression of Snail-1

After transfection of HEC-1A cells with Snail-1 specific siRNA, it was observed that the mRNA expression of Snail-1 was downregulated after 24 hrs (Fold change = -0.76, P= 0.0081), 48 hrs (Fold change = -0.71, P= 0.006), and 72 hrs (Fold change = -0.61, P= 0.00011) compared with the negative control group (Figure 1.a). On the other side, as the dose of Snail-1 specific siRNA increased, the mRNA expression of Snail-1 was decreased in HEC-1A cells; in a dose dependent pattern, the expression of Snail-1 mRNA was downregulated in 40 pmol of Snail-1 specific siRNA (Fold change = -0.81, P= 0.021), 60 pmol of Snail-1 specific siRNA (Fold change = -0.63, P= 0.00021), and 80 pmol of Snail-1 specific siRNA (Fold change = -0.59 1, P= 0.00035) in Snail-1 transfected HEC-1A cells compared with the negative control cells (Figure 1.b).

2.2. Snail-1 protein level

Figure 2.a indicates the intensity of the protein bands after electrophoresis after transfection of HEC-1A cells with Snail-1 specific siRNA. According to the figure 2.b, transfected HEC-1A cells demonstrated a statistically significant downregulation of the Snail-1 protein expression in 60 pmol of Snail-1 specific siRNA (Fold change = -0.80, P= 0.0044) and 80 pmol of Snail-1 specific siRNA (Fold change = -0.49, P= 0.00059) compared with the negative control group.

2.3 Expression of target genes and miR-34a

Snail-1 specific siRNA transfection resulted in significant downregulation of MMP-9 mRNA in 40 pmol of siRNA (Fold change = -0.82, P= 0.0045), 60 pmol of siRNA (Fold change = -0.69, P= 0.00088), and 80 pmol of siRNA (Fold change = -0.48, P= 0.000036) compared with the negative control (Figure 3.a). Transfection of Snail-1 specific siRNA eventuated in significant downregulation of mRNA expression of vimentin in HEC-1A cells after adding 40 pmol of siRNA (Fold change = -0.77, P= 0.025), 60 pmol of siRNA (Fold change = -0.71, P= 0.00012), and 80 pmol of siRNA (Fold change = -0.47, P= 0.00055) compared with the negative control group (Figure 3.b). On the other hand, it was seen that transfection of Snail-1 specific siRNA into HEC-1A cells resulted in significant

 doi:10.20944/preprints201907.0105.v1

upregulation of E-cadherin mRNA after adding 40 pmol of siRNA (Fold change = 1.9, P= 0.00031), 60 pmol of siRNA (Fold change = 2.5, P= 0.00065), and 80 pmol of siRNA (Fold change = 3.1, P= 0.00023) compared with the negative control group (Figure 3.c). Moreover, an upregulation of miR-34a was detected in HEC-1A cells upon transfection with 40 pmol of siRNA (Fold change = 1.5, P= 0.0089), 60 pmol of siRNA (Fold change = 1.9, P= 0.00071), and 80 pmol of siRNA (Fold change = 2.2, P= 0.00064) compared with the negative control group (Figure 3.d).

2.4 Apoptosis rate

It was detected that HEC-1A transfection with adding 80 pmol siRNA Snail-1 specific siRNA led to statistically significant promotion in the rate of apoptotic cells after 48 hrs (P= 0.034), 72 hrs (P= 0.014), and 96 hrs (P= 0.0088) in comparison to the negative control group (Figure 4).

2.5 Migration of HEC-1A cells

According to the wound healing assay, it was seen that transfection of Snail-1 specific siRNA resulted in a significant reduction in the number of migrated HEC-1A cells to the scratched region after 24 hrs (P= 0.00054), 48 hrs (P= 0.00066), and 72 hrs (P= 0.00066) compared with the negative control group (Figure 5).

2.6. Figures, Tables

Table 1. The primer sequences exerted in the Real-time expression analysis in HEC-1A cells.

Gene name	Strand	Sequences
E-cadherin	Forward	5'-TCCATTTCTTGGTCTACG CC-3'
	Reverse	5'-CACCTTCAGCCAACCTGTTT-3'
Snail-1	Forward	5'-GGTTCTTCTGCGCTACTGCTG-3'
	Reverse	5'-GTCGTAGGGCTGCTGGAAGG-3'
MMP-9	Forward	5'-ATTTCTGCCAGGACCGCTTCTAC-3'
	Reverse	5'-ATCCGGCAAACTGGCTCCTTC-3'
Vimentin	Forward	5'-CAGGCAAAGCAGGAGTCCA-3'
	Reverse	5'-AAGTTCTCTTCCATTTCACGCA-3'
β-actin	Forward	5'-TCCCTGGAGAAGAGCTACG-3'
	Reverse	5'-GTAGTTTCGTGGATGCCACA-3'
Let-7a	Target sequence	5'-UGAGGUAGUAGGUUGUAUAGUU-3'
miR-34a	Target sequence	5'-UGGCAGUGUCUUAGCUGGUUGU-3'

 doi:10.20944/preprints201907.0105.v1

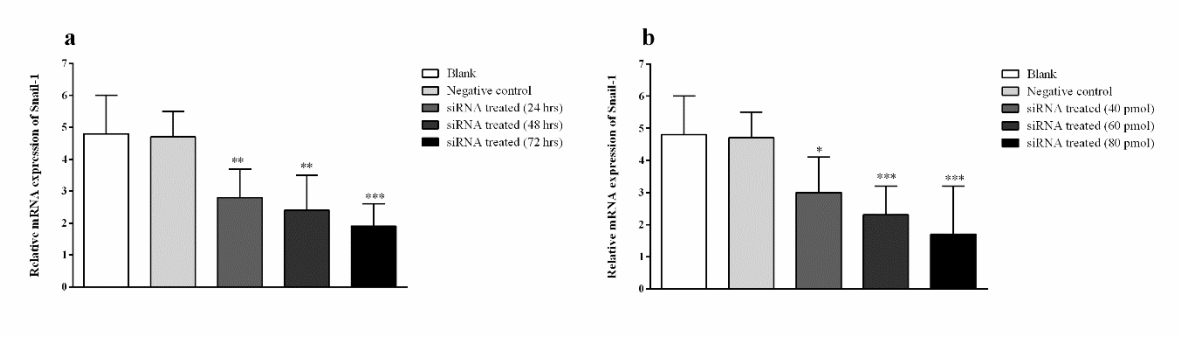

Figure 1. Transfection of HEC-1A cells by Snail-1 specific siRNA and aftermath expression of Snail-1. a; mRNA expression of Snail-1 in HEC-1A cells after of 24, 48 and 72 hrs from transfection of HEC-1A cells with 80 pmol of Snail-1 specific siRNA. b; Snail-1 mRNA expression after 48 hrs since transfection of HEC-1A cells with three doses of 40, 60, and 80 pmol of Snail-1 specific siRNA (Data are represented as mean ± SD; * indicates $P < 0.05$, ** indicates $P < 0.001$, and ***$P < 0.0001$).

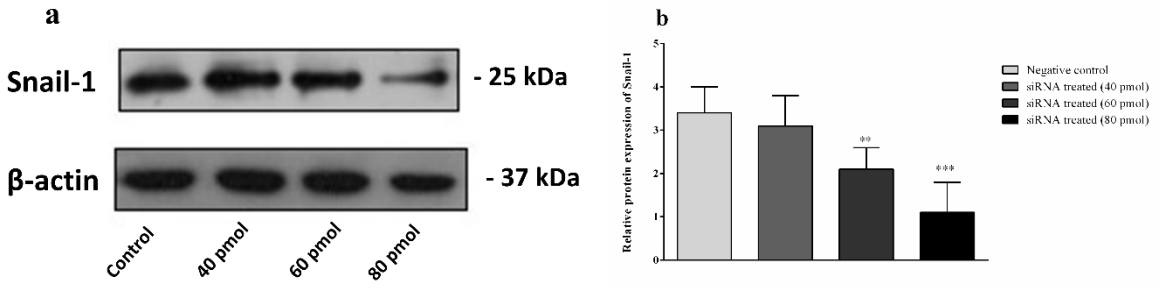

Figure 2. Expression of Snail-1 protein in HEC-1A cells transfected by Snail-1 specific siRNA. HEC-1A cells were transfected with three doses of 40 pmol, 60 pmol, and 80 pmol of Snail-1 specific siRNA. a; Expression level of each band was identified via densitometry and color density of each band was normalized to the β-actin protein level. Electrophoretic bands of the Snail-1 and β-actin proteins. b; protein expression of Snail-1 was decreased after transfection of 40 pmol, 60 pmol, and 80 pmol of Snail-1 specific siRNA into HEC-1A cells (Data are represented as mean ± SD; ** indicates $P < 0.01$ and *** indicates $P < 0.001$).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 July 2019 doi:10.20944/preprints201907.0105.v1

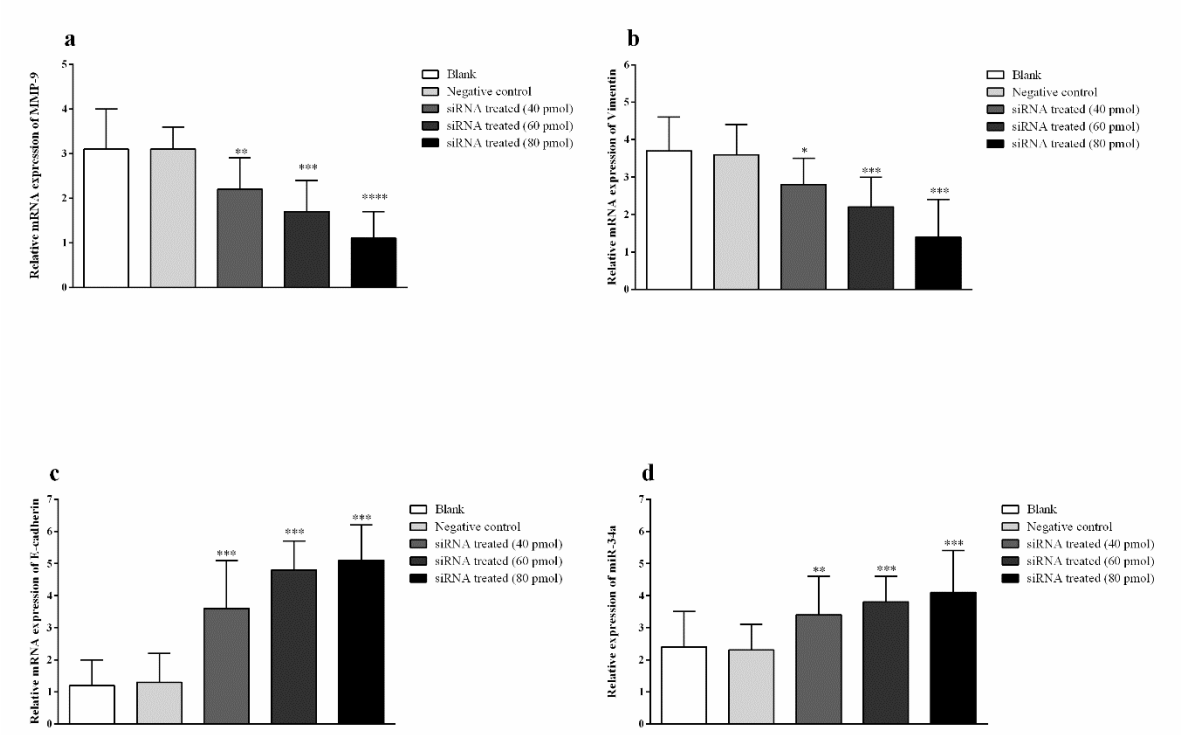

Figure 3. Bar graphs shows the expression levels of metastatic-related genes and miR-34a after transfection of HEC-1A cells by Snail-1 specific siRNA after 48 hrs. mRNA levels of a; MMP-9, b; Vimentin, c; E-cadherin, and d; miR-34a were evaluated after transfection of HEC-1A cells by three doses of 40 pmol, 60 pmol, and 80 pmol of Snail-1 specific siRNA compared with the negative control using quantitative Real-time PCR (Data are represented as mean ± SD; * indicates P<0.05, ** indicates P<0.01, *** indicates P<0.001, and **** indicates P< 0.0001).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 July 2019 doi:10.20944/preprints201907.0105.v1

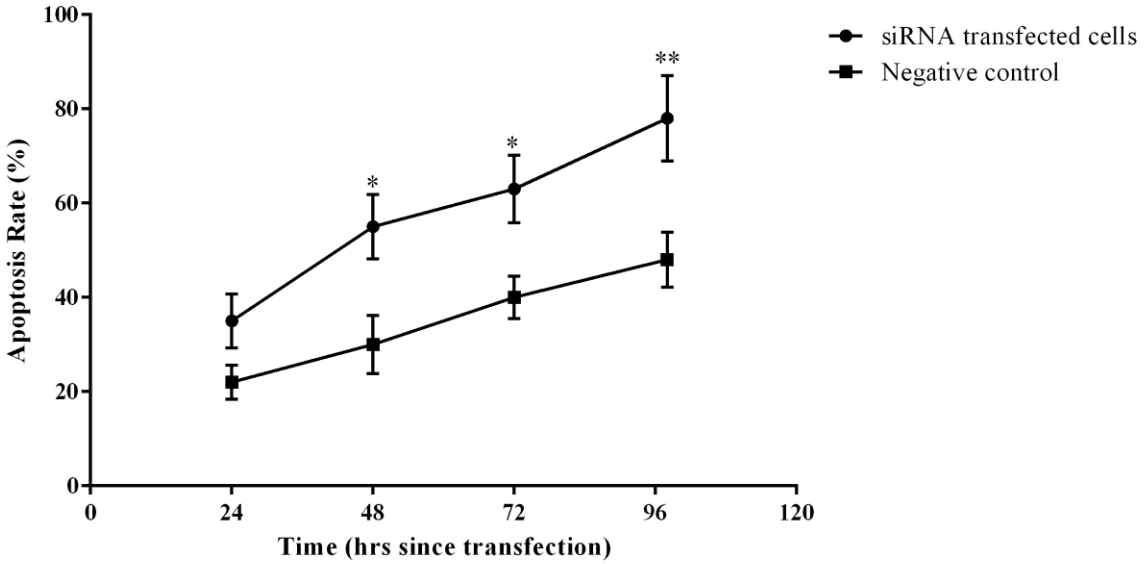

Figure 4. The apoptosis rate of HEC-1A cells after transfection with 40 pmol Snail-1 specific siRNA. Apoptosis of HEC-1A cells was determined by MTT assay after 24 hrs, 48 hrs, 72 hrs, and 96 hrs after transfection (Data are represented as mean ± SD; * indicates P< 0.05 and ** indicates P< 0.01).

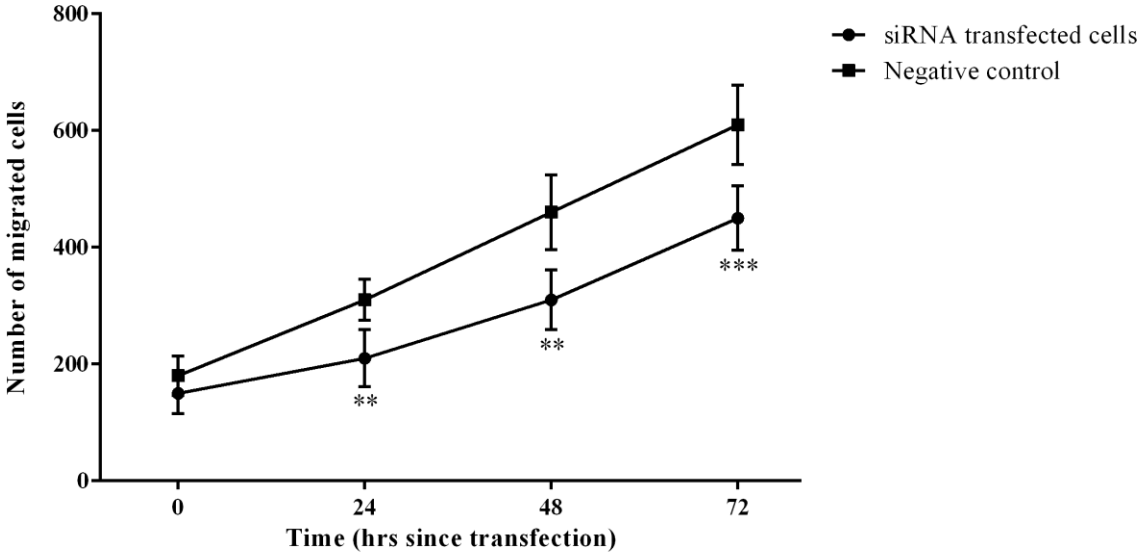

Figure 5. Migration of HEC-1A cells after transfection with Snail-1 specific siRNA since 0 hrs, 24 hrs, 48 hrs, and 72 hrs. A monolayer of HEC-1A cells seeded on wells was transfected by Snail-1 specific siRNA and a scratched was generated on the plate surface, and the filling of gaps was evaluated after 0 hrs, 24 hrs, 48 hrs, and 72 hrs (Data are presented as means ± SD; ** indicates P<0.01 and *** indicates P<0.001).

 doi:10.20944/preprints201907.0105.v1

3. Discussion

Phenotypic as well as molecular transformation of epithelial cells to a mesenchymal phenotype having migratory ability is defined as EMT, which takes an active part in the progression of malignancies [17-19]. Numerous investigations suggest the EMT is the underlying mechanism of cancerous behaviors, such as invasion, migration, and metastasis of malignant cells [20]. As a consequence, it would be of interest to evaluate the detailed molecular mechanisms of invasion and metastasis underlying EMT in EC. A bulk of surveys has established the involvement of Slug/Snail zinc-finger proteins in the tumor behaviors of malignant cells by means of suppression of the adhesive properties of epithelial cells [21-23]. Hence, Snail family proteins, particularly snail-1, are potential targets for the designing of therapeutic strategies.

Gene silencing vi RNA interference (RNAi) has been a rapidly growing concept as a personalized strategy in the treatment of malignancies. Small interfering RNAs (siRNAs) are effector molecules that have the potential silence important genes involved in the development of malignancies [24]. The currently available therapeutic strategies for EC therapy are hormonal therapy, surgery, chemotherapy, immunotherapy, radiation therapy, or a combination of these methods [25]. That notwithstanding, these strategies have not fully been successful in the therapy of EC patients. Therefore, in this study we hypothesized to evaluate the potential of Snail-1 silencing by siRNA in amelioration the invasive and metastatic features of EC *in vitro*.

Snail-1 expression has been established to be in immediate association with the metastatic feature of tumor cells. *In vitro* experiments have demonstrated that Snail is required for metastasis of tumor cells to the lymph node [26]. In addition, increased mRNA expression of Snail was reported in the metastatic lesions obtained from ovarian cancer [27]. As well, it was reported that the nuclear expression of Snail and Slug were positive in 16.9% and 3.7%, respectively, in EC tumors. Furthermore, nuclear expression of Snail, representing EMT, was significantly associated with myometrial invasion, FIGO stage, positive peritoneal cytology as well as survival of EC patients [28]. Studies have also indicated that Snail regulates the expression of vimentin and MMP-9 in the glioma cell lines [29]. In line with these reports, we contemplated that Snail-1 expression silencing can decrease the metastatic behaviors of EC cells. We observed that targeting Snail-1 in HEC-1A cells led to underexpression (MMP-9 and vimentin) and overexpression (E-cadherin) of EMT-related gens.

Although the Snail-1 expression level has been previously evaluated in EC [28], little studies have evaluated the impression of Snail-1 knockdown on the apoptosis and migration of EC tumor cells. However, it has previously been indicated that that that Snail-1 silencing led to promotion of apoptosis in tumor cells [13, 30, 31]. Here in the current study, transfection of Snail-1 specific siRNA transfection increased the apoptosis of HEC-1A cells.

Expression of vimentin and fibronectin as the mesenchymal markers is decreased during EMT, while expression of the epithelial markers like Mucin-1 and E-cadherin is increased. It has been reported that Snail knockdown resulted in the decreased expression of vimentin in the breast cancer cell lines [26]. Moreover, E-cadherin expression has been demonstrated to be reduced in EC that was associated with EMT [28]. Our experiments indicated that Snail-1 silencing resulted in upregulation of E-cadherin, alongside with downregulation of vimentin and MMP-9, in HEC-1A cells, which was associated with a marked decrease of cell migration and metastasis.

Protease enzymes participate in the development of EMT by means of degradation of extracellular matrix, facilitating the migration and metastasis of malignant cells [32]. MMPs have been reported to play a role in increasing the migration and metastasis of malignant cells [33-35]. Invasion of hepatoma cells has been established to be under the impression of Snail function by means of MMP upregulation [36]. Alternately, the generation of MMP-9 and vimentin was increased by Snail in the glioma cell lines [29]. Our experiments revealed that silencing of Snail-1 culminated in underexpression of metastasis-related gene MMP-9 and the number of migrated cells was decreased.

miR-34a has been implicated as a tumor suppressor that is involved in the pathogenesis of various malignancies [37, 38], as well as EC [39, 40]. miR-34a was reported to be downregulated in EC tissues and negatively correlated with Notch1 expression. Moreover, miR-34a suppressed the proliferation, migration, invasion, EMT-associated phenotypes by downregulating the Notch1 expression in EC cells. Furthermore, upregulation of miR-34a repressed the tumor growth in nude mice [41]. Furthermore, underexpression of miR-34a was reported in the esophageal squamous-cell carcinomas. However, upregulation of miR-34a culminated in the increased apoptosis of cancer cells, while downregulated the MMP-2 and MMP-9 expressions, which in turn, repressed invasive and migrative properties of cancer cells [38]. Here, our experiments revealed that transfection of HEC-1A cells by Snail-1 specific siRNA eventuated in upregulation of miR-34a and downregulation of MMP-9, conferring a decreased ability of HEC-1A cells to migrate.

Taking all the findings together, this was the first investigation, to our best knowledge, to evaluate the role of Snail-1 silencing by siRNA in impressing the EMT of EC HEC-1A cells. Snail-1 specific siRNA declined the expression of Snail-1 mRNA in EC HEC-1A cells. Moreover, the apoptosis rate of HEC-1A cells was increased. As well, migration potential of HEC-1A cells was decreased upon transfection, alongside with downregulation of MMP-9 and vimentin as well as upregulation of E-cadherin and miR-34a. As a result, silencing of Snail-1 by specific siRNA suggests a therapeutic strategy for EC therapy. That notwithstanding, arming with further data about the molecular regulation of EC by Snail-1 might contribute to the successful EC treatment.

4. Materials and Methods

4.1. Cell culture

The EC related HEC-1A cell line was chosen for the transfection that was maintained and cultured in the Roswell Park Memorial Institute (RPMI) 1640 culture medium (Sigma-Aldrich, St. Louis, MO, USA), which was supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, MO, USA), 1% penicillin/streptomycin antibiotics (Gibco Inc., Paisley, UK). The culture conditions were 37 °C temperature, 5% CO2, and 95% humidity. Every 24 hrs, the culture medium was exchanged, and passaging was performed when the confluency was approximately 80–90%.

4.2. siRNA transfection of HEC-1A cells

DNA oligonucleotides (siRNAs) targeting specifically the Snail-1 contain three different pooled siRNA duplexes sequences (first sequence, sense strand of 3'-GGACUUUGAUGAAGACCAUtt-5' and antisense strand of 3'-AUGGUCUUCAUCAAAGUCCtt-5'; second sequence, sense strand of 3'-CACGAGGUGUGACUAACUAtt-5' and antisense strand of 3'-UAGUUAGUCACACCUCGUGtt-5'; third sequence, sense strand of 3'-GCGAGCUGCAGGACUCUAAtt-5' and antisense strand of 3'-UUAGAGUCCUGCAGCUCGCtt-5'). In addition, the negative scrambled control siRNA (Santa Cruz Biotechnology, Inc) was also transfected to the control HEC-1A group. For transfection, 2×105 HEC-1A cells/well was cultured in 6-well plates. After 18 hrs, different doses of transfection reagent and siRNA was added into the cells with at least 70% confluency. Cell harvesting was conducted after 24, 48, and 72 hrs since transfection, and then RNA and protein content of cells were isolated.

4.3. Extraction of RNA, synthesis of cDNA

Using the Trizol (Qiagen, Germany), the total RNA was extracted from the HEC-1A cells, according to the manufacturer's user manual guidelines. A NanoDrop spectrophotometer (2000c, Thermo Fisher Scientific, USA) device was applied to examine the purity and integrity of isolated RNA. Afterwards, the reverse transcription of extracted RNAs was conducted to complementary DNA (cDNA) by TAKARA cDNA synthesis Kit (TAKARA, Japan), according to the manufacturer's user manual protocol. On the other side, to evaluate the transcript level of miR-34a, miScript II RT Kit (Cat no. 218161, Qiagen, Hilden, Germany) was used for reverse transcription of the extracted RNA to cDNA.

 doi:10.20944/preprints201907.0105.v1

252 *4.4. Quantitative Real-time-PCR*

253 The RealQ Plus Master Mix Green High ROX (AMPLIQON, Odense M, Denmark) and
254 StepOne Plus Real-time PCR device (Applied Biosystems, Foster City, CA, USA) were applied for
255 quantitative Real-time PCR detection of mRNA and miRNA expressions. For performing Real-time
256 PCR, the primers were obtained from Primer Bank (https://pga.mgh.harvard.edu/primerbank/; for
257 more details, see Table 1). Normalization of the transcript levels of the mRNA and miRNA was
258 performed to the expression level of corresponding housekeeping gene β-actin. The widely exerted
259 comparative C_T approach was used to calculate the relative expression levels of target genes using
260 the $2^{-\Delta\Delta CT}$ formula as suggested by Schmittgen and Livak [16].
261
262 *4.5. MTT assay*

263 To determine the proliferation or apoptosis of Snail-1 specific siRNA transfected HEC-1A cells,
264 the methyl-thiazol-tetrazolium (MTT) assay was carried out. HEC-1A cells (5×103) were seeded in
265 three replicates in the 96-well and 100 µl RPMI 1640 medium was added to each well. The
266 transfection of the HEC-1A cells was conducted on the cells as described above. After that, HEC-1A
267 cells from control and transfected groups were cultured for 24, 48, 72, and 96 hrs. Then 100 µl of
268 MTT reagent (Sigma, Germany) with the concentration of 0.0005 gr/ml in PBS was added to each
269 well and incubation of the plates was conducted for 4 hrs. After that, 100 µl of dimethyl sulfoxide
270 (DMSO) was added to each well to stop the formazan crystal production and incubation of plates
271 was conducted for 30 min on the shaker in the room temperature conditions. Finally, the optical
272 density (OD) of each well was measured by an ELISA reader (Tecan Spectra, Austria) device at 570
273 nm wavelength.
274
275 *4.6. Western blot analysis*

276 The RIPA buffer (25 mM Tris HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate,
277 0.1% SDS) was exerted to extract the protein from HEC-1A cells. After that, 100 µg of the extracted
278 total protein was electrophoresed on 12.5% Sodium dodecyl sulfate (SDS) polyacrylamide gel (SDS-
279 PAGE), proceeding by electroblotting on the Polyvinylidene fluoride (PVDF) membranes.
280 Afterwards, 3% bovine serum albumin (BSA) in TBST solution (1× Tris-Buffered Saline, 0.1%
281 Tween-20) was added to block the membranes at the room temperature conditions (overnight). The
282 primary Rabbit polyclonal antibodies were used for detection of protein levels of Snail-1 (1:500, sc-
283 28199, Santa Cruz Biotechnology) and β-actin (1:3000, monoclonal antibody, Abcam), as the
284 housekeeping protein. After that, the membrane was washed and then incubated with horseradish
285 peroxidase (HRP)-conjugated goat anti-rabbit secondary polyclonal antibody (1:3000, Santa Cruz
286 Biotechnology). To evaluate the protein levels, the electrochemiluminescence (ECL) kit (Roche
287 Diagnostics GmbH) was used. Finally, protein level quantification was conducted by the NIH
288 ImageJ 1.63 software.
289
290 *4.7. Wound healing assays*

291 The metastatic and migration potential of HEC-1A cells after transfection with Snail-1 specific
292 siRNA was determined through the wound healing assay. According to this assay, the ability of
293 transfected cells was evaluated via the filling the gap area. First, 10×105 HEC-1A cells per well were
294 seeded in 24-well plates. Subsequently, after reaching a 90% confluency, a scratch was created on
295 the plates across the cell monolayer via a sterile pipette tip to generate a linear gap region. After
296 that, the plate surface was washed with PBS to remove cell debris from the plates. Afterwards,
297 HEC-1A cells were transfected using 80 pmol Snail-1 specific siRNA. The experiments were
298 repeated in a triplicate order. Using light microscopy, an image of the plates was captured at 0, 24,
299 48, and 72 hrs after scratching. The number of migrated cells was calculated by the NIH ImageJ 1.63
300 software.
301
302 *4.8. Statistical analysis*

The statistical analysis of data and plotting of graphs was performed via GraphPad Prism v.7 software (GraphPad Software, La Jolla California USA). The non-parametric Kruskal–Wallis test was conducted to determine the differences between groups. The data were shown as mean ± standard deviation (SD) obtained from three independent experiments and *P* values less than 0.05 were regarded as statistically significant level.

Author Contributions: Conceptualization, J.J and Y.S.; methodology, Y.S.; software, Y.S.; validation, J.J., W.J. and S.Y.; formal analysis, J.J.; investigation, W.J.; resources, X.J.; data curation, W.J.; writing original draft, J.J.; writing review and editing, J.J.; visualization, Y.S.; supervision, X.J.; project administration, J.J.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

EMT	epithelial-to-mesenchymal transition
siRNA	small interference RNA
EC	Endometrial carcinoma
SNAI1	*Snail family transcriptional repressor 1*
MMP	Matrix metalloproteinase
MTT	Methyl-thiazol-tetrazolium
DMSO	Dimethyl sulfoxide
OD	Optical density
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel
PVDF	Polyvinylidene fluoride
BSA	Bovine serum albumin
HRP	Horseradish peroxidase

References

1. 1. Creasman WT, Odicino F, Maisonneuve P, Quinn MA, Beller U, Benedet JL, et al. Carcinoma of the corpus uteri. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet. 2006;95 Suppl 1:S105-43.
2. 2. Rauh-Hain JA, del Carmen MGJTo. Treatment for advanced and recurrent endometrial carcinoma: combined modalities. 2010;15(8):852-61.
3. 3. Thiery JP, Acloque H, Huang RY, Nieto MAJc. Epithelial-mesenchymal transitions in development and disease. 2009;139(5):871-90.
4. 4. Yang J, Weinberg RAJDc. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. 2008;14(6):818-29.
5. 5. Thiery JP, Sleeman JPJNrMcb. Complex networks orchestrate epithelial–mesenchymal transitions. 2006;7(2):131.
6. 6. Halbleib JM, Nelson WJJG, development. Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. 2006;20(23):3199-214.
7. 7. Peinado H, Olmeda D, Cano A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? Nature Reviews Cancer. 2007;7(6):415-28.
8. 8. Francí C, Gallen M, Alameda F, Baro T, Iglesias M, Virtanen I, et al. Snail1 protein in the stroma as a new putative prognosis marker for colon tumours. PLoS One. 2009;4(5):e5595.
9. 9. Kaufhold S, Bonavida B. Central role of Snail1 in the regulation of EMT and resistance in cancer: a target for therapeutic intervention. Journal of Experimental & Clinical Cancer Research. 2014;33(1):62.
10. 10. Taki M, Higashikawa K, Yoneda S, Ono S, Shigeishi H, Nagayama M, et al. Up-regulation of stromal cell-derived factor-1 alpha and its receptor CXCR4 expression accompanied with epithelial-mesenchymal transition in human oral squamous cell carcinoma. Oncology reports. 2008;19(4):993.
11. 11. Sun M, Guo X, Qian X, Wang H, Yang C, Brinkman KL, et al. Activation of the ATM-Snail pathway promotes breast cancer metastasis. Journal of molecular cell biology. 2012;4(5):304-15.

12. 12. Chen X-M, Huang Q-C, Yang S-L, Chu Y-L, Yan Y-H, Han L, et al. Role of micro RNAs in the pathogenesis of rheumatoid arthritis: novel perspectives based on review of the literature. Medicine. 2015;94(31):e1326.
13. 13. Musavi Shenas SMH, Mansoori B, Mohammadi A, Salehi S, Kaffash B, Talebi B, et al. SiRNA-mediated silencing of snail-1 induces apoptosis and alters micro RNA expression in human urinary bladder cancer cell line. Artificial Cells, Nanomedicine, and Biotechnology. 2016:1-6.
14. 14. Dong P, Xiong Y, Watari H, Hanley SJ, Konno Y, Ihira K, et al. MiR-137 and miR-34a directly target Snail and inhibit EMT, invasion and sphere-forming ability of ovarian cancer cells. Journal of Experimental & Clinical Cancer Research. 2016;35(1):132.
15. 15. Mansoori B, Mohammadi A, Shirjang S, Baghbani E, Baradaran B. Micro RNA 34a and Let-7a Expression in Human Breast Cancers is Associated with Apoptotic Expression Genes. Asian Pacific Journal of Cancer Prevention. 1887;17(4).
16. 16. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative CT method. Nature protocols. 2008;3(6):1101-8.
17. 17. Barrallo-Gimeno A, Nieto MA. The Snail genes as inducers of cell movement and survival: implications in development and cancer. Development. 2005;132(14):3151-61.
18. 18. Huber MA, Kraut N, Beug H. Molecular requirements for epithelial–mesenchymal transition during tumor progression. Current opinion in cell biology. 2005;17(5):548-58.
19. 19. Najafi-Hajivar S, Zakeri-Milani P, Mohammadi H, Niazi M, Soleymani-Goloujeh M, Baradaran B, et al. Overview on experimental models of interactions between nanoparticles and the immune system. Biomedicine & Pharmacotherapy. 2016;83:1365-78.
20. 20. Mani SA, Guo W, Liao M-J, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. 2008;133(4):704-15.
21. 21. Peinado H, Marin F, Cubillo E, Stark H-J, Fusenig N, Nieto MA, et al. Snail and E47 repressors of E-cadherin induce distinct invasive and angiogenic properties in vivo. Journal of cell science. 2004;117(13):2827-39.
22. 22. Olmeda D, Jorda M, Peinado H, Fabra A, Cano A. Snail silencing effectively suppresses tumour growth and invasiveness. Oncogene. 2007;26(13):1862-74.
23. 23. Usami Y, Satake S, Nakayama F, Matsumoto M, Ohnuma K, Komori T, et al. Snail-associated epithelial–mesenchymal transition promotes oesophageal squamous cell carcinoma motility and progression. The Journal of pathology. 2008;215(3):330-9.
24. 24. Ahmadzada T, Reid G, McKenzie DRJBr. Fundamentals of siRNA and miRNA therapeutics and a review of targeted nanoparticle delivery systems in breast cancer. 2018;10(1):69-86.
25. 25. Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, et al. Cancer treatment and survivorship statistics, 2016. 2016;66(4):271-89.
26. 26. Olmeda D, Moreno-Bueno G, Flores JM, Fabra A, Portillo F, Cano A. SNAI1 is required for tumor growth and lymph node metastasis of human breast carcinoma MDA-MB-231 cells. Cancer research. 2007;67(24):11721-31.
27. 27. Elloul S, Bukholt Elstrand M, Nesland JM, Tropé CG, Kvalheim G, Goldberg I, et al. Snail, Slug, and Smad-interacting protein 1 as novel parameters of disease aggressiveness in metastatic ovarian and breast carcinoma. Cancer. 2005;103(8):1631-43.
28. 28. Tanaka Y, Terai Y, Kawaguchi H, Fujiwara S, Yoo S, Tsunetoh S, et al. Prognostic impact of EMT (epithelial-mesenchymal-transition)-related protein expression in endometrial cancer. 2013;14(1):13-9.
29. 29. Mahabir R, Tanino M, Elmansuri A, Wang L, Kimura T, Itoh T, et al. Sustained elevation of Snail promotes glial-mesenchymal transition after irradiation in malignant glioma. Neuro-oncology. 2013:not239.
30. 30. Roy HK, Iversen P, Hart J, Liu Y, Koetsier JL, Kim Y, et al. Down-regulation of SNAIL suppresses MIN mouse tumorigenesis: modulation of apoptosis, proliferation, and fractal dimension. Molecular cancer therapeutics. 2004;3(9):1159-65.
31. 31. Vega S, Morales AV, Ocaña OH, Valdés F, Fabregat I, Nieto MA. Snail blocks the cell cycle and confers resistance to cell death. Genes & development. 2004;18(10):1131-43.
32. 32. Crowe DL, Tsang KJ, Shemirani B. Jun N-Terminal Kinase 1 Mediates Transcriptional Induction of Matrix Metal loproteinase 9 Expression. Neoplasia. 2001;3(1):27-32.

33. 33. Gruss C, Herlyn M. Role of cadherins and matrixins in melanoma. Current opinion in oncology. 2001;13(2):117-23.
34. 34. Sheu B-C, Hsu S-M, Ho H-N, Lien H-C, Huang S-C, Lin R-H. A novel role of metalloproteinase in cancer-mediated immunosuppression. Cancer research. 2001;61(1):237-42.
35. 35. Siahmansouri H, Somi MH, Babaloo Z, Baradaran B, Jadidi-Niaragh F, Atyabi F, et al. Effects of HMGA2 siRNA and doxorubicin dual delivery by chitosan nanoparticles on cytotoxicity and gene expression of HT-29 colorectal cancer cell line. Journal of Pharmacy and Pharmacology. 2016;68(9):1119-30.
36. 36. Miyoshi A, Kitajima Y, Kido S, Shimonishi T, Matsuyama S, Kitahara K, et al. Snail accelerates cancer invasion by upregulating MMP expression and is associated with poor prognosis of hepatocellular carcinoma. British journal of cancer. 2005;92(2):252-8.
37. 37. Hemmatzadeh M, Mohammadi H, Jadidi-Niaragh F, Asghari F, Yousefi M. The role of oncomirs in the pathogenesis and treatment of breast cancer. Biomedicine & Pharmacotherapy. 2016;78:129-39.
38. 38. Shi H, Zhou S, Liu J, Zhu J, Xue J, Gu L, et al. miR-34a inhibits the in vitro cell proliferation and migration in human esophageal cancer. Pathology-Research and Practice. 2016;212(5):444-9.
39. 39. Dong P, Xiong Y, Yue J, Hanley SJ, Watari HJO. miR-34a, miR-424 and miR-513 inhibit MMSET expression to repress endometrial cancer cell invasion and sphere formation. 2018;9(33):23253.
40. 40. Choi YS, Lee KEJJocp. The Significance of miR-34a Expression in Endometrial Carcinogenesis: Correlation With Expression of p16 and Ki-67 Proteins in Endometrial Cancers. 2015;20(4):268.
41. 41. Wang Z, Wang W, Huang K, Wang Y, Li J, Yang XJO. MicroRNA-34a inhibits cells proliferation and invasion by downregulating Notch1 in endometrial cancer. 2017;8(67):111258.
Title of Site. Available online: URL (accessed on Day Month Year).