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Regular paper

E-cadherin, Snail, ZEB-1, *DNMT1*, *DNMT3A* and *DNMT3B* expression in normal and breast cancer tissues*

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Objective: Breast cancer is known as one of very important cancers among females, given that a variety of external (i.e., environmental risk factors) and internal factors (i.e., genetics, and epigenetics) are related to the emergence and progression of breast cancer. Among genetic and epigenetic factors, DNA methyltransferase and EMT related genes have critical roles in breast cancer pathogenesis. In the study presented here, we investigated expression of DNA methyltransferases (e.g., *DNMT1*, *DNMT3A* and *DNMT3B*) and EMT-related genes (e.g., E-cadherin, Snail, ZEB-1). Methods and Materials: Tissue samples were collected from 18 cancer and 24 normal breast tissues. We evaluated the expression levels of DNA methyltransferases and EMT related genes using Quantitative real-time PCR (qRT-PCR). Results: Our results indicated that the expression levels of ZEB-1, Snail, and *DNMT3B* were increased in breast cancer subjects in comparison to the control group. On the other hand, there was a significant decrease in E-cadherin expression in breast cancer tissues in comparison to the normal tissues. Moreover, there were no significant changes for *DNMT1* and *DNMT3A* expression in breast cancer tissues when compared to the normal tissues. Conclusion: Taken together, our finding show that up regulation of ZEB-1 and Snail could be associated with down regulation of E-cadherin and results in promotion of cancer cell invasion. Moreover, down regulation of E-cadherin may be related to deterioration of *DNMT3B* inpatients with breast cancer.

Key words: breast cancer, DNA methyltransferases, E-cadherin, Snail, ZEB-1, DNMT

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Abbreviations: C_t, threshold cycle; EMT, epithelial-mesenchymal transition; E-cadherin, Snail, ZEB-1, EMT-related genes; *DNMT1*, *DNMT3a*, *DNMT3b*, DNA methyltransferases related genes; qRT-PCR, quantitative real-time PCR

INTRODUCTION

Breast cancer is one of the main cancers among women, given that more than 25 000 new breast cancer cases were diagnosed in the United States in 2017 (Waks & Winer, 2019). Increasing evidences indicate that breast cancer is a complex disease with a sequence of internal and external factors involved in its pathogenesis. Besides external factors, genetic and epigenetic factors are very important players in the initiation and progression of this cancer. Hence, deregulation of key genes has a central role in breast cancer pathogenesis (Mirzaei *et al.*, 2018; Dyrstad *et al.*, 2015; Brouckaert *et al.*, 2017; Tyrer *et al.*, 2004; Faghihloo *et al.*, 2014; Vaezjalali *et al.*, 2013). Several studies revealed that deregulation of metastasis-related genes is one of the major steps in the development of breast cancer (Kotiyal *et al.*, 2014). Metastasis is a vital biological process which helps cancer cells to migrate to new sites in the body. Epithelial-mesenchymal transition (EMT) is a conserved program which has critical function in carcinogenesis and emergence of metastatic properties of cancer cells *via* promoting invasion, mobility, and resistance to apoptotic stimuli. Furthermore, EMT-derived tumor cells acquire stem cell properties and exhibit a marked therapeutic resistance. Therefore, better understanding of EMT-related genes' expression could introduce new therapeutic platforms and inhibitors for metastasis (Mittal *et al.*, 2018). Multiple lines of evidence confirmed that various genes, such as E-cadherin, Snail, and ZEB-1 are known as EMT-related genes. It has been shown that deregulation of these genes is associated with metastasis and other cancer –related processes (Tavakolian *et al.*, 2019; Brabletz *et al.*, 2018). In a study, Chen *et al.*, indicated that expression of E-cadherin is associated with the metastasized lymph node (Chen *et al.*, 2015).

Besides genetic alterations, epigenetic modifications are important players in breast cancer pathogenesis. Methylations of different genes are associated with cancerous conditions. In this regard, DNA methyltransferases have a pivotal function in expression of various genes, such as cancer related genes (Mirzaei *et al.*, 2018; Karsli-Ceppioglu *et al.*, 2014).

Methylation of genes can induce a noticeable change in gene transcription and may have an effect on the chromosome structure; therefore, it can be related to activation, or silencing of oncogenes (Diego & Richard, 2014). Methyltransferases are a group of enzymes which have a regulatory role in methylation of cancer-related genes, such as E-cadherin. Given that there are several subtypes of these enzymes, *DNMT1*, *DNMT3B* and *DNMT3B* are the most common DNA methyltransferases which are conserved with the same amino acid sequence in mammals (Michalak & Visvader, 2016). These enzymes have an N-terminal domain designated for binding tonucleic acids, or nucleoproteins, and also a C-terminal domain which accounts for the methylation activity (Estève *et al.*, 2005). *DNMT1* is required during replication, and contribute in the methylation pattern procedure in daughter cells and their parents (Schermelleh *et al.*, 2007). *DNMT3A* and *DNMT3B* are expressed in germ cells during embryogenesis. There is a down-ex-

Table 1. Clinical characteristics of 18 cancer and 24 normal breast tissues.

Age >60	67%
Tumor localization	(Left: 45%) – (right: 55%)
Family history	(Absent: 88.1 %) – (Present: 11.9%)
Lymph node metastasis	(Negative: 48%) – (positive: 52%)
Tumor size	(>2cm : 62.8%) – (<2cm: 37.2%)
Tumor stage	(I-II : 45%) – (III-IV : 55%)
Estrogen receptor (ER) status	(Negative: 54.3%) – (positive: 45.7%)
Progesterone receptor (PR) status	(Negative: 46.6%) – (positive: 53.4%)

pression of *DNMT3A* and *DNMT3B* in differentiated cells (Chen *et al*., 2002; Okano *et al*., 1999). Deregulation of these genes is involved in breast cancer pathogenesis (Mirza *et al*., 2013), hence, assessment of these genes' expression could be used for monitoring breast cancer patients. Moreover, better understanding of behaviors of cancer-related genes could contribute to designing and developing new therapeutic platforms (Subramaniam *et al*., 2013).

In the study presented here, we investigated the expression of DNA methyltransferases (e.g., *DNMT1*, *DNMT3A* and *DNMT3B*) and EMT related genes (e.g., E-cadherin, Snail, ZEB-1).

MATERIALS AND METHODS

Sample information. This study was approved by the Shahid Beheshti University of Medical Sciences (IR.SBMU.MSP.REC.1397.552, Grant No14315). We collected 18 cancer and 24 normal breast tissues from Taleghany hospital in Tehran between 2017 and 2018. All tissues were stored in RNAlater solution (Qiagen GmbH, Hilden, Germany) at –20°C. Two pathologists have confirmed the stage of tissues. All sample information was recorded and is summarized in Table 1. The inclusive criteria in this study included having no history of chemotherapy or any kind of cancers.

RNA extraction. All tissues were digested with the use of a homogenizer and 1 ml RNX-plus solution (Cinnagen, Tehran, Iran). After adding chloroform, RNA was precipitated with isopropanol and washed with 70% ethanol. RNA was diluted with DEPC-treated water and its purity was evaluated with Nanodrop.

cDNA synthesis. RNA from cancer and normal tissues was converted into cDNA by combining 10 µl of reverse transcriptase (cDNA kit, BioFACT, Daejeon, South Korea) and 10 µl of RNA; the samples were first incubated at 95°C for 5 minutes, and cDNA was synthesized by incubation at 50°C for 40 minutes. The process was performed with thermo cycler (Bio Intellectica PCR). We then added 20 µl of sterile water to the samples and used them as template DNA.

Quantitative real-time PCR. Real-time PCR primers were used were: ZEB-1 F, ZEB-1 R, Snail 1-F, Snail 1R, E-cadherin-F, E-cadherin-R, *DNMT1* F, *DNMT1* R, *DNMT3A* F, *DNMT3A* R, *DNMT3B* F, *DNMT3B* R. GAPDH F and GAPDH R were used for control. All primer sequences are listed in Table 2.

Quantitative real-time PCR was performed in a final volume of 20 µl, with the use of Rotor-gene 6000 (Corbett life siences, Sydney,Australia), with 36-well Gene Discs. We used 10 µl of BIOFACT™ 2X real-time PCR master mix (for SYBR Green I; BIOFACT, South Korea), 1 µl of forward primer (10 pmol), 1 µl of reverse primer (10 pmol), 2 µl of 1/2 diluted cDNA and 6 µl of sterile water.All samples were run simultaneously in triplicate in order to confirm our results.

Table 2. Primers used for Real-time PCR

Gene name	Real-time PCR primer
ZEB-1-F	GATGATGAATGCGAGTCAGATGC
ZEB-1-R	CTGGTCCTCTTCAGGTGCC
Snail-1-R	CATAGTTAGTCACACCTCGT
Snail-1-F	CAGACCCACTCAGATGTCAA
E-cadherin-F	AGGGGTTAAGCACAACAGCA
E-cadherin-R	GGTATTGGGGGCATCAGCAT
DNMT1-F	CCTTGGAGAACGGTGCTCAT
DNMT1-R	CTTAGCCTCTCCATCGGACT
DNMT3A-F	CTTTTGCGTGGAGTGTGTGG
DNMT3A-R	CGGATGGGCTTCCTCTTCTC
DNMT3B-F	AGGAGTGTGAAGCAAGGAGC
DNMT3B-R	CCGAGCTTTGCAGTTTTCCC
GAPDH-F	ATGTTCGTCATGGGTGTGAA
GAPDH-R	GGTGCTAAGCAGTTGGTGGT

To analyze the level of gene expression, we compared all genes with GAPDH as an internal control at 95°C for 10 minutes; 40 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds.

To analyze genes expression, threshold cycle (C_t) was measured. The C_t shows the cycle number. To normalize data, all gene threshold cycles were compared with C_t of the housekeeping gene (GAPDH) by the $2^{-\Delta\Delta ct}$ method.

Statistical analysis. Graph-Pad Prism software and ANOVA test were used for analyzing all data. The unpaired, two-tailed student's *t*-test was done to analyze the statistical differences between groups using Graph-Pad Prism software. *P*-value less than <0.05 ($P<0.05$) was taken as a statistically significant difference.

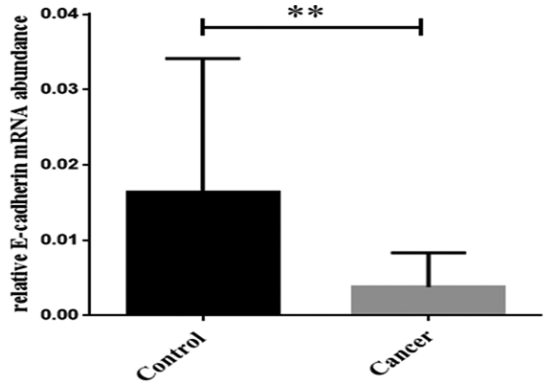

Figure 1. Evaluating the mRNA expression level of E-cadherin in normal and breast cancer tissue.
The results of RT-PCR analysis indicated that there was a decrease in E-cadherin expression in breast cancer tissues when compared to the normal ones. Values are given as mean ± standard deviation of three independent experiments. **$P<0.01$ revealed significant changes in comparison to normal breast tissues.

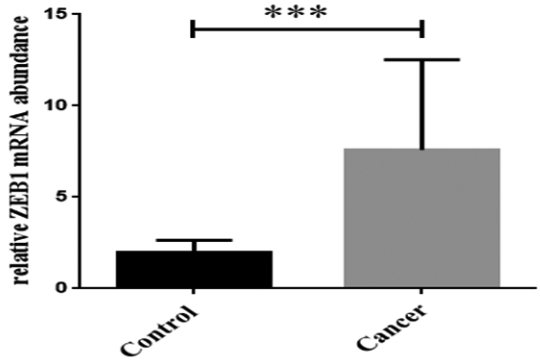

Figure 2. ZEB-1 mRNA expression in the breast cancer tissues compared to normal ones.
The expression level of ZEB-1 had increased significantly in breast cancer tissue. Values are given as mean ± standard deviation of three independent experiments. ***P<0.001 represents significant changes in comparison to normal breast tissues.

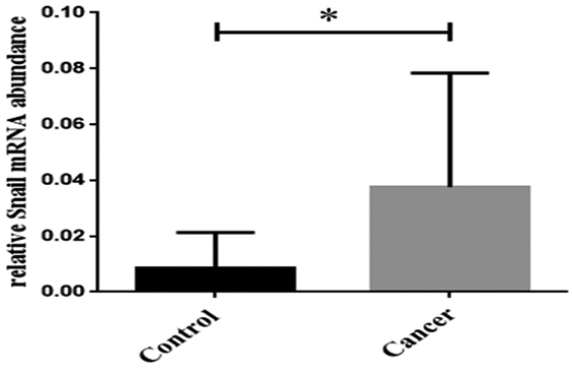

Figure 3. Snail mRNA expression in the breast cancer tissues compared to normal ones.
In this study, there was an up-regulation in Snail expression in the breast cancer tissues in comparison to the normal ones (*P<0.05).

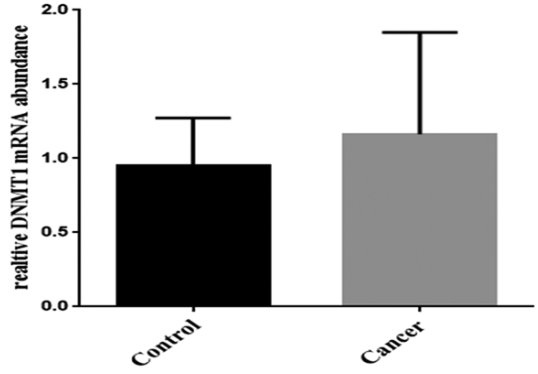

Figure 4. *DNMT1* mRNA expression in the breast cancer tissue.
DNMT1 expression was elevated in the breast cancer tissues in comparison to normal ones, however, not statistically meaningful.

RESULTS

Decrease in E-cadherin expression

One of the EMT genes in our study was E-cadherin which is involved in adhesion between various cells. Our results indicated that there was a significant down regulation of E-cadherin expression in the breast cancer tissue when compared to the normal one (**P<0.01; Fig. 1).

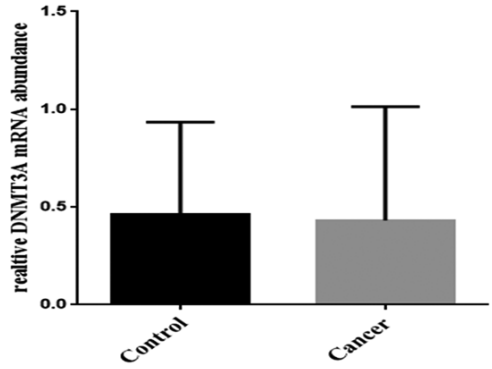

Figure 5. There was no significant difference between the expression level of *DNMT3A* in the tissue samples collected from breast cancer patients and the control ones.
Values are given as mean ±S.D. of three independent experiments.

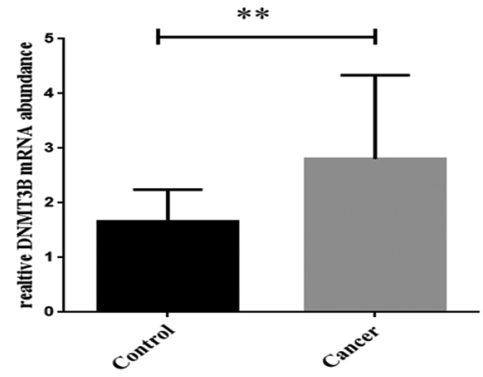

Figure 6. Investigation of the expression level of *DNMT3B* in the breast cancer tissues and the normal ones.
There was a meaningful increase in DNMT3B expression in breast cancer tissues when compared to the normal ones (**P<0.01).

Expression ZEB-1 and Snail

Our results indicated that there was a significant up regulation of ZEB-1 and Snail expression in the cancer tissues when compared to the normal tissues (***P<0.001 and *P<0.05, respectively; Fig. 2, and Fig. 3).

DNMT1, *DNMT3A*, and *DNMT3B* expression in breast tissues

Our data indicated that there was no significant change in gene expression of *DNMT1* and *DNMT3A* (Fig. 4, and Fig. 5). On the other hand, our data indicated that there was a significant up regulation of *DNMT3B* in the breast cancer tissue when compared to the normal tissues (**P<0.01; Fig. 6).

DISCUSSION

Metastasis and the related processes have crucial roles in the progression of breast cancer. In this regard, several studies indicated that a variety of genes are known as metastasis-related genes. For example, EMT-related genes are key players in the invasion properties of breast cancer cells and their deregulation is associated with metastatic properties of breast cancer cells (Faghihloo *et al.*, 2014; Huang *et al.*, 2015). EMT-related proteins play part in adhesion between cells, and without them, cells display metastatic properties. Snail, ZEB-1 and E-cadherin

are the most important genes of this group that could be associated with invasion properties of cells (Faghihloo *et al.*, 2016; Montserrat *et al.*, 2011).

Our results indicated that there was a significant down regulation in E-cadherin expression in the breast cancer tissue when compared to the normal tissue. Moreover, there was a significant up-regulation of ZEB-1 and Snail expression in the cancer tissues when compared to the normal tissues. Many studies have investigated EMT-related genes, such as E-cadherin. Singhai and others (Singhai *et al.*, 2011) had found that E-cadherin can be down-regulated in the breast invasive cancer tissues. Another study had suggested that down-regulation of E-cadherin could be used as as a tumor marker in breast cancer tissues (Younis *et al.*, 2007), while Horne and colleagues (Horne *et al.*, 2018) had demonstrated that down-regulation of E-cadherin is one of the hallmarks of breast cancer tissues. They had shown that E-cadherin in breast cancerous cells is not stable, and there is a down-regulation of this gene at the invasive stage of cancer (Fulga *et al.*, 2015). Yet another study had documented that the tumor cells are able to accumulate all E-cadherin in their cytoplasm, but it is not expressed at the cell membranes (Kowalski *et al.*, 2003). In fact, E-cadherin is one of the most important factors in breast cancer progression (Berx *et al.*, 2001). Also, E-cadherin level could be correlated with a histological type of breast cancer. However, Qureshi and others (Qureshi *et al.*, 2006) has shown that it is not useful as a prognostic biomarker, while Kim and Sahin (Kim & Sahin, 2005) revealed that down regulation of E-cadherin is associated with metastasis.

ZEB-1 is another EMT-related gene which acts as a tumor suppressor and its expression is related to inhibition of cancerous conditions (Zhang *et al.*, 2018). One of the oncogenes, named Ribonucleic acid export 1 (RAE1), is over-expressed in breast cancer due to changes in the level of ZEB-1 (Oh *et al.*, 2019). Yu and others (Yu *et al.*, 2018) documented that the hTERT promoter is stimulated by ZEB-1, leading to triggering of a sequence of breast cancer-related signaling pathways. Another mechanism contributing to breast cancer may involve stimulation of the androgen receptor by ZEB-1 (Graham *et al.*, 2010). Since PTBP3 tends to regulate ZEB-1 in breast cancer, it can be used as a suitable target in cancer therapy (Hou *et al.*, 2018). However, ZEB-1 is able to increase expression of VEGF in breast cancer tissues, and stimulate growth of breast cancer tumor (Liu *et al.*, 2016). Soini and others also found that ZEB-1 is up-regulated in breast cancer (Soini *et al.*, 2011). It is also known that ZEB-1 may be targeted by neurogenin-3, which results in breast cancer progression (Zhou *et al.*, 2017). Moreover, ZEB-1 may be one of the causes of E-cadherin expression repression (Singh *et al.*, 2011; Sánchez-Tilló *et al.*, 2010).

Down-regulation of Snail can reduce breast cancer cell motility. In fact, in breast cancer, Snail increases the RhoA GTPase expression and is associated with initiation of breast cancer (Zhang *et al.*, 2013). Activation of nuclear ERK2 can be achieved by Snail, which is also related to breast cancer (Smith *et al.*, 2014). Ganesan, *et al.* found that the damage of Slug and Snail involved in the activation of phospholipase D (PLD) promoter; therefore, breast cancer progression is stimulated (Ganesan *et al.*, 2016). Lundgren and others (Lundgren *et al.*, 2009) indicated that hypoxia can partially induce Snail expression in the breast cancer tissues. The over-expression of Snail tends to repress p53 at the posttranslational level in breast cancer tissues (Ferrarelli *et al.*, 2016). Also Burton and others (Burton *et al.*, 2015) had demonstrated that the complex of Snail-Cathepsin L can induce breast cancer progression.

Interestingly, it seems that another mechanism of E-cadherin expression repression is related to the Snail expression, which has a potential to reduce E-cadherin expression (Cano *et al.*, 2000).

Our data indicated that there was no significant change in expression of *DNMT1* and *DNMT3A* in the breast cancer tissues. However, we revealed that there was a significant up regulation in *DNMT3B* expression.

Sun and others (Sun *et al.*, 2012) had demonstrated that there is a strong relationship between the heterozygous genotypes of rs16999593 in *DNMT1*, rs2424908 in *DNMT3B* and breast cancer. Probably, in breast cancer tissues, there are some oncogenes, especially MUC1-C, which up-regulate the *DNMT1* and *DNMT3B* expression, and are associated with breast cancer progression (Rajabi *et al.*, 2016). Also, *DNMT1* upregulation may lead to methylation of CpG islands in ERα, and increase cancer progression; thus, it may be possible to diagnose breast cancer by detecting expression of *DNMT1* (Zhang *et al.*, 2016). Shin and co-authors confirmed that there is an extensive effect of *DNMT1* in breast cancer (Shin *et al.*, 2016). Nevertheless, Tang and others (Tang *et al.*, 2014) had shown that the mechanism which contributes to breast cancer may be the effect of miR-185 on E2F6, *DNMT1* and BRCA1. Moreover, *DNMT3B* and *DNMT3B* can reduce the level of E-cadherin expression (Chen *et al.*, 2016).

CONCLUSION

In this study, we found Snail and ZEB-1 to be up-regulated, and there is a decrease in E-cadherin expression in the breast cancer tissue. Taken together, our findings indicate that Snail and ZEB-1 can act as inhibitors of E-cadherin; therefore, they can induce tumor cell metastasis in breast cancer. Furthermore, *DNMT3B* has some effects on this type of cancer. It may be possible that *DNMT3A* and *DNMT3B* can repress E-cadherin expression by methylation of the E-cadherin promoter; therefore, it seems that more assessments for finding the relationship between expression of E-cadherin and *DNMT3A* and *DNMT3B* are needed.

Conflict of Interest

The authors declare no conflicts of interests.

Authors' Contributions

E.F., SH.T, contributed in the study design and performed cell culture and molecular experiments. H.G. performed statistical analyses. All authors read and approved the final version of the manuscript.

REFERENCES

Berx G, Van Roy F (2001) The E-cadherin/catenin complex: an important gatekeeper in breast cancer tumorigenesis and malignant progression. *Breast Cancer Res* **3**: 289–293. https://doi.org/10.1186/bcr309

Karsli-Ceppioglu S, Dagdemir A, Judes G, Ngollo M, Penault-Llorca F, Pajon A, Bignon YJ, Bernard-Gallon D (2014) Epigenetic mechanisms of breast cancer: an update of the current knowledge. *Epigenomics* **6**: 651–664. https://doi.org/10.2217/epi.14.59.

Brabletz T, Kalluri R, Nieto MA, Weinberg RA (2018) EMT in cancer. *Nat Rev Cancer* **18**: 128–134. https://doi.org/10.1038/nrc.2017.118

Brouckaert O, Rudolph A, Laenen A, Keeman R, Bolla MK, Wang Q, Soubry A, Wildiers H, Andrulis IL, Arndt V, Beckmann MW, Ben-

itez J, Blomqvist C, Bojesen SE, Brauch H, Brennan P, Brenner H, Chenevix-Trench G, Choi JY, Cornelissen S, Couch FJ26, Cox A7, Cross SS, Czene K, Eriksson M, Fasching PA, Figueroa J, Flyger H, Giles GG, González-Neira A, Guénel P, Hall P, Hollestelle A, Hopper JL, Ito H, Jones M, Kang D; kConFab, Knight JA, Kosma VM4, Li J, Lindblom A, Lilyquist J, Lophatananon A, Mannermaa A, Manoukian S, Margolin S, Matsuo K, Muir K, Nevanlinna H, Peterlongo P, Pylkäs K, Saajrang S, Seynaeve C, Shen CY, Shu XO, Southey MC, Swerdlow A, Teo SH, Tollenaar RAEM, Truong T, Tseng CC, van den Broek AJ, van Deurzen CHM, Winqvist R, Wu AH, Yip CH, Yu JC, Zheng W, Milne RL5, Pharoah PDP, Easton DF, Schmidt MK, Garcia-Closas M, Chang-Claude J, Lambrechts D, Neven P (2017) Reproductive profiles and risk of breast cancer subtypes: a multi-center case-only study. *Breast Cancer Res* **19**: 119. https://doi.org/10.1186/s13058-017-0909-3.

Burton LJ, Smith BA, Smith BN, Loyd Q, Nagappan P, McKeithen D, Wilder CL, Platt MO2, Hudson T3, Odero-Marah VA (2015) Snail-Cathepsin L signaling in human breast and prostate cancers. *Carcinogenesis* **36**: 1019–1027. https://doi.org/10.1093/carcin/bgv084

Cano A, Perez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, Portillo F, Nieto MA (2000) The transcription factor Snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. *Nat Cell Biol* **2**: 76–83. https://doi.org/10.1038/35000025

Chen L, Jian W, Lu L, Zheng L, Yu Z, Zhou D (2015) Elevated expression of E-cadherin in primary breast cancer and its corresponding metastatic lymph node. *Int J Clin Exp Med* **8**: 11752–11758.

Chen LH, Hsu WL, Tseng YJ, Liu DW, Weng CF (2016) Involvement of DNMT 3B promotes epithelial-mesenchymal transition and gene expression profile of invasive head and neck squamous cell carcinomas cell lines. *BMC Cancer* **16**: 431. https://doi.org/10.1186/s12885-016-2468-x

Chen T, Ueda Y, Xie S, Li E (2002) A novel Dnmt3a isoform produced from an alternative promoter localizes to euchromatin and its expression correlates with active de novo methylation. *J Biol Chem* **277**: 38746–38754. https://doi.org/10.1074/jbc.M205312200

Marzese DM, Scolyer RA, Huynh JL, Huang SK, Hirose H, Chong KK, Kiyohara E, Wang J, Kawas NP, Donovan NC, Hata K, Wilmott JS, Murali R, Buckland ME, Shivalingam B, Thompson JF, Morton DL, Kelly DF, Hoon DS (2014) Epigenome-wide DNA methylation landscape of melanoma progression to brain metastasis reveals aberrations on homeobox D cluster associated with prognosis. *Hum Mol Genet* **23**: 226–238. https://doi.org/10.1093/hmg/ddt420

Dyrstad SW, Yan Y, Fowler AM, Colditz GA (2015) Breast cancer risk associated with benign breast disease: systematic review and meta-analysis. *Breast Cancer Res Treat* **149**: 569–575. https://doi.org/10.1007/s10549-014-3254-6

Estève PO, Patnaik D, Chin HG, Benner J, Teitell MA, Pradhan S (2005) Functional analysis of the N- and C-terminus of mammalian G9a histone H3 methyltransferase. *Nucleic Acids Res* **6**: 3211–3223. https://doi.org/10.1093/nar/gki635.

Faghihloo E, Yavarian J, Jandaghi NZ, Shadab A, Azad TM (2014) Genotype circulation pattern of human respiratory syncytial virus in Iran. *Infect Genet Evol* **22**: 130–133. https://doi.org/10.1016/j.meegid.2014.01.009

Faghihloo E, Sadeghizadeh M, Shahmahmoodi S, Mokhtari-Azad T (2016) Cdc6 expression is induced by HPV16 E6 and E7 oncogenes and represses E-cadherin expression. *Cancer Gene Therapy*. https://doi.org/10.1038/cgt.2016.51

Faghihloo, E, Saremi MR, Mahabadi M, Akbari H, Saberfar E (2014) Prevalence and characteristics of Epstein-Barr virus-associated gastric cancer in Iran. *Arch Iran Med* **17**: 767–770. https://doi.org/0141711/AIM.0010

Ferrarelli L (2016) Snail and HDAC1 target p53 for degradation. *Sci Signal* 9: ec261.

Fulga V, Rudico L, Balica AR, Cimpean AM, Saptefrati L, Margan MM, Raica M (2015) Differential expression of E-cadherin in primary breast cancer and corresponding lymph node metastases. *Anticancer Res* **35**: 759–765. PMID: 25667455

Ganesan R, Mallets E, Gomez-Cambronero J (2016) The transcription factors Slug (SNAI2) and Snail (SNAI1) regulate phospholipase D (PLD) promoter in opposite ways towards cancer cell invasion. *Mol Oncol* **10**: 663–676. https://doi.org/10.1016/j.molonc.2015.12.006

Graham TR, Yacoub R, Taliaferro-Smith L, Osunkoya AO, Odero-Marah VA, Liu T, Kimbro KS, Sharma D, O'Regan RM (2010) Reciprocal regulation of ZEB1 and AR in triple negative breast cancer cells. *Breast Cancer Res Treat* **123**: 139–147. https://doi.org/10.1007/s10549-009-0623-7

Horne HN, Oh H, Sherman ME, Palakal M, Hewitt SM, Schmidt MK, Milne RL, Hardisson D, Benitez J, Blomqvist C, Bolla MK, Brenner H, Chang-Claude J, Cora R, Couch FJ, Cuk K, Devilee P, Easton DF, Eccles DM, Eilber U, Hartikainen JM, Heikkilä P, Holleczek B, Hooning MJ, Jones M, Keeman R, Mannermaa A, Martens JWM, Muranen TA, Nevanlinna H, Olson JE, Orr N, Perez JIA, Pharoah PDP, Ruddy KJ, Saum KU, Schoemaker MJ, Seynaeve C, Sironen R, Smit VTHBM, Swerdlow AJ, Tengström M, Thomas AS, Timmermans AM, Tollenaar RAEM, Troester MA, van Asperen CJ, van Deurzen CHM, Van Leeuwen FF, Van't Veer LJ, García-Closas M, Figueroa JD (2018) E-cadherin breast tumor expression, risk factors and survival: Pooled analysis of 5,933 cases from 12 studies in the Breast Cancer Association Consortium. *Sci Rep* **26**: 6574. https://doi.org/10.1038/s41598-018-23733-4

Hou P, Li L, Chen F, Chen Y, Liu H, Li J, Bai J, Zheng J (2018) PTBP3-mediated regulation of ZEB-1 mRNA stability promotes epithelial-mesenchymal transition in breast cancer. *Cancer Res* **78**: 387–398. doi: https://doi.org/10.1158/0008-5472.CAN-17-0883

Huang J, Li H, Ren G (2015) Epithelial-mesenchymal transition and drug resistance in breast cancer (Review). *Int J Oncol* **47**: 840–848.

Kim E, Sahin A (2005) E-cadherin expression loss in T1 invasive ductal carcinoma of the breast as a predictive marker for lymph node metastasis. *Korean J Pathol* **39**: 187–191.

Kotiyal S, Bhattacharya S (2014) Breast cancer stem cells, EMT and therapeutic targets. *Biochem Biophys Res Commun* **10**: 112–116. https://doi.org/10.1016/j.bbrc.2014.09.069

Kowalski PJ, Rubin MA, Kleer CG (2003) E-cadherin expression in primary carcinomas of the breast and its distant metastases. *Breast Cancer Res* **5**: R217–R222. https://doi.org/10.1186/bcr651

Liu L, Tong Q, Liu S, Cui J, Zhang Q, Sun W, Yang S (2016) ZEB-1 upregulates VEGF expression and stimulates angiogenesis in breast cancer. *PLoS One* **11**: e0148774. https://doi.org/10.1371/journal.pone.0148774

Lundgren K, Nordenskjöld B, Landberg G (2009) Hypoxia, Snail and incomplete epithelial-mesenchymal transition in breast cancer. *Br J Cancer* **101**: 1769–1781. https://doi.org/10.1038/sj.bjc.6605369

Michalak EM, Visvader JE (2016) Dysregulation of histone methyltransferases in breast cancer – Opportunities for new targeted therapies? *Mol Oncol* **10**: 1497–1515. https://doi.org/10.1016/j.molonc.2016.09.003

Mirza S, Sharma G, Parshad R, Gupta SD, Pandya P, Ralhan R (2013) Expression of DNA methyltransferases in breast cancer patients and to analyze the effect of natural compounds on DNA methyltransferases and associated proteins. *J Breast Cancer* **16**: 23–31. https://doi.org/10.4048/jbc.2013.16.1.23

Mirzaei H, Goudarzi H, Eslami G, Faghihloo E (2018) Role of viruses in gastrointestinal cancer. *J Cell Physiol* **233**: 4000–4014. https://doi.org/10.1002/jcp.26194

Mirzaei H, Faghihloo E (2018) Viruses as key modulators of the TGF-β pathway; a double-edged sword involved in cancer. *Rev Med Virol* **28**: e1967. https://doi.org/10.1002/rmv.1967

Mittal V (2018) Epithelial mesenchymal transition in tumor metastasis. *Annu Rev Pathol* **13**: 395–412. https://doi.org/10.1146/annurev-pathol-020117-043854

Montserrat N, Gallardo A, Escuin D, Catasus L, Prat J, Gutiérrez-Avignó FJ, Peiró G, Barnadas A, Lerma E (2011) Repression of E-cadherin by Snail, ZEB-1, and TWIST in invasive ductal carcinomas of the breast: a cooperative effort? *Hum Pathol* **42**: 103–110. https://doi.org/10.1016/j.humpath.2010.05.019

Oh JH, Lee JY, Yu S, Cho Y, Hur S, Nam KT, Kim MH (2019) RAE1 mediated ZEB-1 expression promotes epithelial-mesenchymal transition in breast cancer. *Sci Rep* **9**: 2977.

Okano M, Bell DW, Haber DA, Li E (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for *de novo* methylation and mammalian development. *Cell* **99**: 247–257. https://doi.org/10.1016/s0092-8674(00)81656-6

Qureshi HS, Linden MD, Divine G, Raju UB (2006) E-cadherin status in breast cancer correlates with histologic type but does not correlate with established prognostic parameters. *Am J Clin Pathol* **125**: 377–385. PMID: 16613340

Rajabi H, Tagde A, Alam M, Bouillez A, Pitroda S, Suzuki Y, Kufe D (2016) DNA methylation by DNMT-1 and DNMT3b methyltransferases is driven by the MUC1-C oncoprotein in human carcinoma cells. *Oncogene* **35**: 6439–6445

Sánchez-Tilló E, Lázaro A, Torrent R, Cuatrecasas M, Vaquero EC, Castells A, Engel P, Postigo A (2010) ZEB-1 represses E-cadherin and induces an EMT by recruiting the SWI/SNF chromatin-remodeling protein BRG1. *Oncogene* **29**: 3490–3500. https://doi.org/10.1038/onc.2010.102

Schermelleh L, Haemmer A, Spada F, Rösing N, Meilinger D, Rothbauer U, Cardoso MC, Leonhardt H (2007) Dynamics of DNMT-1 interaction with the replication machinery and its role in postreplicative maintenance of DNA methylation. *Nucleic Acids Res* **35**: 4301–4312. https://doi.org/10.1093/nar/gkm432

Shin E, Lee Y, Koo JS (2016) Differential expression of the epigenetic methylation-related protein DNMT-1 by breast cancer molecular subtype and stromal histology. *J Transl Med* **14**: 87. https://doi.org/10.1186/s12967-016-0840-x

Singh AB, Sharma A, Smith JJ, Krishnan M, Chen X, Eschrich S, Washington MK, Yeatman TJ, Beauchamp RD, Dhawan P (2011) Claudin-1 up-regulates the repressor ZEB-1 to inhibit E-cadherin expression in colon cancer cells. *Gastroenterology* **141**: 2140–2153. https://doi.org/10.1053/j.gastro.2011.08.038

Singhai R, Patil VW, Jaiswal SR, Patil SD, Tayade MB, Patil AV (2011) E-cadherin as a diagnostic biomarker in breast cancer. *N Am J Med Sci* **3**: 227–233

Smith BN, Burton LJ, Henderson V, Randle DD, Morton DJ, Smith BA, Taliaferro-Smith L, Nagappan P, Yates C, Zayzafoon M, Chung LW, Odero-Marah VA (2014) Snail promotes epithelial mesenchymal transition in breast cancer cells in part *via* activation of nuclear ERK2. *PLoS One* **9**: e104987. https://doi.org/10.1371/journal.pone.0104987

Soini Y, Tuhkanen H, Sironen R, Virtanen I, Kataja V, Auvinen P, Mannermaa A, Kosma VM (2011) Transcription factors ZEB-1, twist and snai1 in breast carcinoma. *BMC Cancer* **11**: 73. https://doi.org/10.1186/1471-2407-11-73

Subramaniam D, Thombre R, Dhar A, Anant S (2014) DNA methyltransferases: a novel target for prevention and therapy. *Front Oncol* **4**: 80. https://doi.org/10.3389/fonc.2014.00080

Sun MY, Yang XX, Xu WW, Yao GY, Pan HZ, Li M (2012) Association of DNMT-1 and DNMT3B polymorphisms with breast cancer risk in Han Chinese women from South China. *Genet Mol Res* **11**: 4330–4341

Tang H, Liu P, Yang L, Xie X, Ye F, Wu M, Liu X, Chen B, Zhang L, Xie X (2014) miR-185 suppresses tumor proliferation by directly targeting E2F6 and DNMT-1 and indirectly upregulating BRCA1 in triple-negative breast cancer. *Mol Cancer Ther* **13**: 3185–3197. https://doi.org/10.1158/1535-7163.MCT-14-0243

Tavakolian S, Goudarzi H, Eslami G, Faghihloo E (2019) Transcriptional regulation of epithelial to mesenchymal transition related genes by lipopolysaccharide in human cervical cancer cell line HeLa. *Asian Pac J Cancer Prev* **20**: 2455–2461. https://doi.org/10.31557/APJCP.2019.20.8.2455

Tyrer J, Duffy SW, Cuzick J (2004) A breast cancer prediction model incorporating familial and personal risk factors. *Stat Med* **23**: 1111–1130

Vaezjalali M, Rashidpour S, Rezaee H, Hajibeigi B, Zeidi M, Gachkar L, Aghamohamad S, Najafi R, Goudarzi H (2013) Hepatitis B viral DNA among HBs antigen negative healthy blood donors. *Hepat Mon* 13: e6590. https://doi.org/10.5812/hepatmon.6590

Waks AG, Winer EP (2019) Breast Cancer Treatment: A Review. *JAMA* **321**: 288–300. https://doi.org/10.1001/jama.2018.19323

Younis LK, El Sakka H, Haque I (2007) The prognostic value of E-cadherin expression in breast cancer. *Int J Health Sci (Qassim)* **1**: 43–51. PMCID: PMC3068666

Yu P, Shen X, Yang W, Zhang Y, Liu C, Huang T (2018) ZEB-1 stimulates breast cancer growth by up-regulating hTERT expression. *Biochem Biophys Res Commun* **495**: 2505–2511. https://doi.org/10.1016/j.bbrc.2017.12.139

Zhang A, Wang Q, Han Z, Hu W, Xi L, Gao Q, Wang S, Zhou J, Xu G, Meng L, Chen G, Ma D (2013) Reduced expression of Snail decreases breast cancer cell motility by downregulating the expression and inhibiting the activity of RhoA GTPase. *Oncol Lett* **6**: 339–346. https://doi.org/10.3892/ol.2013.1385

Zhang W, Chang Zh, Shi K, Song L, Cui L, Ma Zh, Li X, Ma W, Wang L (2016) The correlation between DNMT-1 and ERα expression and the methylation status of ERα, and its clinical significance in breast cancer. *Oncology* **11**: 1995–2000. https://doi.org/10.3892/ol.2016.4193

Zhang X, Zhang Z, Zhang Q, Zhang Q, Sun P, Xiang R, Ren G, Yang S (2018) ZEB-1 confers chemotherapeutic resistance to breast cancer by activating ATM. *Cell Death Dis* **9**: 57. https://doi.org/10.1038/s41419-017-0087-3

Zhou Ch, Jiang H, Zhang Zh, Zhang G, Wang H, Zhang Q, Sun P, Xiang R, Yang S (2017) ZEB-1 confers stem cell-like properties in breast cancer by targeting neurogenin-3. *Oncotarget* **8**: 54388–54401. https://doi.org/10.18632/oncotarget.17077