RESEARCH ARTICLE

# *BMI1* and *TWIST1* Downregulated mRNA Expression in Basal Cell Carcinoma

**Fatemeh Vand Rajabpour[1,2], Reza Raoofian[2,3], Leila Youssefian[2], Hassan Vahidnezhad[2], Mostafa Mirshams Shahshahani[4], Hamidreza Fathi[4], Pedram Noormohammadpour[4], Kambiz Kamyab Hesari[4], Morteza Hashemzadeh-Chaleshtori[1], Mina Tabrizi[2]***

## Abstract

**Background: *BMI1*, *TWIST1* and *SNAI2/SLUG* have been implicated in aggressive behavior of squamous cell carcinoma (SCC) and melanoma and *BMI1* expression could identify subtypes of Merkel cell carcinoma (MCC). However, *BMI1*, *TWIST1* and *SNAI2* expression levels in basal cell carcinomas (BCCs) have not been elucidated. We hypothesized BCC could be a good model system to decipher mechanisms which inhibit processes that drive tumor metastasis. The aim of this study was to examine the mRNA expression level of *BMI1*, *TWIST1*, and *SNAI2* in BCCs. Materials and Methods: Thirty-five fresh non-metastatic BCC tissue samples and seven fresh normal skin tissue samples were evaluated by real-time RT-PCR. Results: *BMI1* and *TWIST1* demonstrated marked down-regulation (p<0.00l, p=0.00l respectively), but *SNAI2* showed no significant change (p=0.12). Conclusions: Previous literature has clearly demonstrated a positive association between *BMI1* and *TWIST1* expression and metastatic BCC, aggressive SCC and melanoma. Here, we demonstrated a negative association between *BMI1* and *TWIST1* mRNA expression level and BCC.**

**Keywords:** *BMI1* - *TWIST1* - *SNAI2/SLUG* - basal cell carcinoma - epithelial mesenchymal transition - metastasis

*Asian Pac J Cancer Prev,* **15 (8)**, 3797-3800

## Introduction

Basal Cell Carcinoma is the most common type of skin cancer and probably one of the most common cancers in the world. BCC is well known for its slow growing feature within the epidermis of skin (Moad et al., 2012). BCC is locally invasive and rarely metastatic with a high rate of recurrence. Why does BCC rarely metastasize in comparison with other skin cancers such as Melanoma and Squamous Cell Carcinoma (SCC)? The Hedgehog (Hh) pathway has been proven to be involved in pathogenesis of BCC, but this pathway cannot solely explain metastatic inefficiency and restriction to local invasion (Schwartz and Pirrotta, 2007). Many studies have been conducted on molecular causes of cancer metastasis and have revealed certain transcription factors which act in Epithelial Mesenchymal Transition (EMT) pathway such as *TWIST1*, *SNAIL* and *SNAI2* (Zhu et al., 2013). In EMT, epithelial cells undergo transition into mesenchymal phenotype and are believed to play a critical role in aggressiveness and metastasis (Zhu et al., 2013). Most EMT related molecules show up-regulation in metastatic cancer and have been investigated especially in SCC (Mikheeva et al., 2010; Guo et al., 2012; Yang et al., 2010; Reinisch et al., 2007; Mihic et al., 2007; Gao et al., 2013; Wang et al., 2013)). Since BCC rarely metastasizes, it can be a proper model to study the inhibitory mechanisms of metastasis. Therefore, we decided to test the expression of transcription factors which have been demonstrated to have a major role in aggressiveness of other skin cancers.

*SNAI2* and *TWIST1* cooperatively with *BMI1* are demonstrated to be the important transcription factors in EMT and most of these transcription factors regulate each other. *BMI1* (B lymphoma Mo-MLV insertion region 1 homolog) is a component of the polycomb repressive complex 1 which epigenetically silences genes (Vander et al., 1994). *BMI1* was first identified as an oncogene in mouse lymphomas (Yang et al., 2010; Casas et al., 2011). It has a role in proliferation and self-renewal (Vander et al., 1994). *BMI1* expression has been demonstrated to be regulated by *TWIST1* in Squamous Cell Carcinoma (SCC) cell lines (Guo et al., 2011). It was shown that

*[1]Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, [2]Medical Genetics Department, Tehran University of Medical Sciences, [4]Tumor Clinic, Pathology Department and the Department of Plastic and Reconstructive Surgery, Razi Dermatology Hospital, Tehran University of Medical Sciences, Tehran, [3]Medical Genetics Department, Mashhad University of Medical Sciences, Mashhad, Iran *For correspondence: tabrizi@tums.ac.ir*

*BMI1* and *TWIST1* cooperatively silence the E-cadherin locus which is a marker of epithelial cells (Guo et al., 2011). *TWIST1* also regulates *SNAI2/SLUG* (Yang et al., 2004). Interestingly, it has been proven that *SNAI2* is essential for *TWIST1* function (Yang et al., 2004). The *BMI1-TWIST1-SNAI2* axis has a role in EMT (Mihic et al., 2007; Reinisch et al., 2007; Mikheeva et al., 2010; Yang et al., 2010; Guo et al., 2012). The significance of this axis in Basal Cell Carcinoma (BCC) was not previously explored though there is one study, to the best of our knowledge, documenting elevated *BMI1* expression via immunostaining in sixteen BCC cases (Bachmann et al., 2008). In two separate studies, expression of *BMI1* in Melanoma was reported with conflicting results, but ample evidence provides strong support for *BMI1* involvement in endowment of aggressive behavior to cancerous cells. Recently, one study demonstrated use of *BMI1* expression in identification of Merkel Cell Carcinoma (MCC) subtypes, a rare and highly aggressive skin cancer (Kouzmina et al., 2012). However, mRNA expression level of *BMI1, TWIST1,* and *SNAI2* was not previously quantitated in a large group of BCC cases in comparison with normal skin.

Since BCC rarely metastasizes and is one of the least dangerous cancers in the world, we hypothesized BCC would be a good model system to elucidate mechanisms which inhibit processes that drive tumor metastasis. The aim of this pilot study was to examine the mRNA expression level of *BMI1, TWIST1* and *SNAI2/SLUG* in basal cell carcinoma to generate a more complete picture of these genes in a non-metastatic and non-aggressive cancer.

## Materials and Methods

*Patients*

We collected thirty-five fresh tissue samples from individuals diagnosed with basal cell carcinoma in 2010 at the Tumor Clinic and Reconstructive Surgery Center of the Razi Dermatology Hospital, Tehran, Iran. The procedures followed were in accordance with the ethical standards of Tehran University of Medical Sciences and the Helsinki Declaration of 1975, as revised in 1983. Our collection of BCC cases included twelve cases of nodular, four cases of superficial, five cases of infiltrative, nine cases of micro-nodular, three cases of meta-typical and two cases of the basosquamous type tumor. The thirty-five BCC cases contained twenty-eight males and seven females with an average age of 65±2. Seven fresh normal skin tissues were obtained from cosmetic surgeries such as face lifts. All cases were reviewed by a dermatopathologist to confirm the diagnosis and the subclassification.

*Real-time RT-PCR*

RNA was extracted from tissue samples using the Tripure isolation reagent (Roche, Mannheim, Germany) according to the manufacturer's instructions. cDNA synthesis was performed using the PrimeScript RT reagent (Takara Bio Inc, Shiga, Japan) according to the manufacturer's protocols. For expression analysis, primers were designed using the NCBI. Primer-design tool (Table 1). Quantitative Real-time PCR was carried out using the SYBR Premix Ex Taq TM (Takara Bio Inc, Shiga, Japan) on rotor gene 6,000 corbette detection system. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene expression was used as a housekeeping gene for normalization of data. All experiments were performed in duplicates.

*Statistical analysis*

Statistical analysis was performed using the SPSS statistical package (SPSS, Chicago, IL, U.S.A.). The statistical importance of differences was assessed using the $\chi^2$ test. p values less than 0.05 were considered as statistically significant. The specificity and sensitivity of data was determined by the Receiver Operating Characteristic (ROC) curve.

## Results

Mean Relative Expression (MRE) of all samples is reported in Figure 1. BCC samples were divided into two groups based on invasive and aggressive behavior as evaluated by a dermatopathologist; highly invasive subtypes were included in the high risk group (micro-nodular, infiltrative and meta-typical) and the remaining subtypes were included in the low risk group. Although *BMI1* and *TWIST1* mRNA expression decreased in BCC samples compared to controls (p<0.001 and p=0.001, respectively), there seems to be no significant *BMI1*, *TWIST1* and *SNAI2* MRE difference between high risk and low risk BCC groups. *SNAI2* expression did not demonstrate a significant difference between BCC and controls. Expression pattern of *BMI1* and *TWIST1* seems to be corroborative and in line with previous studies demonstrating *TWIST1* regulation of *BMI1*.

The ROC curve was used to assess the sensitivity and

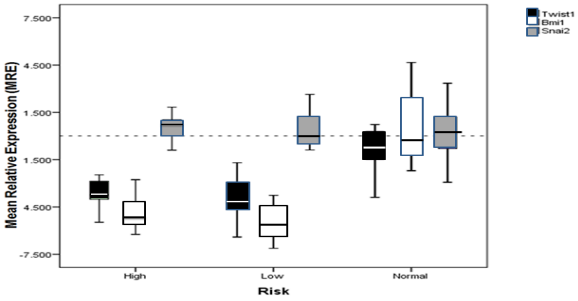


**Figure 1. Mean Relative Expression (MRE) of *TWIST1, BMI1* and *SNAI2* in High Risk BCC, Low Risk BCC and Controls**

**Table 1. Primer Sequence of *BMI1*, *TWIST1*, and *SNAI2* for Real-time PCR**

| Target genes | Sequence (5` - 3`) | Product length (bp) |
|---|---|---|
| *TWIST1* | F: GGCCGGAGACCTAGATG | 187 |
| (NM_000474.3) | R: ACGGGCCTGTCTCGCTTTCT | |
| *BMI1* | F: CTGGTTGCCCATTGACAGCG | 145 |
| (NM_005180.8) | R:AAATCCCGGAAAGAGCAGCC | |
| *SNAI2* | F: GACACATTAGAACTCACACGGG | 200 |
| (NM_003068.4) | R: GTGTGCTACACAGCAGCCAG | |
| GAPDH | F: GGTCGGAGTCAACGGATTTG | 180 |
| (NM_002046.4) | R: CCGTTCTCAGCCTTGACGGT | |

**Table 2. *BMI1*, *TWIST1* and *SNAI2* Sensitivity and Specificity for BCC Detection**

| | Cutoff | Sensitivity | Specificity | p* |
|---|---|---|---|---|
| TWIST1 | -2.09 | 94% | 80% | <0.001* |
| BMI1 | -2.48 | 94% | 100% | <0.001* |
| SNAI2 | -0.45 | 85% | 43% | 0.12 |
| Model | 2.63 | 94% | 100% | <0.001* |

the specificity of *TWIST1* and *BMI1*. *BMI1* can strongly differentiate control from BCC with 100 % specificity and 94% sensitivity (Table 2). In addition, *TWIST1* with 94% sensitivity, has a lower specificity of 80% in comparison with *BMI1*.

## Discussion

Primary tumors are responsible for 10% of deaths but metastases are responsible for 90% of cancer deaths. *BMI1*, as a stemness marker, and *TWIST1*, as a mesenchymal marker, have been implicated in aggressive behavior of cancers. Stemness markers and mesenchymal markers are involved in Epithelia-Mesenchymal Transition which occurs in cancer metastasis. Strong evidence has established a positive association between *BMI1* and *TWIST1* expression and metastatic cancers such as metastatic skin cancers. On the flip-side of the coin is Basal Cell Carcinoma (BCC), well known as one of the least aggressive cancers in the world. BCC is also a rare example of cancers which rarely metastasize. Thus, BCC provides a unique natural human model to monopolize in trying to understand regulatory steps involved in cancer metastasis prevention. We hypothesized BCC could be a good model system to elucidate mechanisms which inhibit processes that drive tumor metastasis.

The results confirm the study hypothesis. A stemness marker, *BMI1*, and a mesenchymal marker, *TWIST1*, clearly shown to be up-regulated in aggressive cancers are shown to be down-regulated in Basal Cell Carcinoma, our proposed model for studying natural intrinsic inhibitory metastatic steps utilized in non-aggressive cancers.

The initial utility of these results in clinical settings could be that down-regulation of *BMI1* and *TWIST1* mRNA could forecast metastatic inefficiency status of a patient and could be used for follow-up purposes. Further biochemical studies on signaling pathways involving and connected to *BMI1* and *TWIST1* could provide more details to the molecular mechanism of cancer metastasis responsible for 90% of cancer deaths.

*TWIST1* which is a major mesenchymal mediator, with *BMI1*, a stemness molecule, cooperatively have an important role in EMT none of our BCC tumor samples were metastatic, but previous studies have demonstrated *BMI1, TWIST1* and *SNAI2* up-regulation in aggressive SCC (Patel et al., 2007; Yang et al., 2010), *SNAI2* up-regulation in melanoma patient tumor samples (Shirley et al., 2012), and *TWIST1* up-regulation in one case of metastatic BCC (Majima et al., 2012). In the present study, *BMI1* and *TWIST1* demonstrate marked down-regulation whereas *SNAI2* expression shows no significant change in BCC tumors compared to controls. The limited data generated in this small group of BCC cases demonstrates *BMI1* and *TWIST1* dichotomy of action in locally invasive BCC in contrast to metastatic BCC, aggressive SCC, and melanoma. Local invasion and metastatic inefficiency of BCC shows no correlation with *SNAI2*.

*BMI1* is a potential mediator of BCC carcinogenesis downstream the Hedgehog signaling pathway demonstrating down-regulation in the present study of non-metastatic BCC cases (Vander et al., 1994). It seems that *BMI1* up-regulation has a role in aggressive behavior of metastatic cancerous cells. In the present study, with a small sample of non-metastatic, slow growing BCC cases, *BMI1* and *TWIST1* cooperatively demonstrate down-regulation. This may provide a clue for molecular differentiation between metastatic and non-metastatic cancerous cells, as perfectly illustrated for MCC subtyping via *BMI1* expression (Kouzmina et al., 2012). However, more studies need to clarify these findings.

Our results suggest *BMI1* as a potential biomarker for distinguishing controls from BCC tumor samples. The *BMI1-TWIST1-SNAI2* axis we initially proposed was confirmed in this pilot study with limited number of cases, but further studies need to be conducted on greater number of BCC cases including metastatic BCC alongside SCC and melanoma cases. It may be a possibility that suppressed or elevated *BMI1* and *TWIST1* expression can distinguish locally invasive behavior from metastatic behavior. In conclusion, we have shown decreased *BMI1* and *TWIST1* mRNA expression level in non-metastatic BCC tissue samples in comparison with normal skin tissue samples. *SNAI2* mRNA expression did not show significant change in BCC compared with controls.

## References

Bachmann IM, Puntervoll HE, Otte AP, Akslen LA (2008). Loss of BMI1 expression is associated with clinical progress of malignant melanoma. *Modern Pathology*, **21**, 583-90.

Casas E, Kim J, Bendesky A, et al (2011). Snail2 is an essential mediator of TWIST1-induced epithelial mesenchymal transition and metastasis. *Cancer Res*, **71**, 245-54.

Gao XH, Yang XQ, Wang BC, et al (2013). Overexpression of twist and matrix metalloproteinase-9 with metastasis and prognosis in gastric cancer. *Asian Pac J Cancer Prev*, **14**, 5055-60.

Guo BH, Feng Y, Zhang R, et al (2011). *BMI1* promotes invasion and metastasis, and its elevated expression is correlated with an advanced stage of breast cancer. *Mol Cancer*, **10**, 10.

Guo W, Keckesova Z, Donaher JL, et al (2012). *SNAI2* and Sox9 cooperatively determine the mammary stem cell state. *Cell*, **148**, 1015-28.

Kouzmina M, Hayry V, Leikola J, et al (2012). *BMI1* expression identifies subtypes of merkel cell carcinoma. *Virchows Archiv*, **461**, 647-53

Majima Y, Hirakawa S, Kito Y, et al (2012). *TWIST1* as a possible biomarker for metastatic basal cell carcinoma. *Acta dermato-venereologica*, **92**, 585-63.

Mihic Probst D, Kuster A, Kilgus S, et al (2007). Consistent expression of the stem cell renewal factor *BMI1* in primary and metastatic melanoma. *Int J Cancer*, **121**, 1764-70.

Mikheeva SA, Mikheev AM, Petit A, et al (2010). *TWIST1* promotes invasion through mesenchymal change in human glioblastoma. *Mol Cancer*, **9**, 194.

Moad AI, Tan ML, Kaur G, Mabruk M (2012). Lack of Increased P15INK4B protein expression in basal cell carcinomas. *Asian Pac J Cancer Prev*, **13**, 6239-44.

Patel KJ, Rhodus N, Patel D, et al (2007). Role of *SNAI2* in head and neck squamous cell carcinoma. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, **103**, 792-7.

Reinisch CM, Uthman A, Erovic BM, Pammer J (2007). Expression of *BMI1* in normal skin and inflammatory and neoplastic skin lesions. *J Cutan Pathol*, **34**, 174-80.

Schwartz YB, Pirrotta V (2007). Polycomb silencing mechanisms and the management of genomic programmes. *Nat Rev Genet*, **8**, 9-22.

Shirley SH, Greene VR, Duncan LM, et al (2012). *SNAI2* expression during melanoma progression. *Am J Pathol*, **180**, 2479-89.

Vander Lugt NM, Domen J, Linders K, et al (1994). Posterior transformation, neurological abnormalities, and severe hematopoietic defects in mice with targeted deletion of *BMI1* proto-oncogene. *Genes Dev*, **8**, 757-69.

Wang WS, Yu SL, Yang XS, et al (2013). Expression and significance of twist and E-cadherin in ovarian cancer tissues. *Asian Pac J Cancer Prev*, **14**, 669-72.

Yang HW, Menon LG, Black PM, et al (2010). *SNAI2*/*SLUG* promotes growth and invasion in human gliomas. *BMC Cancer*, **10**, 301.

Yang J, Mani SA, Donaher JL, et al (2004). Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell*, **117**, 927-96.

Yang MH, Hsu DS, Wang HW, et al (2010). *BMI1* is essential in *TWIST1*-induced epithelial-mesenchymal transition. *Nat Cell Biol*, **10**, 982-92.

Zhu QC, Gao RY, Wu W, Qin HL (2013). Epithelial-mesenchymal transition and its role in the pathogenesis of colorectal cancer. *Asian Pac J Cancer Prev*, **14**, 2689-98.