Decreased Sox2 Messenger RNA Expression in Basal Cell Carcinoma

Reza Ahmadi-Beni, Fatemeh Vand-Rajabpour, Mohamadreza Ahmadifard, Maryam Daneshpazhooh, Pedram Noormohammadpour, Javad Rahmati, Kambiz Kamyab Hesari, Mehdi Yaseri, Mina Tabrizi

Abstract

Background: Sox2, zeb1, and p21 have been implicated in aggressive behavior of squamous cell carcinoma (SCC) and melanoma. However, their expression level in basal cell carcinoma (BCC) has not been elucidated. We hypothesized BCC, contrary to SCC, and melanoma, could be a suitable model to study mechanisms which attenuate tumor metastasis. The aim of this study was to examine the messenger RNA (mRNA) expression levels of sox2, zeb1, and p21 in BCC.

Materials and Methods: Twenty-seven nonmetastatic BCC and twelve normal skin samples were evaluated using real-time reverse transcriptase polymerase chain reaction. Results: The stemness marker sox2 demonstrated marked down-regulation, but zeb1 and p21 showed no significant change.

Conclusions: Here, we report a negative association between sox2 mRNA expression level and nonmetastatic BCC, thus, providing a likely explanation for the fact that normal skin is more reliant on sox2 than BCC. BCC may be using decreased sox2 mRNA to remain incognito from metastatic potential.

Key Words: Basal cell carcinoma, metastasis, sox2, squamous cell carcinoma

Introduction

Basal cell carcinoma (BCC) is the most common human cancer in the world, but it is rarely metastatic.[1] BCC etiology is attributed to epidermal stem cells of the hair follicle in the outer root sheath.[2] Main molecular pathways have been identified which work in BCC,[3] BCC is slow growing with a low infiltration rate and rare metastasis. Attenuated growth and infrequent metastasis invite exploration of possible key embryonic and metastatic genes.[4] Implicated signaling pathways and associated target genes cannot solely explain the low proliferation rate and lack of metastasis in BCC.[4] Multiple mechanisms contribute to metastasis formation, and metastasis master genes are the focus of many researchers.[4,5]

Sox2 is an iconic transcription factor for the maintenance of cancer stem cells (CSCs) and acts as a fast reset button for stem cell induction and metastasis.[4-6] Interestingly, silencing sox2 reverses epithelial-mesenchymal transition (EMT).[9] CSCs are engaged in progression, metastasis and relapse after treatment among the bulk of cancer cells.[9] EMT and CSCs are interrelated and have led researchers to investigate a possible link between the EMT and the CSC phenotype.[11] Epithelial cells attain the mesenchymal phenotype by several EMT-related molecules. Importantly, key regulatory transcription factors, such as zinc-finger E-box-binding homeobox 1 (zeb1), promote the early steps of metastasis for local invasion and subsequent dissemination of cancer cells.[12] The missing links between EMT and CSCs could be regulatory molecules-like the differentiation/anti-proliferation factor p21,[13,14] As an inhibitor, p21 stops the cell cycle, and associated target genes cannot solely explain the low proliferation rate and lack of metastasis in BCC.[11] Multiple mechanisms contribute to metastasis formation, and metastasis master genes are the focus of many researchers.[4,5]
growth and apoptosis, and as a transcription factor, p21 decreases the expression of cell cycle progression genes and increases the expression of senescence-inducing genes; p21 has been shown to inhibit EMT by interacting with zeb1.[15] Another cell cycle-independent function of p21 is its ability to interact with sox2 to suppress the generation and expansion of induced pluripotent stem cells.[16]

To avoid the undesirable spread of cancer cells, elucidation of mechanisms and molecules responsible for rare metastasis in BCC may be helpful for the development of new therapeutic targets. Here, an exploration for rational explanations of the following question began: Why do all BCC types have a low rate of metastasis in comparison with other skin cancers such as melanoma and squamous cell carcinoma (SCC)? Therefore, we examined the expression of transcription factors implicated in invasive behavior of skin cancers in the rarely metastatic BCC.

Materials and Methods

Patients

Twenty-seven BCC specimens otherwise destined for disposal were collected in 2015 at the Tumor Clinic and the Reconstructive Surgery Center of the Razi Dermatology Hospital, Tehran, Iran. Information was provided by dermatologists after patient consent was obtained. This study conformed to the Ethics Committee of Tehran University of Medical Sciences and the Helsinki Declaration of 1975, as revised in 2013. Our BCC samples included nineteen male and eight female patients, with an average age of 65 ± 16, and the samples consisted of twelve cases of nodular, four cases of superficial, five cases of infiltrative, three cases of micronodular, one case of adenoid, one case of metatypical, and one case of sclerotic BCC. Twelve normal skin tissues were received from cosmetic surgeries such as blepharoplasty and rhinoplasty.

Real-time reverse transcriptase polymerase chain reaction

Total RNA was extracted using the tripure isolation reagent (Roche, Mannheim, Germany). RNA concentration was determined by the Nanodrop (Thermo Fisher, DE, USA). cDNA was treated by DNase I (Promega, Madison, USA). cDNA was synthesized from 1 μg of RNA using the PrimeScript™ RT reagent (Takara Bio Inc., Shiga, Japan). Design and bioinformatics analysis of the quantitative polymerase chain reaction (PCR) primers were conducted by the public Web service for primer design of NCBI Primer-design tool [Table 1]. Quantitative real-time PCR was conducted in triplicates on a rotor gene 6000 (Corbett Robotics, Sydney, Australia) with SYBR® Premix Ex Taq™ from Takara according to the manufacturer’s protocol. According to the Pfaffl method, normalization of relative expression levels was obtained with respect to the selected housekeeper gene gapdh by REST-RG software.[17]

Statistical analysis

Multivariate analysis of variance was performed by R (R Core Team, Vienna, Austria) to compare messenger RNA (mRNA) expressions between cancer patients and controls simultaneously. The receiver operator characteristic (ROC) curve determined the sensitivity and specificity of the data. A value of P < 0.05 was considered as statistically significant.

Results

Normalized results of the expression, relative to the expression level of gapdh mRNA, are presented for two distinct invasive/noninvasive BCC groups, compared with reference normal skin [Figure 1]. BCC samples were divided into two groups based on locally invasive behavior and aggressive behavior as evaluated by a dermatopathologist; highly invasive subtypes were included in the high-risk group (infiltrative, micro-nodular, sclerotic, and metatypical) and the remaining subtypes were included in the low-risk group. Decreased sox2 mRNA expression was significant in both low-risk group and high-risk group compared with controls; although, zeb1 and p21 mRNA expressions did not represent a significant difference in the low-risk group and the high-risk group compared with controls. Basal sox2 mRNA expression level in the human skin was confirmed in this study.[18,19] Expression pattern of sox2 appeared to confirm and be concordant with prior studies of sox2; sox2 could strongly differentiate control from BCC according to the ROC curve [Figure 2].

Table 1: Primer sequences

| Primers         | Sequence (5'-3')                                                                 | Length (bp) |
|-----------------|---------------------------------------------------------------------------------|-------------|
| sox2 (NM_003106.3) | F: CTCGCAGACCTACATGAACGGR: TGGAGTGGAGGAAAGAGGTAAAC                                         | 149         |
|                 | R: TTCCAGGACTGAGCGCCTC                                                        |             |
| p21 (NM_001291549.1) | F: CCTGTCACTGTCTTGTACCTTG                                                      | 190         |
|                 | R: TTTGGCCGGTGTAGAATCCAG                                                    |             |
| zeb1 (NM_003068.4) | F: CTCAACTACGGTCAGCCCTG                                                       | 145         |
|                 | R: CGGTTCAGGCCCTTG                                                           |             |
| gapdh (NM_0011519648.1) | F: GGTCGGAGTCGAACGGATTTG                                                      | 180         |
|                 | R: CCGTTCAGGCCCTTG                                                          |             |
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Discussion

Ninety percent of cancer deaths are related to metastasis. BCC is one of the few cancers with rare metastasis and thus an appropriate model for investigation of innate metastatic checkpoints monopolized in BCC for survival. [4] Sox2 is a potential mediator of BCC carcinogenesis downstream of the hedgehog and the epidermal growth factor receptor (EGFR) signaling pathways. [3] Sox2 mRNA demonstrated down-regulation in the present study of nonmetastatic BCC cases. These results help to confirm the CSC theory and can propel search to further understand the causes, processes, and functions of sox2 mRNA decreased expression in conjunction with its partners in spectrum of skin cancers. In addition, this data rationalize the use of further techniques for the investigation of protein expression to clarify the molecular mechanisms yielding EMT, stemness, and poor metastatic ability in BCC to be potentially exploited in cancers with the high rates of metastasis. Genes involved in controlling stem cell self-renewal, uncontrolled expression of which are of great importance in cancer progression, have been introduced as a new class of cancer molecular markers [6-8,10,12].

Previous studies have demonstrated up-regulation of mRNA and protein expression level of zeb1 in the tongue SCC by real-time PCR and immunohistochemistry [20] and also in the murine model of melanoma and short-term culture of human melanoma cell lines by immunohistochemistry/Western blot and real-time PCR. [21] Stelkovics et al. showed p21 up-regulation in SCC compared with BCC by tissue microarray construction and immunohistochemistry. [22] By immunohistochemistry, Murphy et al. reported earlier and higher levels of p21 protein expression upregulation in patients with BCC after ultraviolet (UV) exposure than observed in normal skin UV exposure. [23]

Sox2 gene expression deregulation has been reported in several types of human malignancies including glioblastomas [7] melanomas [18] and SCC. [16] A direct correlation is reported between sox2 protein expression and invasiveness/metastasis potential of various solid tumors. In line with such findings, it has been shown that sox2 down-regulation can decrease the invasiveness potential of melanomas and gliomas. [7,9] Thus, depleted sox2 expression in BCC suggests the poverty of CSCs in this cancer. A possible mechanism for sox2 down-regulation could be transcriptional suppression marker histone H3 lysine 27 trimethylation (H3K27 me3) found in the sox2 promoter in skin squamous cells. [24]

Stemness gene down-regulation in BCC has been previously reported. [6,5] Down-regulation of sox2 mRNA expression, similar to down-regulation of Bmi1 and Twist1, could prognostically indicate the inefficient metastatic status of a tumor and could be useful for follow-up. [6,5] Our results are in agreement with Patil et al. [19] They distinguished BCC from basaloid SCCs, which is an aggressive and recurrent cancer that metastasizes to regional lymph nodes. Sox2 immunoreactivity was 0% in BCC versus 93% in basaloid SCC. Interestingly, they reported sox2 nuclear staining in the adjacent nonneoplastic squamous epithelium/epidermis with the strongest staining in the basal cell layer and its progressive decrease with the maturing epithelium. They suggested that unlike sox2 potential role as a stemness marker in various cancers, sox2 does not appear to mediate a major role in the regulation of progenitor cells from which BCCs originate. [2,3]

Decrease in sox2 mRNA expression was observed in this study on BCC, a carcinoma-associated with dysregulation of both the sonic hedgehog (SHH) and EGFR pathways. [3] These findings suggest, but certainly do not prove, a correlation between sox2 mRNA decrease and an abnormality of the SHH pathway and further suggest the
hypothesis that sox2 down-regulation might be related to disruption of regulatory transcriptional networks in the maintenance of the stemness state and self-renewal by molecules with metastasis suppressor function.[20] This possibility is intriguing given that both the SHH pathway and sox2 are important in an ectodermal development, and sox2 has previously been shown to decrease p21 transcription, a protein which downregulates both patched and smoothened when exogenously expressed in HaCaT cells.[24] Furthermore, sox2 knockdown in pancreatic cancer cells resulted in cell growth inhibition through cell cycle arrest (not apoptosis) through the transcriptional induction of p21 and p27[21] and linking sox2 to a downstream effector such as p53 opens the possibility that sox2 inactivation may promote cell growth arrest through a p53-dependent pathway. In vitro studies indicate the p53-p21 axis as a negative regulator of sox2 activity. This is a plausible function given the apparent absence of sox2 mRNA expression in several p21 expressing cancers. We and others found no significant deregulation of p21 in BCC.[22]

Distinct tumor-initiating and metastatic cancer cells are sensitive to sox2 inhibition, raising the hope that interfering with sox2 signaling may also improve relapse rates and metastasis.[6,28,29] The sox2 gene is crucial for self-renewal and differentiation processes of embryonic stem cells. Furthermore, it has been shown that sox2 gene expression in tissue stem cells plays a similar role in utero.[28] As sox2 seems to be a biological marker for the stemness state of cancer cells,[10] the considerably greater expression of sox2 in normal skin compared to BCCs provides a likely explanation for the fact that normal skin is more reliant on sox2 than BCC. It is essential to run complementary functional, biochemical, and signal transduction studies to better understand possible sox2 down-regulation and its underlying mechanism of action and possible associations of metastasis suppressor pathways. BCC patient samples did not demonstrate any significant correlation with p21 and zeb1 mRNA expression levels.

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Conflicts of interest
There are no conflicts of interest.

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