PAX3 is a biomarker and prognostic factor in melanoma: Database mining

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Received July 16, 2018; Accepted February 14, 2019

DOI: 10.3892/ol.2019.10155

Abstract. Paired box 3 (PAX3) is a transcription factor and critical regulator of pigment cell development during embryonic development. However, while there have been several studies on PAX3, its expression patterns and precise role remain to be clarified. The present study is an in-depth computational study of tumor-associated gene information, with specific emphasis on the expression of PAX3 in melanoma, using Oncomine along with an investigation of corresponding expression profiles in an array of cancer cell lines through Cancer Cell Line Encyclopedia analysis. Based on Kaplan-Meier analysis, the prognostic value of high PAX3 expression in tissues from patients with melanoma compared with normal tissues was assessed. PAX3 was more highly expressed in male patients with melanoma compared with female patients with melanoma. Using Oncomine and Coexpedia analysis, it was demonstrated that PAX3 expression was clearly associated with SRY-box 10 expression. The survival analysis results revealed that high PAX3 mRNA expression was associated with worse survival rates in patients with melanoma. These results suggested that PAX3 may be a biomarker and essential prognostic factor for melanoma, and provided an important theoretical basis for the development of melanoma treatments.

Introduction

Since the 1980s, the incidence of melanoma has been increasing at an annual rate of ~2.8% (1). Out of all patients diagnosed with cutaneous malignant melanoma, ~20% will succumb to metastatic disease, and the prognosis is significantly worse for those patients who are diagnosed with regional and distant metastases, with a 10-year survival rate of 64 and 16%, respectively (2,3).

Paired box 3 (PAX3) protein is known to be involved in the development of cancer (4). PAX3 protein contains two DNA binding domains; a paired domain and a homeodomain, which may function alone or in combination to bind downstream target genes (5-8).

Medic *et al* (9) suggested that the traditional developmental roles of PAX3 in regulating differentiation, proliferation, cell survival and migration are retained in melanoma cells. In melanoma, PAX3 expression is evident at all stages of disease progression, including primary lesions, circulating melanoma cells and metastatic lesions (10-14).

Previous studies have demonstrated that PAX3 can drive and activate C-X-C motif chemokine receptor 4 (CXCR4)/MET proto-oncogene receptor tyrosine kinase expression, and may promote melanoma metastasis and rapid tumor growth (15,16). E3 ligase APC/C (Cadherin 1) promotes ubiquitination-mediated PAX3 proteolysis and inhibits the proliferation of melanoma cells and melanoma growth (17). In addition, phosphorylation of PAX3 affects the melanoma phenotype (18). These findings may contribute to the further diagnosis, prognosis and potential treatment of melanoma.

In the present study, large databases of melanoma genetic information were analyzed to investigate the expression pattern of PAX3 in melanoma compared with normal tissues, and its association with characteristic molecular markers and their corresponding prognostic value in melanoma.

Materials and methods

Oncomine analysis. Oncomine (http://www.oncomine.org) is a gene chip-based database and integrated data mining platform in which conditions can be set for filtering and mining data. In the present study, the following screening conditions were used: i) Cancer type: Melanoma; ii) gene: PAX3; iii) data type: mRNA; iv) analysis type: Cancer vs. normal analysis; v) clinical outcome: Survival status; vi) outlier analysis: Overall survival follow-up time (days); and vii) threshold

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Key words: paired box 3, melanoma, database mining, prognostic value, biomarker

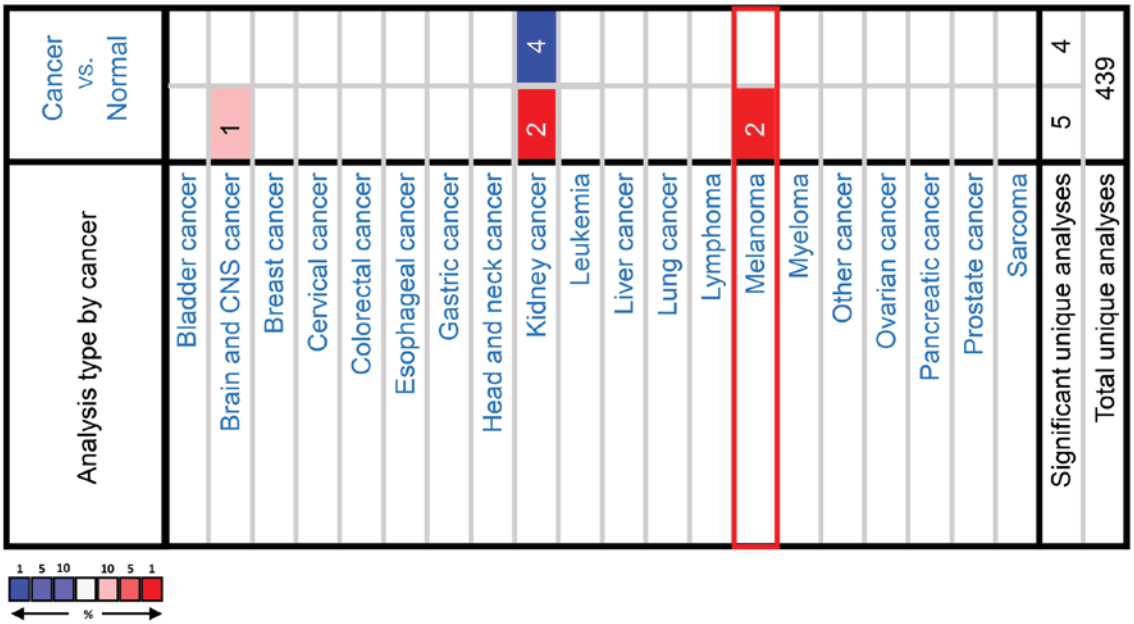

Figure 1. mRNA expression pattern of paired box 3 in melanoma. Number of datasets with statistically significant mRNA overexpression (red) or down-regulation (blue) of the target gene (cancer vs. normal tissue). The P-value threshold was set as 0.01. The number in each cell represents the number of analyses that met the threshold specific to a type of cancer. The gene rank was analyzed by percentile of the target gene in all genes measured in each study. Cell color is determined by the best gene rank percentile for the analyses within the cell. Melanoma is framed in red. CNS, central nervous system.

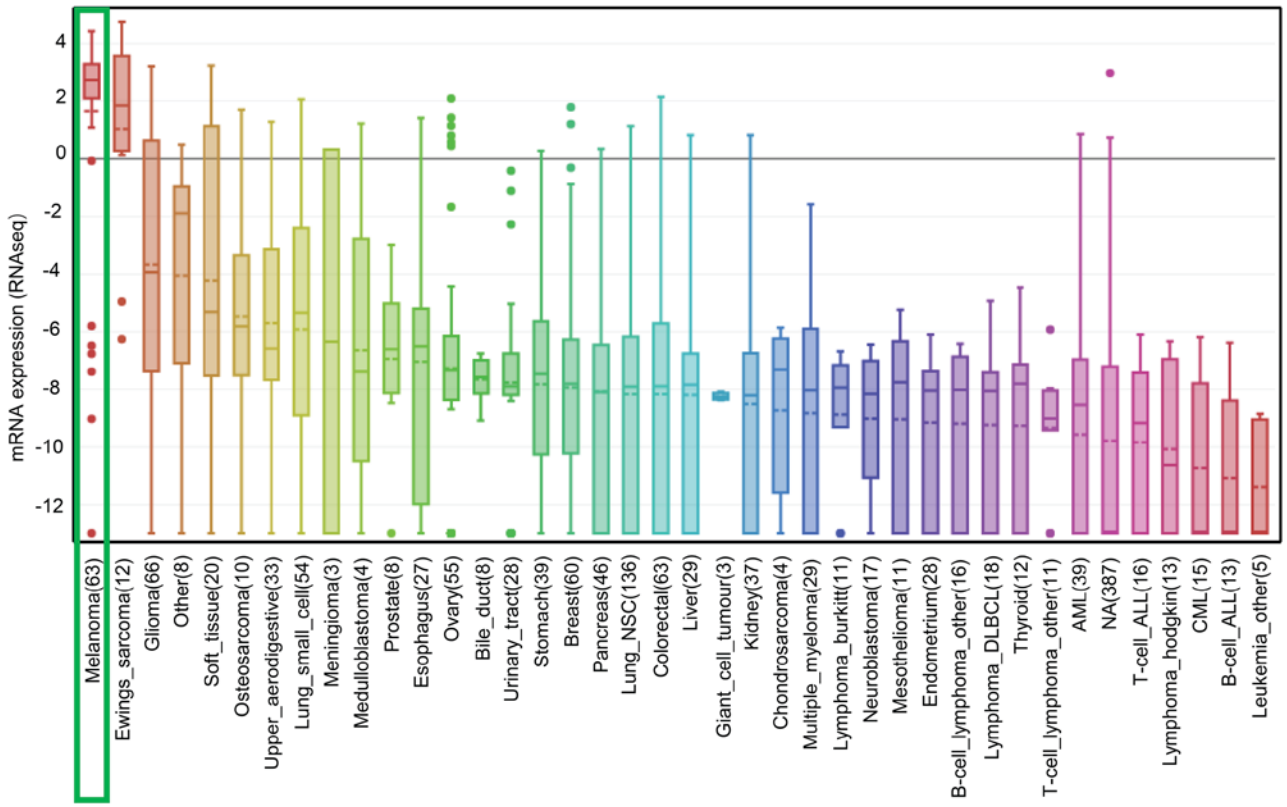

Figure 2. PAX3 is highly expressed in melanoma cell lines according to Cancer Cell Line Encyclopedia analysis. PAX3 mRNA expression was ranked highly in a variety of cancer cell lines (melanoma shown in green frame). The numbers in parentheses indicate the sample size and the y-axis number indicates mRNA expression (RNAseq). PAX3, paired box 3; AML, acute myeloid leukemia; B-cell_ALL, B-cell acute lymphocytic leukemia; CML, chronic myeloid leukemia; Lung_NSC, non-small cell lung cancer; Lymphoma_DLBCL, diffuse large B-cell lymphoma; NA, nasopharyngeal carcinoma; RNAseq, RNA sequencing; T-cell_ALL, T-cell acute lymphocytic leukemia.

setting conditions (P<0.0001; fold change >2; gene rank <10%). The data output was saved in Excel format.

Cancer Cell Line Encyclopedia (CCLE) analysis. The mRNA expression levels of PAX3 and SRY-box 10 (SOX10) in various

types of cancer were analyzed using CCLE (https://portals.broadinstitute.org/ccle/home), which is an online database of gene expression, chromosomal copy number and massively parallel sequencing data from 1,000 human cancer cell lines, aimed to facilitate the identification of genetic lineages and predictors of drug sensitivity.

Coexpedia analysis. Co-expression of PAX3 was analyzed using the Coexpedia database (http://www.coexpedia.org/), which is a database of context-associated co-expression networks inferred from individual series of microarray samples for human and mouse samples based on Gene Expression Omnibus data (https://www.ncbi.nlm.nih.gov/geo/). The generated network was a filtered network for the medical subject heading term 'melanoma'. The score for each gene is a summation of edge-weights (Log likelihood score) to all connected genes in the network.

Statistical analysis. Differences in PAX3 expression between normal tissue and melanoma tissue were examined by unpaired t-test, and survival analysis for different groups was performed using the Kaplan-Meier method with log-rank test. The median of all sample expression values was calculated using descriptive statistical analyses. The data were expressed as the mean ± standard deviation. All data were analyzed using GraphPad Prism v7 software (GraphPad Software, Inc., La Jolla, CA, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression levels of PAX3 in all tumor types. A total of 439 different study results were analyzed in the Oncomine database (Fig. 1). Among them, there were nine studies with statistically significant differences in PAX3 expression, five studies with increased PAX3 expression and four with reduced PAX3 expression. In kidney cancer, there were two studies identifying increased expression and four studies identifying decreased expression. In melanoma, there were two studies identifying increased expression and no studies identifying decreased expression. Furthermore, the CCLE analysis was consistent with the Oncomine analysis, which indicates that the expression of PAX3 was increased in melanoma cell lines (Fig. 2).

Oncomine analysis revealed that in a dataset from Haqq *et al* (19), which included 25 melanoma, nine non-neoplastic nevus and three normal skin samples analyzed on cDNA microarrays, PAX3 mRNA expression in melanoma samples, including all subtypes, was increased 10.168-fold ($P=3.92\times10^{-5}$) and in non-neoplastic nevus it was increased 7.654-fold ($P=1.08\times10^{-5}$) compared with in normal tissues (Fig. 3A and B). In a dataset from Riker *et al* (20), which included 14 cutaneous melanoma and four normal skin samples analyzed on Affymetrix HG U133 Plus 2.0 microarrays, PAX3 expression in cutaneous melanoma samples was increased 3.902-fold ($P=1.76\times10^{-4}$) compared with in normal tissue (Fig. 3C). Additionally, in a study by Talantov *et al* (21), which included 45 cutaneous melanoma, 18 benign melanocytic skin nevus and seven normal skin samples analyzed on Affymetrix U133A microarrays, PAX3 mRNA expression in benign melanocytic skin nevus samples was increased 4.230-fold (P=0.003) and was increased 3.650-fold (P=0.005) in cutaneous melanoma samples compared with in normal tissues (Fig. 3D and E). These results suggested that PAX3 may serve a unique role in the development of melanoma.

Co-expression analysis of PAX3. Since PAX3 was identified to be specific to melanoma, the potential role of PAX3 in melanoma was further investigated. In a dataset from Wagner *et al* (22), Coexpedia co-expression analysis suggested that SOX10 ranked first with a score of 2.778 (Table I and Fig. 4). In Oncomine co-expression analysis, and the dataset from Pratilas *et al* (23), PAX3 expression was identified to be significantly associated with SOX10 (r=0.806; Table II and Fig. 5). As shown in Tables I and II, co-expression analysis data indicated that PAX3 expression may be clearly associated with SOX10 expression. Additionally, similar results were obtained in the CCLE analysis, in which the mRNA expression level of SOX10 was ranked highest in melanoma cell lines (Fig. 6). In addition, SOX10 overexpression was observed in melanoma cell lines with high PAX3 expression, while low expression was observed in melanoma cell lines with low PAX3 expression (P=0.0017; Fig. 7). The aforementioned results suggested that SOX10 may be a co-expressed gene of PAX3.

PAX3 predicts a worse survival rate in patients with melanoma. In a study by Xu *et al* (24), the overall survival rate was statistically analyzed using the Kaplan-Meier method and a log-rank test was used to compare high and low expression groups. In the present study the median of all sample expression values was calculated. The cut-off value was -0.921135. If the sample expression value was less than the median, it was considered to belong to the low expression group, otherwise it was considered to belong to the high expression group. Low mRNA expression levels of PAX3 were associated with a significantly longer survival time in all patients with melanoma [hazard ratio (HR)=2.274, P=0.0086; Fig. 8A]. High mRNA expression levels of PAX3 were significantly associated with shorter survival time (HR=2.454, P=0.0252; Fig. 8B). Low mRNA expression levels of PAX3 were significantly associated with longer survival time in patients with superficial spreading melanoma (HR=2.454, P=0.0252; Fig. 8B). Low mRNA expression of PAX3 was not significantly associated with longer survival time in nodular melanoma (HR=3.262, P=0.0792) (Fig. 8C). In addition, compared with female patients with melanoma, male patients with melanoma exhibited higher PAX3 expression (P=0.0286, t=2.232; Fig. 8D). These results suggested that PAX3 may serve a unique role in the development of melanoma.

Discussion

During previous years, the understanding of melanoma development and biology has improved. It has become clear that the progression from premalignant lesions to fully developed melanoma does not represent a single evolutionary pattern. Each melanoma subtype can develop from different precursor lesions and may exhibit different stages of gene mutation and transformation (25). However, some patients relapse with disseminated disease, and ~10% of melanoma cases are

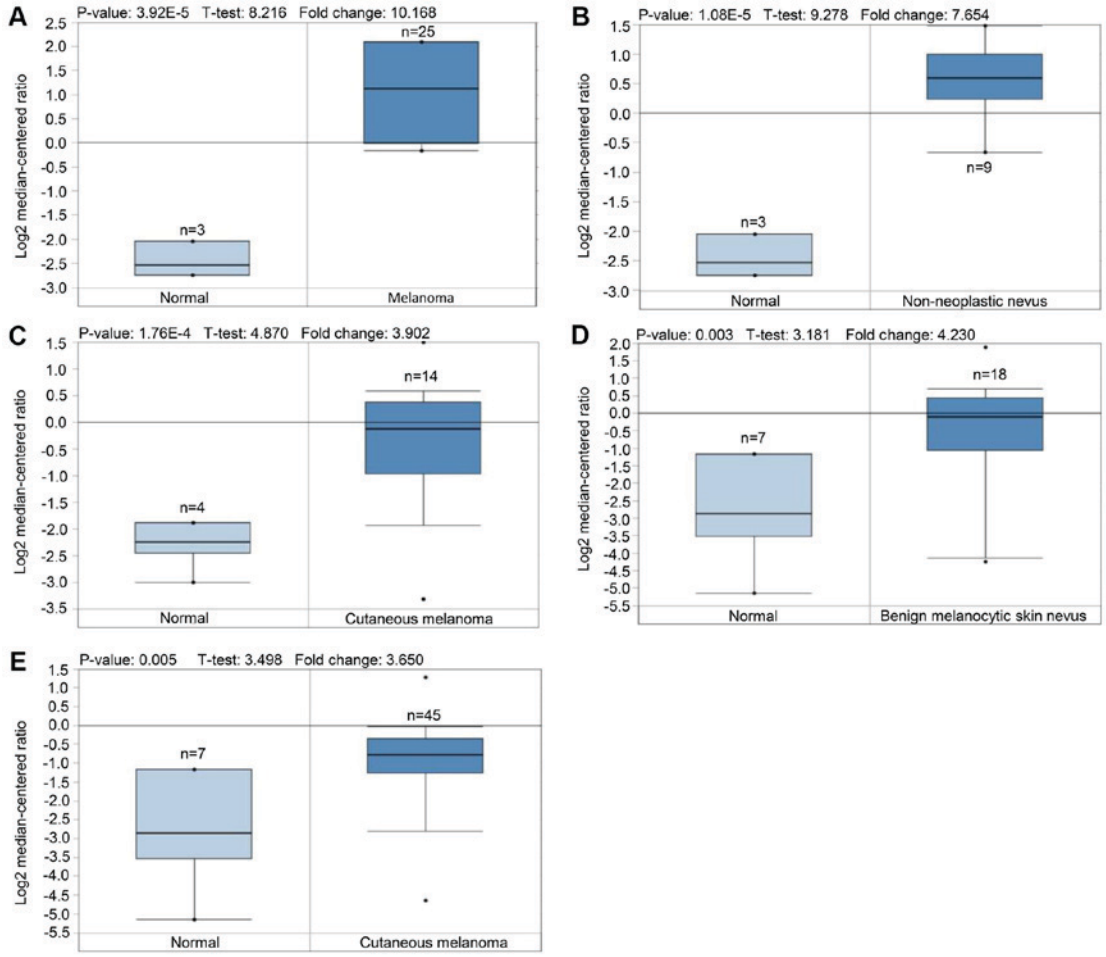

Figure 3. Box plots derived from gene expression data in Oncomine comparing PAX3 mRNA expression in normal and melanoma tissue. The P-value was set at 0.01 and fold change was defined as 2. (A) Comparison of melanoma, including all subtypes and normal tissue (19). (B) Comparison of non-neoplastic nevus and normal tissue (19). (C) Comparison of cutaneous melanoma and normal tissue (20). (D) Comparison of benign melanocytic skin nevus and normal tissue (21). (E) Comparison of cutaneous melanoma and normal tissue (21). PAX3, paired box 3.

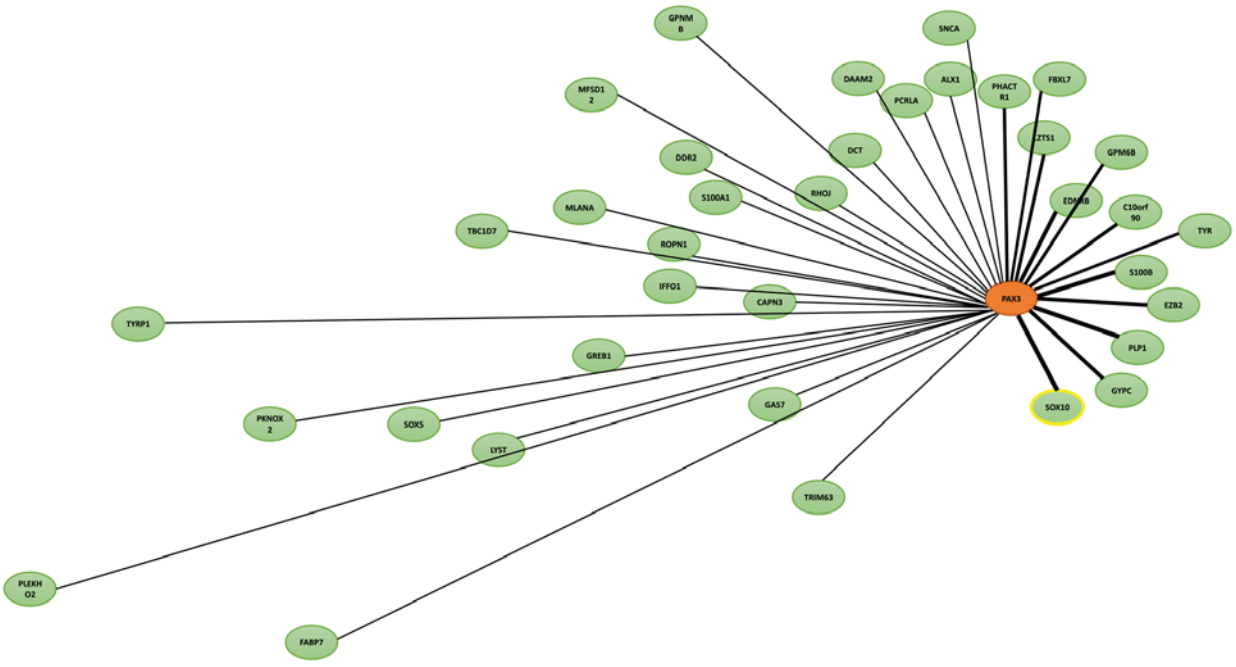

Figure 4. Filtered network for the medical subject heading term 'Melanoma'. This was obtained by Coexpedia analysis. The score for each gene is the summation of edge-weights (Log likelihood score) to all connected genes in the network. The thicker the line the closer the association with PAX3. SOX10 ranked highest with a score of 2.778 (shown in yellow frame). PAX3, paired box 3; SOX10, SRY-box 10.

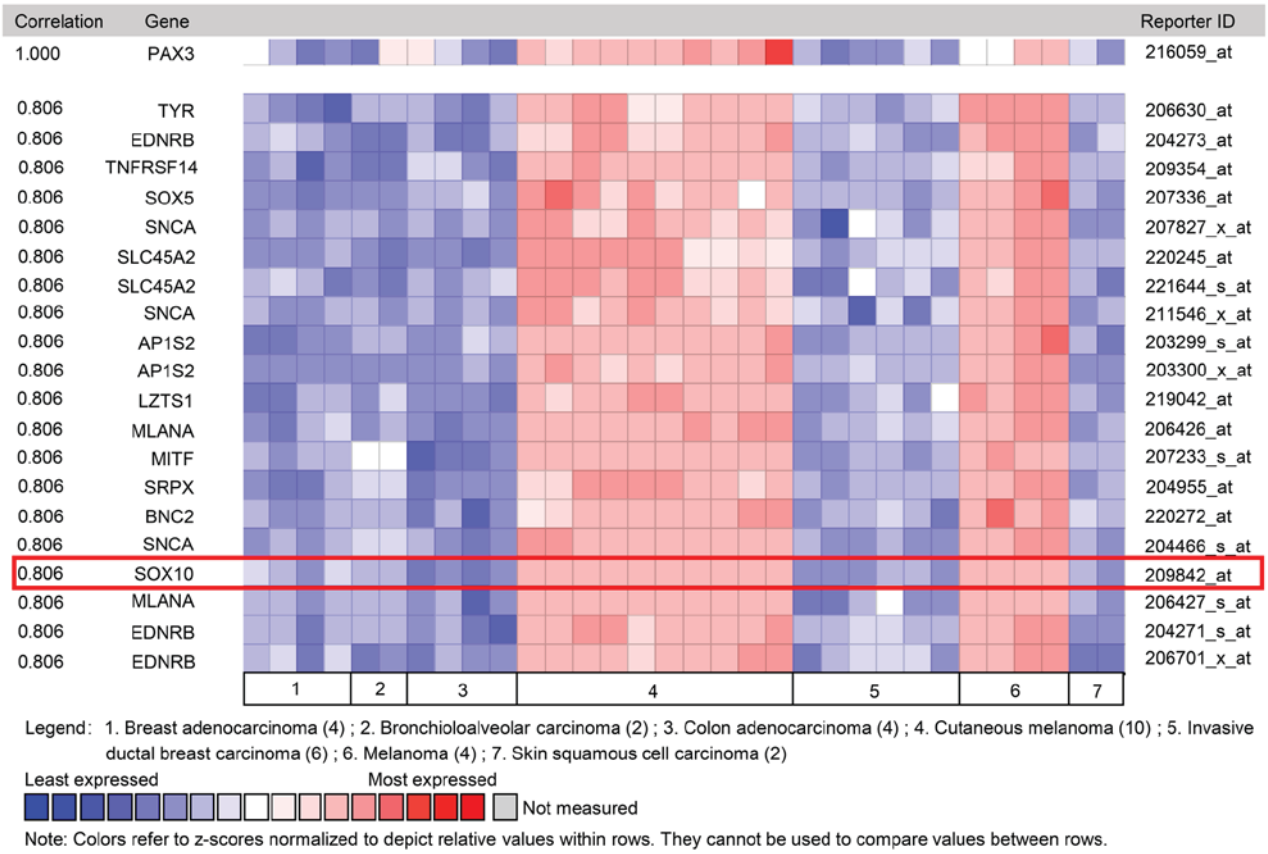

Figure 5. According to Oncomine analysis, PAX3 expression is significantly associated with SOX10 expression (shown in red frame). The cancer cell lines were derived from different tumors. PAX3, paired box 3; SOX10, SRY-box 10.

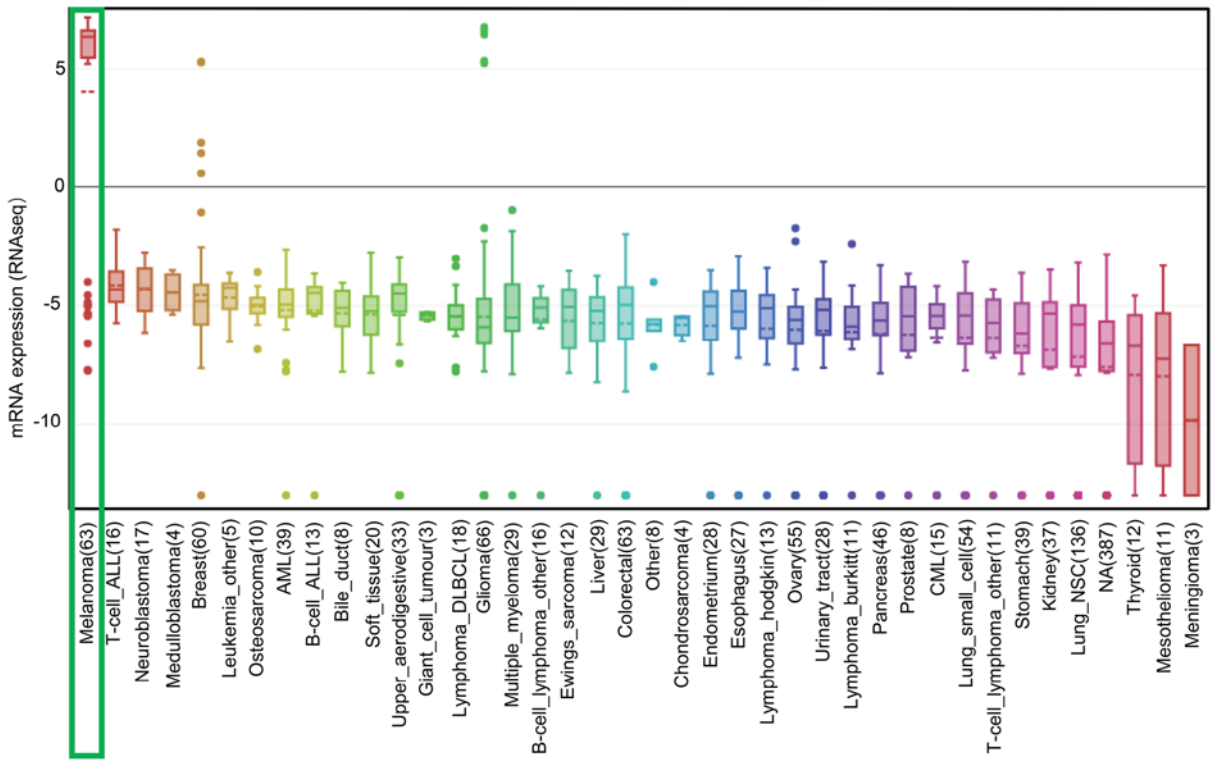

Figure 6. SOX10 mRNA expression in different tumor cells according to Cancer Cell Line Encyclopedia analysis. The mRNA expression level of SOX10 was ranked highest in melanoma cell lines (shown in green frame). The numbers in parentheses indicate the sample size and the y-axis indicates mRNA expression (RNAseq). PAX3, paired box 3; SOX10, SRY-box 10; AML, acute myeloid leukemia; B-cell_ALL, B-cell acute lymphocytic leukemia; CML, chronic myeloid leukemia; Lung_NSC, non-small-cell lung cancer; Lymphoma_DLBCL, diffuse large B-cell lymphoma; NA, nasopharyngeal carcinoma; RNAseq, RNA sequencing; T-cell_ALL, T-cell acute lymphocytic leukemia.

Table I. Co-expression analysis of PAX3 using Coexpedia.

Rank	Gene	Score
1	SOX10	2.778
2	PLP1	2.549
3	GYPC	2.498
4	EDNRB	2.489
5	S100B	2.457
6	C10orf90	2.377
7	ZEB2	2.367
8	LZTS1	2.221
9	GPM6B	2.174
10	TYR	2.038
11	PHACTR1	1.928
12	FBXL7	1.875
13	DCT	1.873
14	RHOJ	1.842
15	ALX1	1.827
16	FCRLA	1.821
17	CAPN3	1.746
18	DAAM2	1.709
19	GAS7	1.707
20	SNCA	1.688

Filtering was performed for the medical subject heading term 'Melanoma' and the score for each gene is the summation of edge-weights (Log likelihood score) to all connected genes in Coexpedia analysis.

Table II. Co-expression analysis of PAX3 using Oncomine.

Gene	Correlation	Reporter ID
PAX3	1.000	216059_at
SOX10	0.806	209842_at
TYR	0.806	206630_at
EDNRB	0.806	204273_at
TNFRSF14	0.806	209354_at
SOX5	0.806	207336_at
SNCA	0.806	207827_x_at
SLC45A2	0.806	220245_at
SLC45A2	0.806	221644_s_at
SNCA	0.806	211546_x_at
AP1S2	0.806	203299_s_at
AP1S2	0.806	203300_x_at
LZTS1	0.806	219042_at
MLANA	0.806	206426_at
MITF	0.806	207233_s_at
SRPX	0.806	204955_at
BNC2	0.806	220272_at
SNCA	0.806	204466_s_at
MLANA	0.806	206427_s_at
EDNRB	0.806	204271_s_at

The correlation (log2 median-centered intensity) in the table represents genes co-expressed with PAX3 in the Pratilas cell line (23) (GSE10087) derived from melanoma in Oncomine analysis.

diagnosed during late stages and are either unresectable or have metastasized (26). Therefore, a number of studies have investigated the development of melanoma to improve targeted therapies (26-28).

PAX3 serves a vital regulatory role in pigment cell development during embryonic development (29). Medic *et al* (9) suggested that there is no statistically significant difference in the expression of PAX3 in melanocytes and melanoma cells; however, Bailey *et al* (30) demonstrated that PAX3 expression is significantly inhibited in adult melanocytes and the expression of PAX3 mRNA in melanoma cells is 200-times that of normal skin (31). Notably, PAX3, particularly PAX3E, significantly inhibits the proliferation and increases chemosensitivity of melanoma cells (15,32-35), meanwhile, treatment with PAX3 inhibitors resulted in a significant decrease in PAX3 expression in melanoma cells, whereas PAX3 expression had no change in melanocytes (36). PAX3 may not only drive the expression of genes that promote cellular metastasis and invasion, but may also regulate the mRNA expression levels of genes involved in melanoma differentiation, proliferation and survival (9,34,37). Reid *et al* (14) revealed that PAX3 expression is evident at all stages of melanoma progression, including primary lesions, circulating melanoma cells and metastatic lesions. Medic *et al* (9) suggested that PAX3 directly targets the transforming growth factor β1 promoter in metastatic melanoma cell lines, as well as other genes associated with cell migration, including melanoma cell adhesion molecule, chondroitin sulfate proteoglycan 4 and CXCR4. Additionally, PAX3

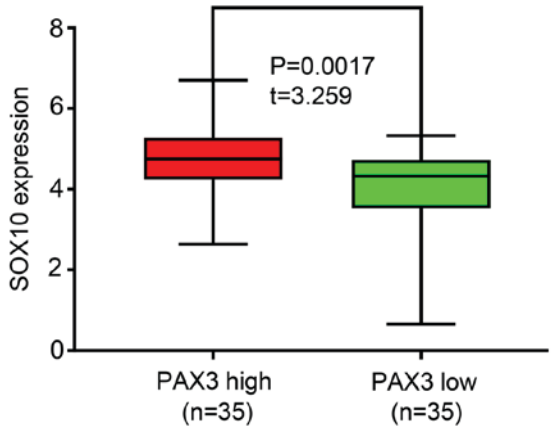

Figure 7. Cancer Cell Line Encyclopedia analysis reveals that SOX10 is overexpressed in melanoma cell lines with high expression levels of PAX3, but is downregulated in those with low PAX3 expression. PAX3, paired box 3; SOX10, SRY-box 10.

may drive CXCR4 expression to promote melanoma metastasis (18). In addition, silencing PAX3 with RNA interference can inhibit proliferation and induce terminal differentiation and apoptosis, according to the activation of caspase-3 and p53 in melanoma cells (38-40). Notably, >2.76 copies/μl of PAX3d mRNA in the bloodstream predicts recurrence of cutaneous malignant melanoma (41). Furthermore, the human PAX3 gene may serve a role in other human malignancies,

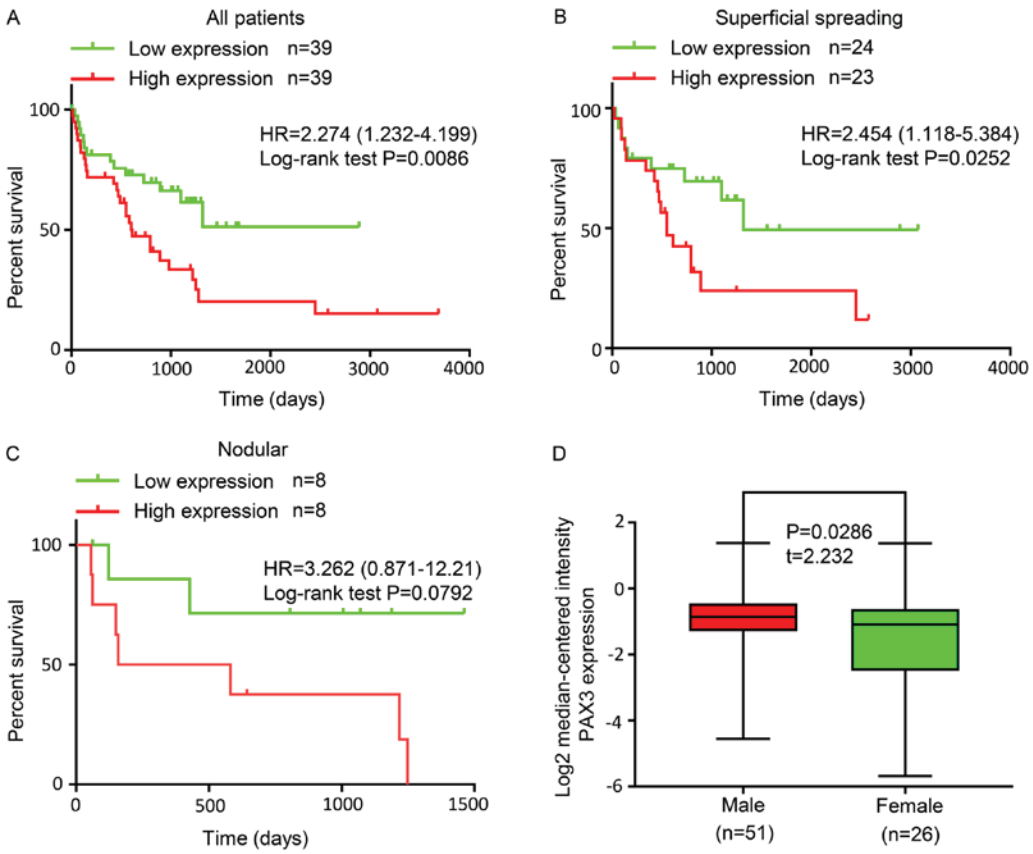

Figure 8. Prognostic value of PAX3 mRNA expression in melanoma. (A) High mRNA expression levels of PAX3 were associated with a shorter survival time in all patients with melanoma. (B) High mRNA expression levels of PAX3 were associated with a shorter survival time in patients with superficial spreading melanoma. (C) High mRNA expression levels of PAX3 were associated with a shorter survival time in patients with nodular melanoma. (D) Comparison of PAX3 mRNA expression in males and females. PAX3, paired box 3.

including rhabdomyosarcoma and Ewing's sarcoma (42). Co-expression analysis using Oncomine revealed a positive association between PAX3 expression and SOX10 expression. The findings of Bondurand *et al* (43) are consistent with the complex functional roles of PAX3 and SOX10 in neural crest stem cell-derived melanocyte development, and SOX10 has been demonstrated to markedly activate melanocyte inducing transcription factor (MITF) expression in cultured cell lines, while PAX3 synergistically transactivates the promoter of MITF with SOX10 to influence the maintenance of melanocyte stem cells (43-45). Additionally, a small number of PAX3 transcriptional cofactors have been identified, but only SOX10 and ETS proto-oncogene 1 transcription factor have been verified within melanoma cells (46). Furthermore, there is increasing evidence that signaling proteins tend to form interaction networks rather than simple linear pathways, meaning PAX3 and SOX10 may use specific interaction networks to regulate the proliferation, differentiation and migration of melanocyte precursors (47-51).

In the present study, the incidence rate of melanoma was identified to be higher in men than in women. Using Oncomine analysis, the expression of PAX3 in male patients with melanoma was significantly higher than in female patients (P=0.0286, t=2.232). Some studies have suggested that this increased male susceptibility may be associated with androgens (52-54) and hyperandrogenism may result in variants in melanoma-associated pigmentary genes (55-58). According to Kocarnik *et al* (59), the solute carrier family 45 member 2 single nucleotide polymorphism rs16891982, which has a non-synonymous mutation (F374L) located in exon 5, may be responsible for imparting a higher melanoma risk in men, possibly through alterations in pigmentation and melanogenesis (60). Therefore, the present study demonstrated that the differential expression of PAX3 and its downstream targets may be a potential predictor of sex-specific genetic risks in melanoma.

In conclusion, PAX3 was highly expressed in melanoma and predicted a worse survival rate for patients with melanoma. PAX3 expression was positively associated with SOX10 expression, and the expression of PAX3 in male patients with melanoma was significantly higher than that in female patients. The aforementioned evidence indicated that PAX3 may act as a potential modulator in melanoma.

Acknowledgements

The authors would like to thank Dr H. Nikki March, for editing the English text of a draft of this manuscript.

Funding

This study was partly supported by the National Natural Science Foundation of China (grant no. 81704087), the National Disease Research Program of TCM (grant

no. 20160524), the Innovative Talents Promotion Project-Key Scientific and Technological Innovation Team Project (grant no. 2017KCT-27) and the Shaanxi Province Clinical Medical Research Center Project (grant no. 2016LCZX-12).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YL and SC designed the study and drafted the manuscript. WL and YZ were primarily dedicated to collecting and statistically analyzing data. XY and JX supervised the scientific work, interpreted the data, revised the manuscript, provided financial support and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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