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
LIU et al: PAX3 IS A BIOMARKER AND PROGNOSTIC FACTOR IN MELANOMA

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 cancers were analyzed. 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.
Coexpression analysis. Coexpression of PAX3 was analyzed using the Coexpression database (http://www.coexpression.org/), which is a database of context-associated coexpression 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=3.92x10^{-5}) and in non-neoplastic nevus it was increased 7.654-fold (P=1.08x10^{-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=1.76x10^{-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.

Coexpression 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 coexpression analysis suggested that SOX10 ranked first with a score of 2.778 (Table I and Fig. 4). In Oncomine coexpression 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, coexpression 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 coexpressed 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, r=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_DLBC, diffuse large B-cell lymphoma; NA, nasopharyngeal carcinoma; RNAseq, RNA sequencing; T-cell_ALL, T-cell acute lymphocytic leukemia.
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 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.
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 tran-
scriptional 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 melano-
genesis (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 mela-

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.

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
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.

References

1. Little EG and Eide MF: Update on the current state of melanoma incidence. Dermatol Clin 30: 355-361, 2012.
2. Buzaid AC and Atkins M: Practical guidelines for the management of biochemotherapy-related toxicity in melanoma. Clin Cancer Res 7: 2611-2619, 2001.
3. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T and Thun MJ: Cancer statistics, 2008. CA Cancer J Clin 58: 71-96, 2008.
4. Robson EJ, He SJ and Eccles MR: A PANorama of PAX genes in cancer and development. Nat Rev Cancer 6: 52-62, 2006.
5. Corry GN and Underhill DA: PAX3 target gene recognition occurs through distinct modes that are differentially affected by disease-associated mutations. Pigment Cell Melanoma Res 26: 67-77, 2013.
6. Chalepakis G and Gruss P: Identification of DNA recognition sequences for the PAX3 paired domain. Gene 162: 267-270, 1995.
7. Chalepakis G, Jones FS, Edelman GM and Gruss P: PAX-3 contains domains for transcription activation and transcription inhibition. Proc Natl Acad Sci USA 91: 12745-12749, 1994.
8. Epstein DJ, Vogan KJ, Trasler DG and Gros P: A mutation within intron 3 of the Pax-3 gene produces the clinically splice-variants of PAX3 and PAX7 forms in myogenic and neural tumor cell lines. Cancer Res 59: 5432-5436, 1999.
9. Medic S, Rizos H and Ziman M: Differential PAX3 functions in normal skin melanocytes and melanoma cells. Biochem Biophys Res Commun 411: 832-837, 2011.
10. Barber TD, Barber MC, Cloutier TE and Friedman TB: PAX3 gene structure, alternative splicing and evolution. Gene 237: 311-319, 1999.
11. Barr FG, Fitzgerald JC, Ginsberg JP, Vanella ML, Davis RJ and Bemecelli JL: Predominant expression of alternative PAX3 and PAX7 forms in myogenic and neural tumor cell lines. Cancer Res 59: 5443-5448, 1999.
12. Takeuchi H, Morton DL, Kuo C, Turner RR, Elashoff D, Elashoff R, Tsaback B, Fujimoto A and Hoon DS: Prognostic significance of molecular upstaging of paraffin embedded sentinel lymph nodes in melanoma patients. J Clin Oncol 22: 2671-2680, 2004.
13. Galibert MD, Yavuzer U, Dexter TJ and Goding CR: PAX3 and regulation of the melanocyte-specific tyrosinase-related protein-1 promoter. J Biol Chem 274: 26904-26900, 1999.
14. Reid LL, Millward P, Pardoe R, Lee M, Frank MH, Ireland A, Monshizadeh L, Rai T, Heenan P, Medic S, et al: Markers of circulating tumour cells in the peripheral blood of patients with melanoma correlate with disease recurrence and progression. Br J Dermatol 168: 85-92, 2013.
15. Kopic JD, Little EC, Lui JW, Izuka T and Lang D: PAX3 and ETS1 synergistically activate MET expression in melanoma cells. Oncogene 34: 4964-4974, 2015.
16. Kubic JD, Lui JW, Little EC, Ludvik AE, Kondo S, Salgia R, Aplin AE and Lang D: PAX3 and FOXD3 promote CXCR4 expression in melanoma. J Biol Chem 290: 21901-21914, 2015.
17. Cao J, Dai X, Wan L, Wang H, Zhang J, Goff PS, Sviderskaya EV, Xuan Z, Xu Z, Xu X, et al: The E3 ligase APC/C(Cdh1) promotes ubiquitination-mediated proteolysis of PAX3 to suppress melanocyte proliferation and melanoma growth. Sci Signal 8: r87, 2015.
18. Eyangar AS, Miller PJ, Loupe JM and Hollembach AD: Phosphorylation of PAX3 contributes to melanoma phenotypes by affecting proliferation, invasion, and transformation. Pigment Cell Melanoma Res 27: 846-848, 2014.
19. Haqq C, Nosrati M, Sudilovsky D, Crothers J, Khodakabakhsh D, Pulliam BL, Federman S, Miller JR III, Allen RE, Singer ML, et al: The gene expression signatures of melanoma progression. Proc Natl Acad Sci USA 102: 6092-6097, 2005.
20. RIker AI, Enkemann SA, Fodstad O, Liu S, Ren S, Morris C, Xi Y, Howell P, Metge B, Samant RS, et al: The gene expression profiles of primary and metastatic melanoma yields a transition point of tumor progression and metastasis. BMC Genomics 1: 13, 2008.
21. Talantov D, Mazumder A, Yu JX, Briggs T, Jiang Y, Backus J, Atkins D and Wang Y: Novel genes associated with malignant melanoma but not benign melanocytic lesions. Clin Cancer Res 11: 7234-7242, 2005.
22. Lu X, Meng X, Sun G, Lu Z, Xu X, Wang Z, Wang J, Wang H, Wang C and Wang D: PAX3 and FOXD3 synergistically activate MET expression in melanoma cells. J Exp Clin Cancer Res 34: 100, 2015.
23. Pulliam BL, Federman S, Miller JR III, Allen RE, Singer ML, et al: The gene expression signatures of melanoma progression. Proc Natl Acad Sci USA 102: 6092-6097, 2005.
35. Liu F, Cao J, Wu J, Sullivan K, Shen J, Ryu B, Xu Z, Wei W and Cui R: Stat3-targeted therapies overcome the acquired resistance to vemurafenib in melanomas. J Invest Dermatol 133: 2041-2049, 2013.

36. Smith MP, Ferguson J, Arozarena I, Hayward R, Marais R, Chapman A, Hurlstone A and Wellbrock C: Effect of SMURF2 targeting on susceptibility to MEK inhibitors in melanoma. J Natl Cancer Inst 105: 33-46, 2013.

37. Barlett D, Boyle GM, Ziman M and Medic S: Mechanisms contributing to differential regulation of PAX3 downstream target genes in normal human epidermal melanocytes versus melanoma cells. PLoS One 10: e0124154, 2015.

38. He S, Li CG, Slobbe L, Glover A, Marshall E, Baguley BC and Eccles MR: PAX3 knockdown in metastatic melanoma cell lines does not reduce MITF expression. Melanoma Res 21: 24-34, 2011.

39. He SJ, Stevens G, Braithwaite AW and Eccles MR: Transfection of melanoma cells with antisense PAX3 oligonucleotides additionally complements cisplatin-induced cytotoxicity. Mol Cancer Ther 4: 996-1003, 2005.

40. Scholl FA, Kamarasev J, Murrmann OV, Geertsen R, Dummer R and Schilder BW: PAX3 is expressed in human melanomas and contributes to tumor cell survival. Cancer Res 61: 823-826, 2001.

41. Autilio C, Paolillo C, Laveri MM, Pocino K, De Paolis E, Di Stasio E, Marchetti P, Gian Carlo CA and Capoluongo E: PAX3-d mRNA over 2.76 copies/µl in the bloodstream predicts cutaneous malignant melanoma relapse. Oncotarget 8: 85479-85491, 2017.

42. Wang Q, Fang WH, Krupinski J, Kumar S, Slevin M and Kumar P: Pax genes in embryogenesis and oncogenesis. J Cell Mol Med 12: 2281-2294, 2008.

43. Bondurand N, Pinguault V, Goericke DE, Lemort N, Sock E, Le Caignec C, Wegner M and Goossens M: Interaction among MITF, SNAI2, KIT, EDN3 and EDNRB genes. Postepy Hig Med Dosw (Online) 67: 1109-1118, 2013 (In Polish).

44. Pingault V, Ente D, Dastot-Le Moal F, Goossens M, Marlin S and Bondurand N: Review and update of mutations causing Waardenburg syndrome. Hum Mutat 31: 391-406, 2010.

45. Otręba M, Rok J, Buszman E and Wrześniok D: Regulation of melanogenesis: The role of CAMP and MITF. Postepy Hig Med Dosw (Online) 66: 33-40, 2012 (In Polish).

46. Lin JY and Fisher DE: Melanocyte biology and skin pigmentation. Nature 445: 843-850, 2007.

47. Li WQ, Cho E, Weinstock MA, Mashiq H and Qureshi AA: Epidemiological assessments of skin outcomes in the nurses' health studies. Am J Public Health 106: 1677-1683, 2016.

48. Zhang M, Qureshi AA, Geller AC, Frazier L, Hunter DJ and Han J: Use of tanning beds and incidence of skin cancer. J Clin Oncol 30: 1588-1593, 2012.

49. Li WQ, Qureshi AA, Ma J, Goldstein AM, Giovannucci EL, Stampfer MJ and Han J: Personal history of prostate cancer and increased risk of incident melanoma in the United States. J Clin Oncol 31: 4394-4399, 2013.

50. Nair-Shalliker V, Egger S, Chrzanowska A, Mason R, Waite L, Le Couteur D, Seibl MJ, Handelsman DJ, Cumming R, Smith DP and Armstrong BK: Associations between sun sensitive pigmentary genes and serum prostate specific antigen levels. PLoS One 13: e0193893, 2018.

51. Chia SE, Wong KY, Cheng C, Lau W and Tan PH: Sun exposure and the risk of prostate cancer in the singapore prostate cancer study: A case-control study. Asian Pac J Cancer Prev 13: 3179-3185, 2012.

52. Nair-Shalliker V, Smith DP, Egger S, Hughes AM, Kaldor JM, Clements M, Kricker A and Armstrong BK: Sun exposure may increase risk of prostate cancer in the high UV environment of New South Wales, Australia: A case-control study. Int J Cancer 131: E726-E732, 2012.

53. Bonilla C, Gilbert R, Kemp JP, Timpson NJ, Evans DM, Donovan JL, Hamdy FC, Neal DE, Fraser WD, Davey SG, et al.: Using genetic proxies for lifecourse sun exposure to assess the causal relationship of sun exposure with circulating vitamin d and prostate cancer risk. Cancer Epidemiol Biomarkers Prev 22: 597-606, 2013.

54. Kocarnik JM, Park SL, Han J, Dumitrescu L, Cheng I, Wilkens LR, Schumacher FR, Kolonel L, Carlson CS, Crawford DC, et al.: Replication of associations between GWAS SNPs and melanoma risk in the Population Architecture Using Genomics and Epidemiology (PAGE) Study. J Invest Dermatol 134: 2049-2052, 2014.

55. Hernandez B, Ibarrola-Villava M, Fernandez LP, Peña-Chile M, Llorca-Cardeñosa M, Oltra SS, Alonso S, Boyano MD, Martinez-Cadenas C and Ribas G: Sex-specific genetic effects associated with pigmentation, sensitivity to sunlight, and melanoma in a population of Spanish origin. Biol Sex Differ 7: 17, 2016.