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ORIGINAL ARTICLE

Immunohistochemical evaluation of vitamin D receptor (VDR) expression in cutaneous melanoma tissues and four VDR gene polymorphisms

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ABSTRACT

Objective: Vitamin D receptor (VDR) mediates vitamin D activity. We examined whether VDR expression in excised melanoma tissues is associated with VDR gene (*VDR*) polymorphisms.

Methods: We evaluated VDR protein expression (by monoclonal antibody immunostaining), melanoma characteristics, and carriage of *VDR*-FokI-rs2228570 (C>T), *VDR*-BsmI-rs1544410 (G>A), *VDR*-ApaI-rs7975232 (T>G), and *VDR*-TaqI-rs731236 (T>C) polymorphisms (by restriction fragment length polymorphism). Absence or presence of restriction site was denoted by a capital or lower letter, respectively: "F" and "f" for FokI, "B" and "b" for BsmI, "A" and "a" for ApaI, and "T" and "t" for TaqI endonuclease. Seventy-four Italian cutaneous primary melanomas (52.1±12.7 years old) were studied; 51.4% were stage I, 21.6% stage II, 13.5% stage III, and 13.5% stage IV melanomas. VDR expression was categorized as follows: 100% positive *vs*. <100%; over the median 20% (high VDR expression) *vs*. ≤20% (low VDR expression); absence *vs*. presence of VDR-expressing cells.

Results: Stage I melanomas, Breslow thickness of <1.00 mm, level II Clark invasion, Aa heterozygous genotype, and AaTT combined genotype were more frequent in melanomas with high *vs*. low VDR expression. Combined genotypes BbAA, bbAa, AATt, BbAATt, and bbAaTT were more frequent in 100% *vs*. <100% VDR-expressing cells. Combined genotype AATT was more frequent in melanomas lacking VDR expression (odds ratio=14.5; *P*=0.025). VDR expression was not associated with metastasis, ulceration, mitosis >1, regression, tumor-infiltrating lymphocytes, tumoral infiltration of vascular tissues, additional skin and non-skin cancers, and melanoma familiarity.

Conclusions: We highlighted that *VDR* polymorphisms can affect VDR expression in excised melanoma cells. Low VDR expression in AATT carriers is a new finding that merits further study. VDR expression possibly poses implications for vitamin D supplementation against melanoma. VDR expression and *VDR* genotype may become precise medicinal tools for melanoma in the future.

KEYWORDS Vitamin D receptor; VDR protein expression; VDR polymorphism; cutaneous melanoma; metastatic melanoma; skin cancer; predictive biomarkers; FokI polymorphism

Introduction

Cutaneous melanoma incidence continually increases in developed countries, particularly in fair-skinned individuals[1-4]. Recent data[5] indicated that prevalence of melanoma in Northern Italy is two fold higher than in Southern Italy and with Central Italy showing prevalence at intermediate level. High incidence rates were particularly registered in the Northeast Friuli-Venezia Giulia region (19.6/100,000/year in men; 16.4/100,000/year in women)[6], implying that geographically detailed studies should be performed regarding melanoma risk factors[7].

Melanoma is the leading cause of mortality among skin cancer patients with low survival rates[8]. Although new therapeutic treatments are available[9], early detection and surgery remain the main treatment options. Therefore, every new discovery on melanoma biological pathways presents opportunity in improving management and treatment options. Considerable preclinical and epidemiologic data suggest that vitamin D may play an important role in cancer

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pathogenesis and progression[10]. Numerous preclinical studies specifically indicated that exposure of cancer cells to high concentrations of vitamin D metabolites halts progression through the cell cycle, induces apoptosis, and slows down or stops tumor growth[11]. Vitamin D also enhances antitumor activity of some cytotoxic anticancer agents in *in vivo* preclinical models[12]. Anti-proliferative effects of vitamin D for cancer prevention and treatment were explored in several malignancies, including skin, breast, prostate, colorectal, and other cancers[8,13-18]. Numerous epidemiological studies supported the hypothesis that individuals with lower serum vitamin D levels feature higher exposure risk to different cancers[12]. Current literature suggests the chemopreventive role of vitamin D by acting against initiation and progression of tumorigenesis[8,12-18]. Despite the stronger consensus on protective role of vitamin D against cancer, particularly by reduction of mortality rate, its therapeutic function for cancer patients remains debatable[10,19]. Remarkably, a recent meta-analysis showed that vitamin D supplementation minimally affects total cancer incidence, even when total cancer mortality is significantly reduced[20].

Vitamin D receptor (VDR) is a nuclear transcription factor belonging to the nuclear receptor superfamily that binds 1α, 25-dihydroxyvitamin D (calcitriol) with high affinity and specificity[19]. Upon binding to the active form of vitamin D, VDR translocates from cytoplasm into the nucleus and binds to vitamin D responsive elements (VDREs), thus up- or down-regulating hundreds of genes directly controlled by vitamin D[19,21,22]. Increasing evidence showed pleiotropic hormonal effects of vitamin D on calcium and skeletal metabolism, immunological responses, detoxification, oxidative stress, cancer-related metabolic pathways, proliferation, and cell differentiation[18,19,21].

VDR is abundantly expressed in the skin[19,21]. Some VDR expression was also reported to occur in cultured melanoma cells[11,13,14,23]. Intermittent sun exposure or ultraviolet (UV) radiation and sunburns are known environmental risk factors for melanoma[24,25]. However, chronic and continuous UV radiation exposure activates vitamin D biosynthesis, which in turn can develop a protective action against tumoral proliferation[8,12,16,25]. Four recently discovered mechanisms may underlie actions of VDR as a tumor suppressor in the skin[10].

Human *VDR* gene is located on chromosome 12q12-q14 and comprises 11 exons and 11 introns[26]. Genetic variants of *VDR* may modulate its actions, with FokI, BsmI, ApaI, and TaqI being the most studied single-nucleotide polymorphisms (SNPs)[26-28]. *VDR*-FokI polymorphism is a functional SNP that extends lengths of the receptor protein from 424 to 427 amino acid residues. BsmI, ApaI, and TaqI polymorphisms are located in the 3′ terminal region of the *VDR* gene and do not affect protein sequence of the VDR receptor. FokI polymorphism is reported not to be in linkage disequilibrium with the other three polymorphisms. Instead, BsmI, ApaI, and TaqI polymorphisms are reported to be in linkage disequilibrium to a variable extent; thus, combined genotypes including two or three of these polymorphisms were investigated in literature[26,28,29]. Some evidence suggested that genotypes FF, BB, tt, and the combined genotype BBAAtt may be associated with increased expression of VDR, which in turn regulates actions of vitamin D[26-28]. Roles of *VDR* polymorphisms in melanoma were evaluated in some recent studies and meta-analyses[30-37]. However, associations of *VDR* polymorphisms with skin cancer risk remain insufficiently characterized[30,31,36].

At present, no study examined *VDR* polymorphisms and VDR expression in melanoma cells of excised tissues from patients.

Thus far, only one cohort of 69 Polish melanoma patients was investigated by two studies for VDR expression in tumor tissues[38,39]. VDR expression progressively decreases from normal skin to melanocytic nevi to melanomas[38], suggesting the relationship between VDR expression and melanoma prognosis[39]. Brożyna and colleagues[38] observed reduced expression levels of VDR in skin surrounding nevi and melanomas as opposed to normal skin.

Advances in melanoma treatment can be achieved through developments in understanding of melanoma risk factors, genomics, and molecular pathogenesis[31,40].

By immunohistochemical staining of primary cutaneous melanoma tissues, we investigated VDR expression in relation to characteristics, melanoma histological grading, and metastatic stage of patients. We also explored the association of four *VDR* SNPs- FokI-rs2228570 C>T located in exon 2, BsmI-rs1544410 G>A located in intron 8, ApaI-rs7975232 T>G located in intron 8, and TaqI-rs731236 T>C located in exon 9- with VDR expression levels in cutaneous malignant melanoma tissues.

Patients and methods

Patients

Enrolment and clinical visits of all study participants were performed at the Udine University-Hospital Dermatology Clinic. Diagnostic procedures were conducted according to routine protocols. All participants signed a written informed

consent. The Udine Institutional Ethical Committee approved the study protocol in accordance to the Declaration of Helsinki.

Seventy-four (39 males, 35 females, age range: 29-82 years) unrelated patients (hospitalized or outpatients) who consecutively underwent surgical excision of cutaneous melanoma were enrolled based on a retrospective design. Inclusion criteria were as follows: melanoma different from that in *in situ* only, absence of mucosal melanomas, patient is a resident of Friuli-Venezia Giulia region (Northern Italy), and absence of major chronic diseases, such as autoimmune diseases, and type 1 diabetes.

Assessment of melanoma diagnosis and patient stage classification were performed by clinical/histological findings as described by Balch et al.[41]. For patients with multiple melanomas, we examined only the first main melanoma according to histological assessment of major primary tumor grading, and primary melanoma characteristics were accounted for study analyses.

Questionnaires were used to collect information from each participant; data obtained included demographic and lifestyle characteristics and medical and family history of melanoma. Body mass index (BMI) was determined by ratio of weight (kg) to squared height (m).

Immunohistochemical staining and evaluation of VDR expression

Slides stained with hematoxylin and eosin were reviewed for each case from formalin-fixed and paraffin-embedded blocks and were selected for VDR immunohistochemical staining. Immunohistochemistry was performed on 5 μm thick paraffin sections as follows: after dewaxing, rehydration and endogenous peroxidase quenching with 3% v/v H_2O_2 in methanol for 15 min, antigen retrieval in 0.01 M citrate buffer at 98°C water bath for 40 min, application and incubation of primary antibody (VDR mouse monoclonal D-6, sc-13133, Santa Cruz Biotechnology, Texas, USA) at 1:200 dilution 1 h at room temperature[42,43], incubation with peroxidase-based EnVision+ /Horseradish peroxidase (Dako A/S, Glostrup, Denmark) for 30 min at room temperature, and treatment with diaminobenzidine for 3 min[44]. The sections were then counterstained with Mayer's hematoxylin, dehydrated, and mounted. VDR expression was evaluated on tumor cells (nuclear and cytoplasmic staining) of the whole section. Immunolabeled sections were viewed under Nikon Eclipse 80i light microscope at 25× magnification, and a semi-quantitative evaluation was performed to determine VDR expression levels in malignant melanoma cells (nuclear and cytoplasmic staining). Staining of sweat gland cells, which consistently showed strong and diffused positivity throughout all samples, was used as positive reference. The term "emboli" indicates tumoral invasion of vascular cells observed in slide specimens. Melanoma specimens were reviewed by two pathologists involved in the study but were unaware of all other clinical and molecular data during evaluation. Our categorization choices were based on the following considerations: A) when melanomas feature 100% VDR-positive cells, all tumor cells are possibly responsive to vitamin D stimulation; B) when 0% cells are positive for VDR expression, virtually all tumor cells do not respond to vitamin D stimulation; C) aside from extreme conditions, a cut off at median percentage of positive cells can be reasonably used for evaluating the half of samples with higher *vs*. the half of samples with lower VDR expression. Therefore, results were ranked based on percentage of cells positive for VDR expression (irrespective of staining intensity). Cytoplasmic VDR expression ranged from 0% to 100%, with a median value at 20.0%. We categorized variables as follows: 100% cytoplasmic VDR-expression-positive cells versus all remaining melanomas; >20% cytoplasmic VDR-expression-positive cells (high VDR expression) *vs*. ≤20% positive cells (low VDR expression); and absent (0% positive cells) *vs*. present (>0% positive cells) VDR expression. Only 11 out of 74 cases (14.9%) showed nuclear VDR-positive immunostaining, and all these cases were categorized into >20% cytoplasmic VDR-expression-positive cells. Given the low number of melanoma specimens showing VDR expression detected in nuclei, this parameter was not further analyzed.

Determination of *VDR* gene polymorphisms

Determination of SNP *VDR*-FokI (C>T), *VDR*-BsmI (G>A), *VDR*-ApaI (T>G), *VDR*-TaqI (T>C) was performed as previously described[29,45] after extraction of genomic DNA from ethylenediaminetetraacetic-acid -venous blood samples[46]. Absence or presence of restriction site was denoted by a capital or lower letter, respectively: "F" and "f" for FokI, "B" and "b" for BsmI, "A" and "a" for ApaI, and "T" and "t" for TaqI endonucleases[28,29]. FokI, BsmI, ApaI and TaqI polymorphisms of *VDR* were studied using previously tested primers[29,45] to amplify appropriate DNA fragments. FokI enzyme (Euroclone, Milano, Italy) digestion of 265 bp amplified DNA was used to determine FokI restriction fragment length polymorphism (RFLP) yielding 196 and 69 bp fragments in the presence of f allele[46]. To analyze BsmI polymorphism, the resulting amplified 825 bp polymerase

chain reaction (PCR) fragment was digested with BsmI restriction enzyme (Euroclone, Milano, Italy), generating 650 and 175 bp fragments in the presence of b allele[29]. ApaI digestion of the 740 bp amplified DNA was used to determine both ApaI and TaqI RFLP, generating 530 and 210 bp fragments in the presence of a allele. Digestion with TaqI of the 740 bp PCR fragment generated 290, 245, and 205 bp fragments in the presence of t allele and 495 and 245 bp fragments in its absence (T allele) owing to an additional monomorphic TaqI site[29]. DNA fragments were separated by polyacrylamide gel electrophoresis.

Statistical analysis

Kolmogorov-Smirnov test was used to assess normal data distribution. Percentage of VDR-positive cells by immunohistochemical staining was not normally distributed. Thus, median values and ranges were reported for this variable. Mann-Whitney U test was used to assess differences between groups. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to determine association of groups with different VDR expression and melanoma characteristics, alleles, genotypes, and combined genotypes. Our study was explorative as no previous investigation determined frequencies of VDR polymorphisms in melanoma patients according to immunohistochemical findings of VDR expression Prior to study enrolment, we evaluated that a number of 70 subjects fitted the 80% power at an alpha level of 0.05 to detect differences between high (above the median) and low VDR expression melanoma groups whether OR value equals 3 or more for a SNP site[47]. Deviation tests from Hardy-Weinberg equilibrium (HWE) were performed using a separate Chi-square distribution for each SNP[29]. Linkage disequilibrium (LD) between SNPs was determined as described[29]. Two-sided significance level was set at 0.05, and P values $\leq$ 0.10 were considered as a tendency to be significant. Statistical software SPSS (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Results

VDR immunohistochemical staining

A significant variability in VDR immunohistochemical staining was observed in cytoplasm of melanoma cells, with 16.2% (12/74) of patients tested positive for 100% melanoma cell staining, whereas 20.3% (15/74) were tested negative. Median percentage value of VDR-positive staining reached 20.0%. Figures 1, 2, and 3 display representative images of VDR immunohistochemical staining in excised tissues from patients with primary cutaneous melanoma, showing 100% staining, intermediate staining, and no staining, respectively.

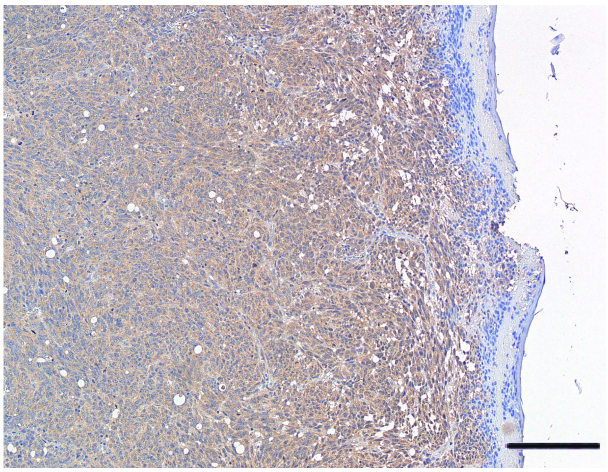

Figure 1 Representative image of VDR protein expression in cutaneous melanoma tissue. Melanoma showing diffuse positivity for VDR expression. Eroded epidermis (H&E staining, 25×. Bar indicates 50 μm).

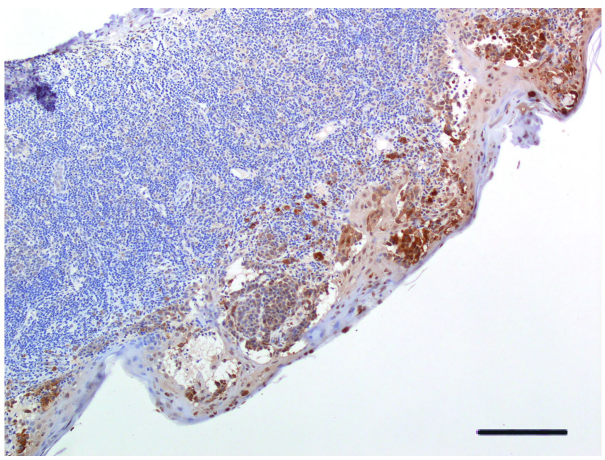

Figure 2 Representative image of VDR protein expression in cutaneous melanoma tissue. Melanoma featuring tumor-infiltrating lymphocytes (TILs). Intraepidermal component of lesion (at the bottom of image) shows strong positivity for VDR expression (H&E staining, 25×. Bar indicates 50 μm).

Patients and melanoma characteristics according to VDR expression

All 74 (35 females and 39 males) cutaneous melanoma patients were white residents in Northern Italy. Average age at melanoma diagnosis was 52.1±12.7 years, with 27%

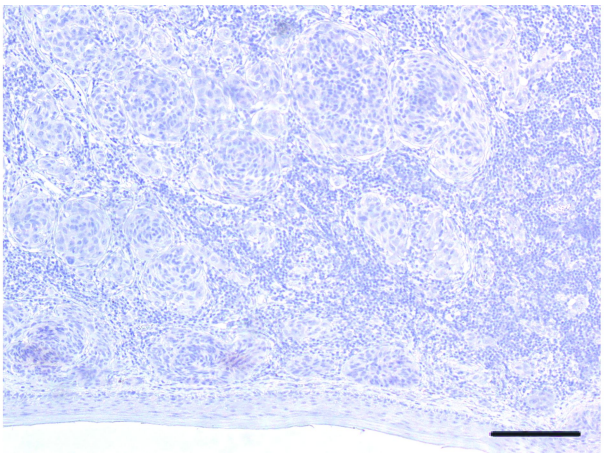

Figure 3 Representative image of VDR protein expression in cutaneous melanoma tissue. Melanoma featuring TILs. In this case, melanoma cells nesting in the dermis are negative throughout the whole lesion (H&E staining, 25×. Bar indicates 50 µm).

(20/74) of patients exhibiting metastatic melanoma (i.e., stages III and IV), and all showed Clark level higher than Clark I invasion.

Table 1 shows the main clinical characteristics of melanoma patients and compares three different binary categories according to percentage of tumor cells positive for VDR staining: a) 100% positive *vs.* <100% positive, b) over 20% (median value) positive (high VDR expression) *vs.* ≤ 20% positive (low VDR expression), and c) absence *vs.* presence of VDR-positive cells. Complete data on ORs and CIs are reported on **Table S1**. Age of melanoma diagnosis, gender, BMI, and smoking did not differ between compared groups. Differences were not significant in comparison of 12 subjects with 100% VDR-positive cells *vs.* 62 subjects with <100% VDR-positive cells. The following statically significant differences were observed during comparison of 36 melanomas with high VDR expression *vs.* 38 melanomas with low VDR expression: stage I was more frequent in the former (63.9% *vs.* 39.5%, OR=2.71, CI=1.06–6.95, *P*=0.036), whereas stage II was less frequent (11.1% *vs.* 31.6%, OR=0.27, CI=0.08–0.94, *P*=0.033); the former presents a lower mean Breslow thickness (1.23±0.88 *vs.* 2.27±1.97 mm, *P*=0.008) and more frequent Breslow thickness of <1.00 mm (50.0% *vs.* 26.3%, OR=2.80, CI=1.06–7.41, *P*=0.036) and less frequent Breslow thickness ≥1.01 mm (50.0% *vs.* 77.8%, OR=0.36, CI=0.13–0.95, *P*=0.036). A higher frequency of Clark II invasion (38.9% *vs.* 15.8%, OR=3.39, CI=1.13–10.2, *P*=0.025) was observed in high- than low-VDR-expression group. Superficial spreading was present in 61.1% of melanomas with high VDR expression *vs.* 39.5% of low-VDR-expression melanomas, where this difference did not reach significant *P* values (*P*=0.063). In comparing absence *vs.* presence of VDR-positive immunohistochemical staining of melanoma cells, the only significant finding was a higher frequency of stage II A in the former *vs.* the latter (26.7% *vs.* 5.1%, OR=6.79, CI=1.33–34.7, *P*=0.028) (all sub-stage data are shown in **Table S1**).

Overall, as shown in **Table 1**, none of the tumor markers commonly associated with severe prognosis and metastatic stage were associated with VDR immunohistochemical staining; these markers included ulceration, mitosis >1, absence of tumor-infiltrating lymphocytes (TILs), emboli, and epithelioid variants. The presence of multiple melanomas, additional skin, non-skin cancers, and melanoma familiarity did not correlate with VDR immunohistochemical staining.

By further analysis, median VDR expression did not differ between 20 metastatic melanomas (median: 17.5%, range: 0%–100% VDR-positive cells) and 54 non-metastatic melanomas (median: 25.0%, range: 0%–100% VDR-positive cells), with *P*=0.796. Significant *P* value (*P*=0.095) was not observed in differences in median values of VDR expression of stage I melanomas compared with those of stages II+III+IV. Median values of VDR expression of Clark II (in our cohort, none of melanomas showed Clark I invasion) melanomas were significantly higher compared with Clark III+IV+V levels (median: 70.0%, range: 0%–100% *vs.* median: 10.0%, range: 0%–100% VDR-positive cells, *P*=0.019). A significant *P* value was not observed in differences in median values of VDR expression in superficially spreading melanomas compared with the remaining ones (*P*=0.075).

Patient and melanoma characteristics according to *VDR* polymorphisms

Table 2 shows *VDR* polymorphism genotypes, alleles, and combined genotype frequencies in all 74 patients and in groups of melanomas categorized according to VDR immunohistochemical staining. Each *VDR* polymorphism of FokI, BsmI, ApaI, and TaqI was in HWE. FokI SNP was not in LD with other SNPs. BsmI was in LD with ApaI and TaqI, and ApaI was in LD with TaqI. Thus, for further analyses, we considered binary and ternary combination of genotypes comprising BsmI, ApaI and TaqI polymorphisms. As observed in other studies[26,29], not all theoretically possible binary and ternary combination of genotypes were observed; thus, **Table 2** reports only combined genotypes with at least one confirmed finding.

Differences between VDR expression groups were not

Table 1 Clinical characteristics of 74 consecutively enrolled melanoma patients and comparison between groups of 100% VDR-positive cells (*n*=12) *vs*. <100% (*n*=62); over the median (>20%) VDR-positive cells (*n*=36) *vs*. below or equal the median (≤20%) (*n*=38); and absence of VDR-positive cell (*n*=15) *vs*. remaining cases with detected VDR expression (*n*=59).

Characteristics	All melanoma patients (*n*=74)	100% VDR-positive cells (*n*=12)	<100% VDR-positive cells (*n*=62)	*P*=100% *vs*. <100% VDR-positive	>20%[a] VDR-positive cells (*n*=36)	≤20% VDR-positive cells (*n*=38)	*P*>20% *vs*. ≤ 20% VDR-positive	VDR absence (0% positive) (*n*=15)	VDR presence (>0% positive) (*n*=59)	PVDR absence *vs*. presence
Age at melanoma diagnosis, years, mean ± SD	52.1±12.7	49.8±13.0	52.7±12.9	0.519[c]	51.7±13.9	52.8±12.0	0.770[c]	51.5±14.5	52.5±12.6	0.767[c]
Age <50 years at melanoma diagnosis	31 (41.9)	6 (50.0)	25 (40.3)	0.534	16 (44.4)	15 (39.5)	0.665	7 (46.7)	24 (40.7)	0.675
BMI, kg/m^2	25.8±4.0	25.0±4.6	25.9±3.9	0.412[c]	26.0±4.3	25.6±3.8	0.804[c]	25.7±2.6	25.8±4.3	0.762[c]
Male	39 (52.7)	6 (50.0)	33 (53.2)	0.838	20 (55.6)	19 (50.0)	0.632	9 (60.0)	30 (50.8)	0.526
Smoker	11 (14.9)	2 (16.7)	9 (14.5)	1.000	4 (11.1)	7 (18.4)	0.377	2 (13.3)	9 (15.3)	1.000
Stage I	38 (51.4)	6 (50.0)	32 (51.6)	0.919	23 (63.9)	15 (39.5)	0.036	6 (40.0)	32 (54.2)	0.325
Stage II	16 (21.6)	2 (16.7)	14 (22.6)	1.000	4 (11.1)	12 (31.6)	0.033	6 (40.0)	10 (16.9)	0.077
Stage III	10 (13.5)	3 (25.0)	7 (11.3)	0.351	6 (16.7)	4 (10.5)	0.510	1 (6.7)	9 (15.3)	0.676
Stage IV	10 (13.5)	1 (8.3)	9 (14.5)	1.000	3 (8.3)	7 (18.4)	0.310	2 (13.3)	8 (13.6)	1.000
Metastatic melanoma (Stage III +IV)	20 (27.0)	4 (33.3)	16 (25.8)	0.724	9 (25.0)	11 (28.9)	0.702	3 (20.0)	17 (28.8)	0.746
Trunk	47 (63.5)	5 (41.7)	42 (67.7)	0.108	24 (66.7)	23 (60.5)	0.583	7 (46.7)	40 (67.8)	0.129
Upper limb	6 (8.1)	2 (16.7)	4 (6.5)	0.249	2 (5.6)	4 (10.5)	0.675	3 (20.0)	3 (5.1)	0.093
>Lower limb	14 (18.9)	3 (25.0)	11 (17.7)	0.687	7 (19.4)	7 (18.4)	0.911	4 (26.7)	10 (16.9)	0.463
Hands/feet	5 (6.8)	2 (16.7)	3 (4.8)	0.183	2 (5.6)	3 (7.9)	1.000	0 (–)	5 (8.5)	0.576
Head/neck	2 (2.7)	0 (–)	2 (3.2)	1.000	1 (2.8)	1 (2.6)	1.000	1 (6.7)	1 (1.7)	0.367
Superficial spreading	37 (50.0)	7 (58.3)	30 (48.4)	0.528	22 (61.1)	15 (39.5)	0.063	6 (40.0)	31 (52.5)	0.386
Nodular	31 (41.9)	4 (33.3)	27 (43.5)	0.512	12 (33.3)	19 (50.0)	0.146	8 (53.3)	23 (39.0)	0.314
Acral lentiginous	3 (4.1)	1 (8.3)	2 (3.2)	0.417	1 (2.8)	2 (5.3)	1.000	0 (–)	3 (5.1)	1.000
Spitzoide	2 (2.7)	0 (–)	2 (3.2)	1.000	1 (2.8)	1 (2.6)	1.000	1 (6.7)	1 (1.7)	0.367
Others	3 (4.1)	0 (–)	3 (4.8)	1.000	0 (–)	3 (7.9)	0.240	0 (–)	3 (5.1)	1.000
Breslow thickness, mm, mean ± SD	1.77±1.62	1.34±0.94	1.85±1.71	0.416[c]	1.23±0.88	2.27±1.97	0.008[c]	2.16±1.88	1.66±1.55	0.145[c]

Continued

Continued

Characteristics	All melanoma patients (*n*=74)	100% VDR-positive cells (*n*=12)	<100% VDR-positive cells (*n*=62)	*P*=100% *vs.* <100% VDR-positive	>20%[a] VDR-positive cells (*n*=36)	≤20% VDR-positive cells (*n*=38)	*P*>20% *vs.* ≤ 20% VDR-positive	VDR absence (0% positive) (*n*=15)	VDR presence (>0% positive) (*n*=59)	PVDR absence *vs.* presence
Breslow thickness <1.00 mm	28 (37.8)	4 (33.3)	24 (38.7)	1.000	18 (50.0)	10 (26.3)	0.036	4 (26.7)	24 (40.7)	0.318
Breslow thickness ≥1.01 mm	46 (62.2)	8 (66.7)	38 (61.3)	1.000	18 (50.0)	28 (77.8)	0.036	11 (73.3)	35 (59.3)	0.318
Clark I	0 (–)	0 (–)	0 (–)		0 (–)	0 (–)	–[b]	0 (–)	0 (–)	–[b]
Clark II	20 (27.0)	5 (41.7)	15 (24.2)	0.287	14 (38.9)	6 (15.8)	0.025	2 (13.3)	18 (30.5)	0.328
Clark III	15 (20.3)	1 (8.3)	14 (22.6)	0.439	6 (16.7)	9 (23.7)	0.453	4 (26.7)	11 (18.6)	0.488
Clark IV	36 (48.6)	6 (50.0)	30 (48.4)	0.919	15 (41.7)	21 (55.3)	0.242	8 (53.3)	28 (47.5)	0.684
Clark V	2 (2.7)	0 (–)	2 (3.2)	1.000	0 (–)	2 (5.3)	0.494	1 (6.7)	1 (1.7)	0.367
Ulceration	25 (33.8)	5 (41.7)	20 (32.3)	0.525	11 (30.6)	14 (36.8)	0.568	5 (33.3)	20 (33.9)	0.967
Mitosis >1	51 (68.9)	8 (66.7)	43 (69.4)	1.000	23 (63.9)	28 (73.7)	0.363	12 (80.0)	39 (66.1)	0.365
Regression	12 (16.2)	1 (8.3)	11 (17.7)	0.677	6 (16.7)	6 (15.8)	0.919	4 (26.7)	8 (13.6)	0.248
Brisk positive TILs[c]	25 (33.8)	5 (41.7)	20 (32.3)	0.525	15 (41.7)	10 (26.3)	0.163	5 (33.3)	20 (33.9)	0.967
Non-brisk, TILs[c]	26 (35.1)	3 (25.0)	23 (37.1)	0.522	10 (27.8)	16 (42.1)	0.197	7 (46.7)	19 (32.2)	0.295
TIL absence[c]	23 (31.1)	4 (33.3)	19 (30.6)	1.000	11 (30.6)	12 (31.6)	0.924	3 (20.0)	20 (33.9)	0.365
Emboli	9 (12.2)	2 (16.7)	7 (11.3)	0.633	4 (11.1)	5 (13.2)	1.000	2 (13.3)	7 (11.9)	1.000
Microsatellitosis	2 (2.7)	0 (–)	2 (3.2)		1 (2.8)	1 (2.6)	1.000	0 (–)	2 (3.4)	1.000
Epithelioid variant	11 (14.9)	3 (25.0)	8 (12.9)	0.371	6 (16.7)	5 (13.2)	0.672	0 (–)	11 (18.6)	0.106
Fusate variant	4 (5.4)	0 (–)	4 (6.5)	1.000	3 (8.3)	1 (2.6)	0.351	0 (–)	4 (6.8)	0.576
Small-cell variant	2 (2.7)	0 (–)	2 (3.2)	1.000	1 (2.8)	1 (2.6)	1.000	1 (6.7)	1 (1.7)	0.367
>1 melanoma	9 (12.2)	1 (8.3)	8 (12.9)	1.000	4 (11.1)	5 (13.2)	1.000	2 (13.3)	7 (11.9)	1.000
Additional non-melanoma skin cancer	11 (14.9)	2 (16.7)	9 (14.5)	1.000	6 (16.7)	5 (13.2)	0.672	3 (20.0)	8 (13.6)	0.684
Additional non-skin cancer	14 (18.9)	3 (25.0)	11 (17.7)	0.687	9 (25.0)	5 (13.2)	0.194	3 (20.0)	11 (18.6)	1.000
Melanoma familiarity	13 (17.6)	3 (25.0)	10 (16.1)	0.432	8 (22.2)	5 (13.2)	0.306	1 (6.7)	12 (20.3)	0.282

[a] Two-tailed Mann–Whitney U test. [b] OR uncountable because one or two of compared groups included zero subject. [c] TILs, tumor infiltrating lymphocytes.

Table 2 Genotype and allele of ApaI, VDR-polymorphism, and BsmI-ApaI, ApaI-TaqI, BsmI-ApaI-TaqI combined genotypes of 74 melanoma patients and comparisons between groups of 100% VDR-positive cells (*n*=12) *vs*. <100% (*n*=62); over the median (>20%) VDR-positive cells (*n*=36) *vs*. below or equal the median (≤20%) (*n*=38); and absence of VDR-positive cell (*n*=15) *vs*. remaining cases with detected VDR expression (*n*=59).

VDR genotype or combined genotype	All melanoma patients (*n*=74)	100% VDR-positive cells (*n*=12)	<100% VDR-positive cells (*n*=62)	*P*=100% *vs*.<100% VDR-positive	>20%[a] VDR-positive cells (*n*=36)	≤20% VDR-positive cells (*n*=38)	*P*>20% *vs*. ≤20% VDR-positive	VDR absence (0% positive) (*n*=15)	VDR presence (>0% positive) (*n*=59)	PVDR absence *vs*. presence
ApaI genotype										
AA	30 (40.5)	8 (66.7)	22 (35.5)	0.058	13 (36.1)	17 (44.7)	0.450	8 (53.3)	22 (37.3)	0.258
Aa	36 (48.6)	4 (33.3)	32 (51.6)	0.246	22 (61.1)	14 (36.8)	0.037	5 (33.3)	31 (52.5)	0.184
aa	8 (10.8)	0 (–)	8 (12.9)	0.339	1 (2.8)	7 (18.4)	0.056^	2 (13.3)	6 (10.2)	0.660
A allele	96/148 (64.9)	20/24 (83.3)	76/124 (61.3)	0.038	48/72 (66.7)	48/76 (63.2)	0.655	21/30 (70.0)	75/118 (63.6)	0.509
a allele	52/148 (35.1)	4/24 (16.7)	48/124 (38.7)	0.038	24/72 (33.3)	28/76 (36.8)	0.655	9/30 (30.0)	43/118 (36.4)	0.509
BsmI-ApaI combined genotype										
BBAA	23 (31.1)	5 (41.7)	18 (29.0)	0.498	9 (25.0)	14 (36.8)	0.271	6 (40.0)	17 (28.8)	0.533
BbAA	5 (6.8)	3 (25.0)	2 (3.2)	0.028	4 (11.1)	1 (2.6)	0.194	1 (6.7)	4 (6.8)	1.000
BbAa	31 (41.9)	1 (8.3)	30 (48.4)	0.010	18 (50.0)	13 (34.2)	0.169	5 (33.3)	26 (44.1)	0.452
Bbaa	2 (2.7)	0 (–)	2 (3.2)	1.000	0 (–)	2 (5.3)	0.494	0 (–)	2 (3.4)	1.000
bbAA	2 (2.7)	0 (–)	2 (3.2)	1.000	0 (–)	2 (5.3)	0.494	1 (6.7)	1 (1.7)	0.367
bbAa	5 (6.8)	3 (25.0)	2 (3.2)	0.028	4 (11.1)	1 (2.6)	0.194	0 (–)	5 (8.5)	0.576
bbaa	6 (8.1)	0 (–)	6 (9.7)	0.581	1 (2.8)	5 (13.2)	0.200	2 (13.3)	4 (6.8)	0.595
ApaI-TaqI combined genotype										
AATT	4 (5.4)	0 (–)	4 (6.5)	1.000	0 (–)	4 (10.5)	0.115	3 (20.0)	1 (1.7)	0.025
AATt	14 (18.9)	6 (50.0)	8 (12.9)	0.008	9 (25.0)	5 (13.2)	0.194	4 (26.7)	10 (16.9)	0.463
AAtt	12 (16.2)	2 (16.7)	10 (16.1)	1.000	4 (11.1)	8 (21.1)	0.246	1 (6.7)	11 (18.6)	0.439
AaTT	12 (16.2)	3 (25.0)	9 (14.5)	0.399	9 (25.0)	3 (7.9)	0.046	1 (6.7)	11 (18.6)	0.439
AaTt	24 (32.4)	1 (8.3)	23 (37.1)	0.089	13 (36.1)	11 (28.9)	0.511	4 (26.7)	20 (33.9)	0.761
aaTT	8 (10.8)	0 (–)	8 (12.9)	0.339	1 (2.8)	7 (18.4)	0.056^	2 (13.3)	6 (10.2)	0.660
BsmI-ApaI-TaqI combined genotype										
BBAATT	1 (1.4)	0 (–)	1 (1.6)	1.000	0 (–)	1 (2.6)	1.000	1 (6.7)	0 (–)	0.203
BBAATt	10 (13.5)	3 (25.0)	7 (11.3)	0.351	5 (13.9)	5 (13.2)	1.000	4 (26.7)	6 (10.2)	0.110
BBAAtt	12 (16.2)	2 (16.7)	10 (16.1)	1.000	4 (11.1)	8 (21.1)	0.246	1 (6.7)	11 (18.6)	0.439
BbAATt	4 (5.4)	3 (25.0)	1 (1.6)	0.012	4 (11.1)	0 (–)	0.051	0 (–)	4 (6.8)	0.576
BbAATT	1 (1.4)	0 (–)	1 (1.6)	1.000	0 (–)	1 (2.6)	1.000	1 (6.7)	0 (–)	0.203
BbAaTT	7 (9.5)	0 (–)	7 (11.3)	0.590	5 (13.9)	2 (5.3)	0.255	1 (6.7)	6 (10.2)	1.000
BbAaTt	24 (32.4)	1 (8.3)	23 (37.1)	0.089	13 (36.1)	11 (28.9)	0.511	4 (26.7)	20 (33.9)	0.761
BbaaTT	2 (2.7)	0 (–)	2 (3.2)	1.000	0 (–)	2 (5.3)	0.494	0 (–)	2 (3.4)	1.000
bbAATT	2 (2.7)	0 (–)	2 (3.2)	1.000	0 (–)	2 (5.3)	0.494	1 (6.7)	1 (1.7)	0.367
bbAaTT	5 (6.8)	3 (25.0)	2 (3.2)	0.028	4 (11.1)	1 (2.6)	0.194	0 (–)	5 (8.5)	0.576
bbaaTT	6 (8.1)	0 (–)	6 (9.7)	0.581	1 (2.8)	5 (13.2)	0.200	2 (13.3)	4 (6.8)	0.595

[a] Over the median value of percentage (%) of cells positive for VDR protein. [b] OR uncountable because one or two of compared groups included zero subject. [c] *P* value by Mann–Whitney U test.

significant for single FokI, BsmI, and TaqI genotypes and alleles, and BsmI-TaqI combined genotypes (data shown in **Table S3**).

As reported in **Table 2** (data comprising all ORs and CIs are shown in **Table S2**. **Table S1** to **Table S3** in the supplementary materials, available with the full text of this article at www.cancerbiomed.org), heterozygous Aa genotype was more frequent in melanomas with high than low VDR expression (61.1% *vs*. 36.8%, OR=2.69, CI=1.05–6.90, *P*=0.037). A allele was found in 83.3% of 100% VDR-positive melanomas and in 61.3% of those with <100% VDR-positive cells (OR=3.16, CI=1.02–9.80, *P*=0.038).

By analyzing combined genotypes (**Table 2**), six significant differences were observed after comparing 100% *vs*. <100% VDR-expression-positive groups. Combined genotypes BbAA (OR=10.0, CI=1.46–68.3, *P*=0.028), bbAa (OR=10.0, CI=1.46–68.3, *P*=0.028), AATt (OR=6.75, CI=1.74–26.1, *P*=0.008), BbAATt (OR=20.3, CI=1.90–217, *P*=0.012), and bbAaTT (OR=10.0, CI=1.46–68.3, *P*=0.028) were more frequent in the former *vs*. the latter group, whereas combined genotype BbAa (OR=0.10, CI=0.01–0.80, *P*=0.010) was less frequent in the former group than the latter.

In comparing the >20% *vs*. ≤20% VDR-expression-positive groups, combined genotype AaTT (OR=3.89, CI=0.96–15.8, *P*=0.046) was more frequent in the former than in the latter group.

In comparing absent- *vs*. present-VDR-expression groups, the ApaI-TaqI combined genotype AATT (OR=14.5, CI=1.39–152, *P*=0.025) was more frequent in the melanoma group lacking VDR expression *vs*. the remaining patients.

Considering continuous median percentage values of VDR-expression-positive cells, with 100% VDR-positive cells, significantly higher VDR expression was observed for bbAa (median: 100.0%, range: 15%–100% *vs*. median: 20.0%, range: 0%–100%, *P*=0.029), BbAATt (median: 100.0%, range: 60%–100% *vs*. median: 20.0%, range: 0%–100%, *P*=0.011), bbAaTT (median: 100.0%, range: 15%–100% *vs*. median: 20.0%, range: 0%–100%, *P*=0.029) combined genotype carriers *vs*. non-carriers. By contrast, significantly lower VDR expression was noted for carriers of AATT combined genotype (median: 0.0%, range: 0%–10% *vs*. median; 30.0%, range: 0%–100%, *P*=0.014) *vs*. non-carriers.

Discussion

In our study, VDR expression was predominantly assessed in cytoplasm of melanoma cells (79.7%, 59/74 cases), with few tumors (14.9%, 11/74 cases) displaying VDR positivity in the nucleus. Such findings partially contrast those of other studies performed in a Polish cohort of 69 patients with primary cutaneous melanoma (comprising 35 metastatic melanomas and that were classified as follows: 4 Clark I, 6 Clark II, 23 Clark III, 24 Clark IV, and 12 Clark V stage, where 30 were superficial-spreading, 37 were nodular, and two were acral lentiginous melanomas); these previous studies indicated VDR-positive nuclear immunostaining in 84.1% and cytoplasmic immunostaining in 66.7% of patients[38,39]. The study by Brożyna et al.[39], however, showed percentage of melanoma specimens with high nuclear staining at 17.4% (12/69), which is close to the percentage of nuclear staining in our study. Discrepancies between results of Brożyna et al. and our study probably arise from different antibodies employed[21], diverse histological characteristics, and/or geographical/genetic backgrounds of melanomas in the respective studies. According to European cancer observatory data[3], the estimated age standardized (European) incidence rate (per 100,000/year) of malignant cutaneous melanoma and mortality are 5.6 and 2.8 in Poland and 13.4 and 2.0 in Italy, respectively.

We further analyzed data regarding cytoplasmic VDR immunohistochemical staining. Once the cytoplasmic VDR binds with 1α, 25-dihydroxyvitamin D ligand, and adequate coreceptor protein, retinoid X receptor, it translocates to the nucleus, and by recruitment of coactivators and corepressors modulates transcription of target genes that encode proteins responsible for final activities induced by vitamin D hormonal signaling[18]. Consequently, absence or down-regulation of VDR expression may present implications for vitamin D resistance in melanoma tissues[38,48,49] and potentially modulates effects of vitamin D supplementation on prevention therapy of melanoma patients[49,50].

We did not observe effects of age at melanoma diagnosis and gender on VDR expression in cutaneous melanomas[45]. Roles of BMI and smoking were focuses of previous melanoma research[8,25,51]. In the present investigation, we did not observe the effects of BMI and smoking on VDR expression.

Consistent with studies performed by other authors on a Polish cohort[38,39], we observed that stage I melanomas were more frequent in tumors with high than with low VDR expression. In our study, stage IIA invasion was particularly more frequent in melanomas lacking VDR expression than in melanomas showing VDR expression. However, we did not observe significant data in relation to metastatic stages III and IV. Causes of these findings will require further enlarged studies.

Overall, metastatic melanomas did not exhibit different VDR expression from non-metastatic melanomas. Such results agreed with observations of some studies[13,20,48] but contradicted the findings of other authors[38,39]. Specific geographical/ethnic backgrounds possibly affected results. Thus, enlarged studies in subjects with different ethnicities should be performed in the future to substantiate this issue.

In our study, localization of melanoma on the body was unrelated with VDR expression. We only observed a tendency (P=0.093) for upper limb melanomas to be more frequent in tumors lacking VDR expression. Thus, despite the expected different exposures to sunlight and environmental factors of different parts of the human body, VDR expression in our study is not associated with body regions in which primary melanomas develop.

Lesion-specific characteristics did not correlate with VDR expression; these characteristics include ulceration, number of mitotic figures, regression, absence of TILs, non-brisk or brisk TILs, tumor emboli, and melanoma subtype (epithelioid and small cell). At variance, in Polish patients studied by Brożyna and colleagues[39] cytoplasmic VDR immunostaining was higher in group of brisk TIL-positive *vs*. that of absent and non-brisk TIL melanomas (P=0.01), and VDR expression was lower in melanomas with ulceration. We noted a tendency for higher frequency of superficial spreading (P=0.063) in melanomas with high than low VDR expression. Similarly, Brożyna and colleagues[38] observed higher VDR expression in superficially spreading than nodular melanomas.

Remarkably, VDR expression was related to tumor Breslow thickness and Clark levels in our Italian patients. Melanomas with a thickness below 1.00 mm were more frequently observed in cases with high than low VDR expression, whereas those with thickness of over or equal to 1.01 mm were more frequent in melanomas with low VDR expression. Clark level II (none of the studied melanomas presented a Clark I) was detected more frequently in melanomas with high than low VDR expression (P=0.025). Overall, such findings concur with previous data on Polish patients[38,39].

We observed that VDR expression was unrelated with the presence of multiple melanomas, additional non-melanoma skin cancers and non-skin cancers, and melanoma familiarity. To our knowledge, no previous study assessed these issues.

To our knowledge, our study was the first to investigate the relationship between VDR expression of human melanoma cells in excised tissues of patients and *VDR* polymorphisms. Out of four *VDR* polymorphisms investigated in our study, individual SNPs of FokI, BsmI, and TaqI did not display any relation with expression of VDR in melanoma. Only the ApaI genotype was correlated to VDR expression in melanoma. The heterozygous genotype Aa was identified in 61.1% of melanomas with high VDR expression *vs*. 36.8% of melanomas with low VDR expression (OR=2.69, P=0.037). A allele was more frequent in 100% than <100% VDR-positive cells (OR=3.16, P=0.038). To our knowledge, no research studied the role of *VDR*-ApaI polymorphism in VDR expression in melanoma tissues. *VDR*-ApaI SNP is located in an intron sequence and thus cannot directly modify the amino acid sequence of VDR protein; however, it participates in VDR RNA processing[26]. Recent evidence demonstrated that intronic sites of the VDR gene can function as binding sites of transcriptional regulators, such as p53[52,53]. A meta-analysis study[35] indicated that *VDR*-ApaI polymorphism of the European population features an association with overall skin cancer risk (Aa *vs*. AA, OR=1.27, CI=1.05–1.53; Aa+aa *vs*. AA, OR=1.23, CI=1.04–1.47). In a recent Italian study, the Aa heterozygous genotype was associated with increased risk of lumbar pathologies, especially osteochondrosis[29].

A number of combined genotypes in our study yielded significant findings according to VDR expression. The AaTT combined genotype was more frequent in melanomas with high than low VDR expression. Combined genotypes BbAA, bbAa, AATt, BbAATt, and bbAaTT were more frequent in 100% VDR-positive cells than <100% VDR-positive cells. The AATT combined genotype was much more frequent in subjects without VDR expression (20%) than in those with VDR expression (1.7%) (OR=14.5, P=0.025). No previous study investigated the relationship of VDR expression with *VDR* combined genotypes. Lack of VDR expression in excised melanoma tissues has been associated with reduced overall survival of patients[38,39]. Therefore, melanoma prognosis may be influenced by carrying a *VDR* combined genotype associated to absent or reduced VDR expression. In our melanoma patients, AATT is a rare combined genotype with a frequency of 5.4%, which is similar to the recent finding in an Italian cohort of 518 non-oncological subjects (6.0%)[29]. Further enlarged studies are warranted to assess roles of ApaI, BsmI, and TaqI combined genotypes in VDR expression in melanomas and their prognosis.

Regulation of VDR abundance is an important modulation mechanism of cellular responsiveness to 1α, 25-dihydroxyvitamin D[10]. Mechanisms underlying regulation of VDR abundance include alterations in transcription rate of VDR gene and/or stability of VDR mRNA and epigenetic changes[14,18,54]. Interestingly, treatment with calcitriol can

enhance VDR mRNA in cultured melanoma cells, showing that increasing vitamin D consumption can induce VDR expression[14]. On the other hand, enhanced melanogenesis was associated with downregulation of VDR expression[38,55,56]. Response of melanoma cells to calcitriol corresponds to expression level of VDR mRNA, which in turn may be regulated by VDR miRNAs and by epigenetically modulating drugs[50]. Remarkably, recent evidence suggests that tumor suppressors, such as p53, are implicated on direct regulation of VDR[53]. Molecules other than vitamin D, such as curcumin and vitamin E derivatives, were indicated as novel VDR ligands[19,57], whereas vitamin A derivatives were suggested as modulators of VDR actions[58]. Given the wide variety of positive and negative VDR modulators, each individual expression of VDR is highly dynamic in cells, with nuclear translocation of VDR fluctuating upon instant induction[18,19,22]. We speculate that unresponsiveness of some cutaneous melanomas to anti-proliferative effects of vitamin D possibly resulted from absence or insufficient VDR expression in melanoma and/or melanocytic cells. Further human studies are warranted to assess whether benefits of vitamin D augmentation are modulated by VDR expression in melanoma/melanocytic cells and/or by carriage of a specific *VDR* genotype and/or combined genotype polymorphisms that affect VDR expression. Such factors can modulate dose requirement of vitamin D treatments[59].

Roles of vitamin D in skin cancers still require complete elucidation[10,57]. All vitamin D actions virtually occur through VDR activation. Recent evidence shows that the effects of 1α, 25-dihydroxyvitamin D and VDR are mediated at least in part by cellular calcium levels; thus, calcium possibly contributes to the suppressive ability of VDR on skin cancer[10,57]. Deletion of VDR notably results in an increased susceptibility to tumorigenesis and also reduces ability of keratinocytes to clear UVB-induced DNA mutations[57]. VDR can bind to thousands of VDREs on human genome and up- or downregulate hundreds of genes[10,30,57]. Based on bioinformatic analysis, almost 15,000 sites in human DNA are bound by VDR, and 16%–21% of these putative binding sites are found at gene promoters[10,57,60]. Aside from classical VDRE-mediated mechanisms, increasing evidence point to regulatory contribution of several miRNAs[14,18,54] and long non-coding RNAs[10,57]. A recent study on VDR cistrome demonstrated the unexpected complexity of gene regulation, which was examined on a genome-wide scale in target tissues and cells, including a cross-talk between VDR and immune factors[60,61]. VDR expression is also commonly and significantly down regulated in colon adenocarcinoma. VDR cistrome analyses suggested that reduced VDR expression in colon cancer changes VDR activity by dampening expression of tumor suppressors by either stabilizing or inhibiting down regulation of oncogene expression. In turn, these effects may be associated with severe patient outcomes[62]. Thus, further research should study complex gene interactions and biological pathways related to vitamin D and melanoma and combine clinical evidence with molecular findings to support further progress[57,60,61].

Limitations of our study include the limited number of patients and absence of data on circulating vitamin D levels in patients at the time of melanoma excision. Strengths of this research comprise genetic background restrictions of enrolled patients and determination of demographic, lifestyle, histological, and genetic characteristics.

Given the sample size and multiple comparisons of our study, a validation of independent datasets with larger samples, and multivariable analysis will be necessary to adjust genetic traits for age, sex, tumor location, sun exposure, and smoking. Serum sampling in future studies may also be performed to determine circulating levels of vitamin D at time of melanoma excision.

Conclusions

Current information insufficiently discusses influence of vitamin D oral supplementation to direct VDR modulatory effects in human skin cells, including melanocytes and keratinocytes. Our present findings support the necessity of further studies on this issue by combining clinical and molecular approach.

Our study showed that VDR expression is associated with prognostic parameters of tumor Breslow thickness and Clark level. However, VDR expression was not related to metastatic melanomas. Our immunohistochemical results concur with those of a previous study on VDR expression in colorectal cancer, showing that VDR was not associated with tumor location, stage, and grade[42], and with another lung tumor study which demonstrated high variability of VDR expression[43]. Interestingly, we observed correlation between the Aa genotype and AaTT combined genotype with higher level of VDR expression and between AATT combined genotype and low or absent VDR expression; this new finding will require further validation. Future studies should assess whether VDR expression and *VDR* combined genotypes affect benefits of vitamin D and can drive an appropriate dose and schedule of calcitriol or other active (low calcemic) vitamin D analogs for melanoma treatment. Future set up of personalized nutrition and behavioral interventions will benefit from molecular studies exploring the connection of

biological pathways to bioactive components of food and cancer[63]. Our study suggests that determination of VDR expression in excised tissues of melanoma and/or determination of *VDR* genotypes carriage can be used as personalized tool of precision medicine when considering melanoma patients.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

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