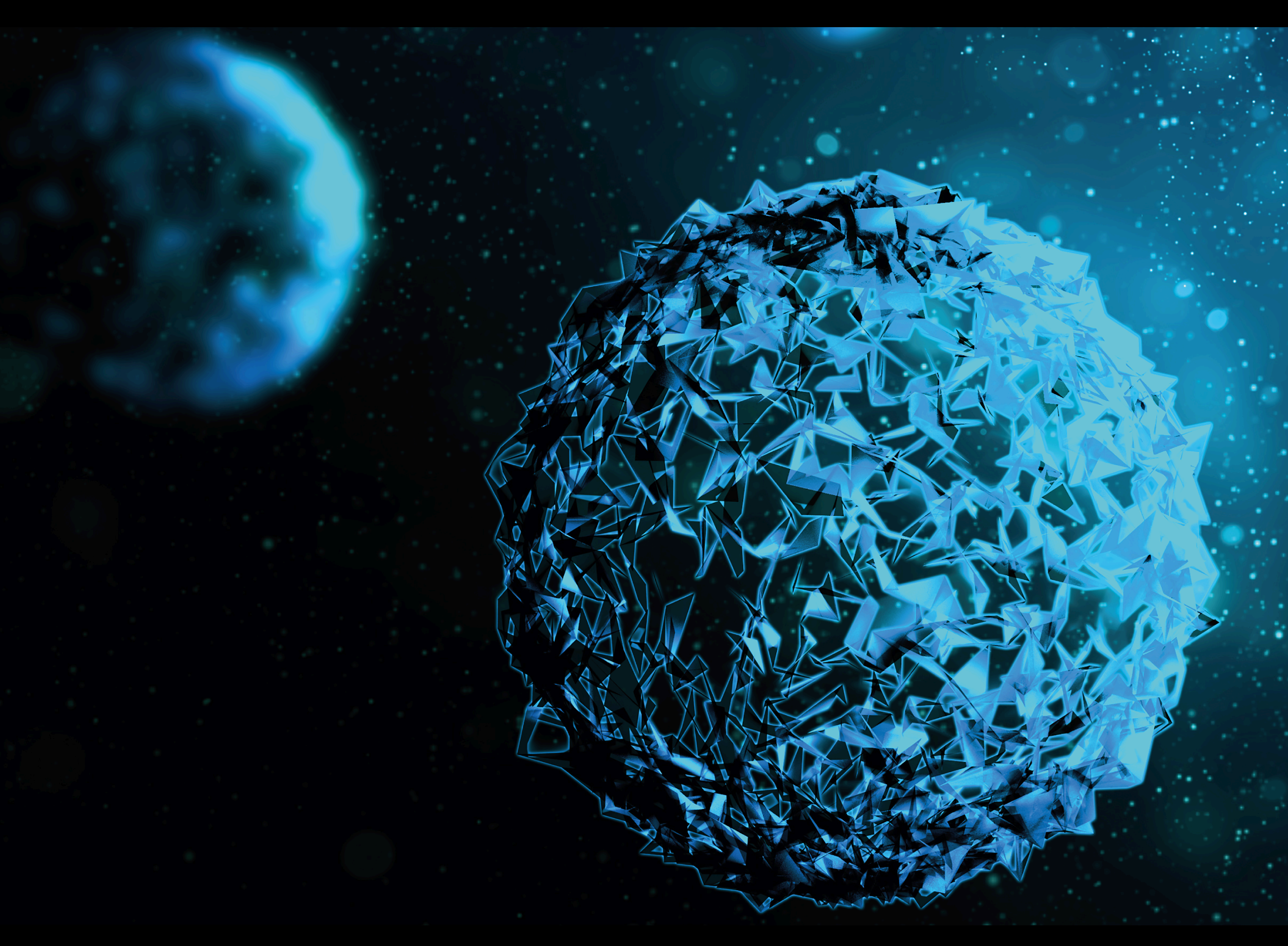
BioMed Research International

Understanding the Molecular Mechanisms of Vitamin D based Therapies in the Treatment of Cancers

Lead Guest Editor: Subbarao Venkata Madhunapantula
Guest Editors: Arun Sharma, Asha Srinivasan, Jessy Abraham, and Olga A. Sukocheva

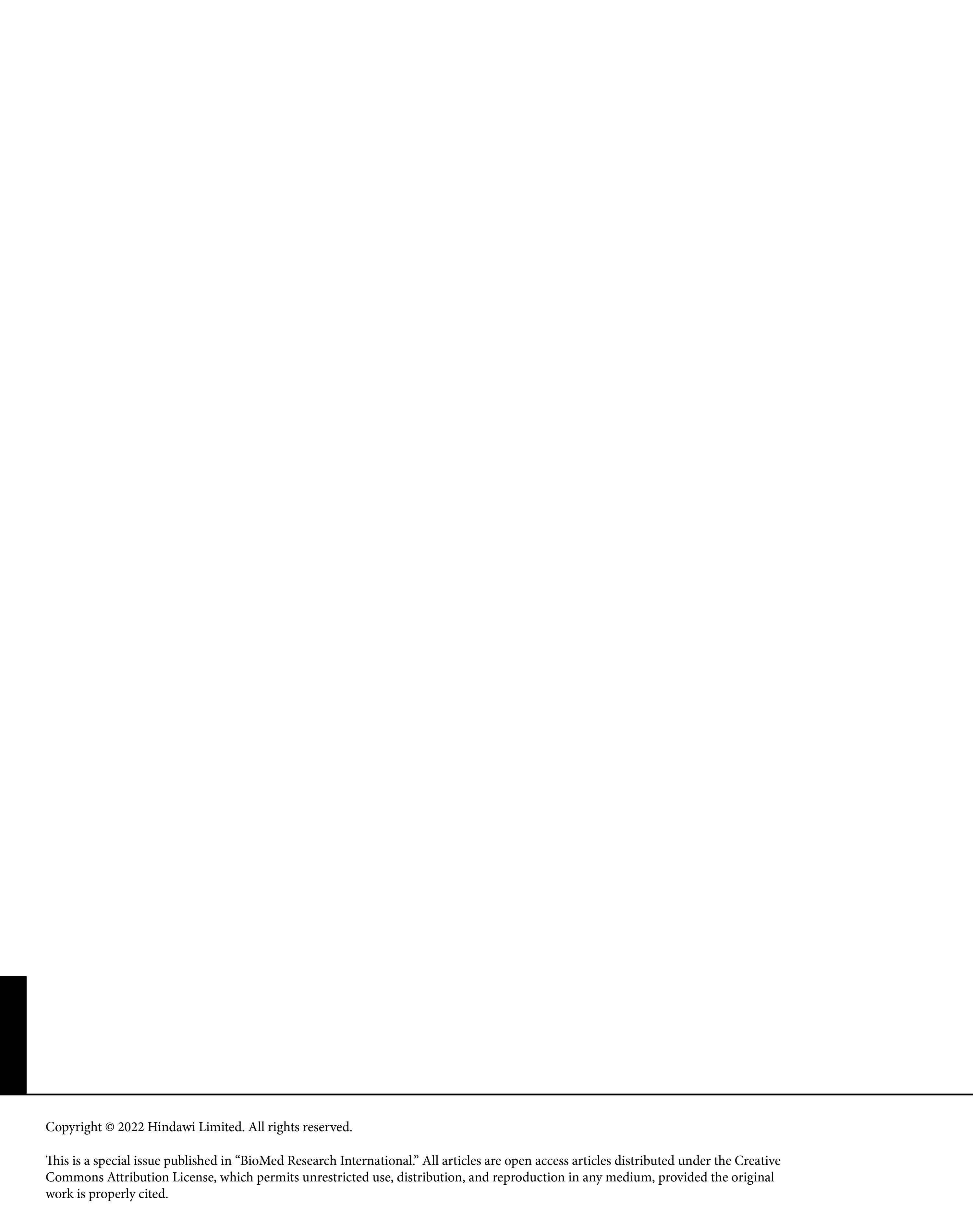


Understanding the Molecular Mechanisms of Vitamin D based Therapies in the Treatment of Cancers

BioMed Research International

Understanding the Molecular Mechanisms of Vitamin D based Therapies in the Treatment of Cancers

Lead Guest Editor: Subbarao Venkata Madhunapantula
Guest Editors: Arun Sharma, Asha Srinivasan, Jessy Abraham, and Olga A. Sukocheva



Copyright © 2022 Hindawi Limited. All rights reserved.

This is a special issue published in "BioMed Research International." All articles are open access articles distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Section Editors

Penny A. Asbell, USA
David Bernardo, Spain
Gerald Brandacher, USA
Kim Bridle, Australia
Laura Chronopoulou, Italy
Gerald A. Colvin, USA
Aaron S. Dumont, USA
Pierfrancesco Franco, Italy
Raj P. Kandpal, USA
Fabrizio Montecucco, Italy
Mangesh S. Pednekar, India
Letterio S. Politi, USA
Jinsong Ren, China
William B. Rodgers, USA
Harry W. Schroeder, USA
Andrea Scribante, Italy
Germán Vicente-Rodriguez, Spain
Momiao Xiong, USA
Hui Zhang, China

Academic Editors

Oncology

Fawzy A.S., Australia
Gitana Maria Aceto, Italy
Amedeo Amedei, Italy
Aziz ur Rehman Aziz, China
Riadh Badraoui, Tunisia
Stergios Boussios, Greece
Alberto Briganti, Italy
Franco M. Buonaguro, Italy
Xianbin Cai, Japan
Melchiorre Cervello, Italy
Winson Cheung, Canada
Somchai Chutipongtanate, Thailand
Kate Cooper, USA
Enoc Mariano Cortes-Malagon, Mexico
Alessandro De Vita, Italy
Hassan, El-Abid, Morocco
Yujiang Fang, USA
Zhien Feng, China
Stefano Gambardella, Italy
Dian Gao, China
Piotr Gas, Poland
Nebu Abraham George, India
Xin-yuan Guan, Hong Kong
Hirotaka Iwase, Japan
Arumugam R. Jayakumar, USA
Mitomu Kioi, Japan
Krzysztof Ksiazek, Poland
Yuan Li, China
Anna Licata, Italy
Wey-Ran Lin, Taiwan
César López-Camarillo, Mexico
João F Mota, Brazil
Rakesh Sathish Nair, USA
Peter J. Oefner, Germany
Mana Oloomi, Iran
Vera Panzarella, Italy
Pierpaolo Pastina, Italy
Georgios G. Pissas, Greece
Kyoung-Ho Pyo, Republic of Korea
Giandomenico Roviello, Italy
Daniele Santini, Italy
Wen Shi, USA
Krzysztof Siemianowicz, Poland
Henrique César Santejo Silveira, Brazil
Himangshu Sonowal, USA
Maurizio Soresi, Italy
Kenichi Suda, Japan
Farzad Taghizadeh-Hesary, Iran
Seyithan Taysi, Turkey
Fernando Toshio Ogata, Sweden
Abhishek Tyagi, USA
Marco A. Velasco-Velázquez, Mexico
Thirunavukkarasu Velusamy, India
Navin Viswakarma, USA
Ya-Wen Wang, China
Hushan Yang, USA
Zongguo Yang, China
Hui Yu, USA
Baotong Zhang, USA
Yi Zhang, China

Eugenio Zoni , Switzerland

Contents

Vitamin D Receptor Gene Polymorphisms and the Risk of CIN2+ in Shanxi Population
Dongyan Li, Yan Liu, Dangyi Kong, Dimitri Papukashvili, Nino Rcheulishvili, Hongwei Zhao, Yinge Li, Chaiyun Hou, Jinfeng Ma, Xiaoqing Lu, and Wenqi Bai
Research Article (9 pages), Article ID 6875996, Volume 2022 (2022)

Bioactive Vitamin D Attenuates MED28-Mediated Cell Growth and Epithelial–Mesenchymal Transition in Human Colorectal Cancer Cells
Chun-Yin Huang, Yu-Ting Weng, Nien-Tsu Hsieh, Po-Chen Li, Tzu-Yi Lee, Chun-I Li, Hsiao-Sheng Liu, and Ming-Fen Lee
Research Article (10 pages), Article ID 2268818, Volume 2022 (2022)

Hindawi
BioMed Research International
Volume 2022, Article ID 6875996, 9 pages
https://doi.org/10.1155/2022/6875996

Research Article

Vitamin D Receptor Gene Polymorphisms and the Risk of CIN2+ in Shanxi Population

Dongyan Li,[1,2] Yan Liu,[2] Dangyi Kong,[2] Dimitri Papukashvili ,[3] Nino Rcheulishvili ,[3] Hongwei Zhao,[1] Yinge Li,[4] Chaiyun Hou,[5] Jinfeng Ma ,[1] Xiaoqing Lu ,[1] and Wenqi Bai [1]

[1]*Shanxi Province Cancer Hospital/Shanxi Hospital Affiliated to Cancer Hospital, Chinese Academy of Medical Sciences/Cancer Hospital Affiliated to Shanxi Medical University, Taiyuan, China*
[2]*The Second Hospital of Shanxi Medical University, Taiyuan 030001, China*
[3]*Department of Pharmacology, School of Medicine, Southern University of Science and Technology, Shenzhen 518000, China*
[4]*Department of Obstetrics and Gynecology, Shuozhou Central Hospital, Shanxi Province, China*
[5]*Department of Obstetrics and Gynecology, Hequ County Hospital, Shanxi Province, China*

Correspondence should be addressed to Jinfeng Ma; mjinfeng99@163.com, Xiaoqing Lu; 982602719@qq.com, and Wenqi Bai; 326498302@qq.com

Received 16 July 2022; Accepted 26 September 2022; Published 16 November 2022

Academic Editor: Olga Sukocheva

Copyright © 2022 Dongyan Li et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Cervical cancer is one of the most common malignancies in women with high morbidity and mortality. Human papillomavirus (HPV) infection is the primary cause of cervical cancer, of which HPV 16 is the predominant. Early detection and effective treatment of cervical precancerous lesions are the key to preventing cervical cancer. Vitamin D receptor (VDR) gene polymorphism is considered to be an important cause of cancer development. Here, we studied the association of VDR polymorphisms (FOKI, BsmI, ApaI, and TaqI) in HPV16-positive cervical intraepithelial neoplasia (CIN)2+ patients. HPV16-positive patients who visited the Colposcopy Clinic of Obstetrics and Gynecology, the Second Hospital of Shanxi Medical University for biopsy due to abnormal HPV and/or Thinprep cytologic test (TCT) from September 1, 2020 to October 1, 2021 were grouped by pathological results. The fasting blood samples were collected and VDR polymorphisms were detected using TaqMan fluorescent probes, and the three sites of BsmI-ApaI-TaqI were subjected to haplotype analysis. FOKI ff genotype ($OR = 2.01$; 95% $CI = 1.12 - 3.59$; $p = 0.019$) and f allele ($OR = 1.48$; 95% $CI = 1.10 - 1.98$; $p = 0.009$) were found to be associated with the risk of CIN2+. TaqI Tt genotype ($OR = 2.03$; 95% $CI = 1.20 - 3.43$; $p = 0.008$), tt genotype ($OR = 2.09$; 95% $CI = 1.09 - 4.02$; $p = 0.028$), and t allele ($OR = 1.35$; 95% $CI = 1.01 - 1.80$; $p = 0.041$) were associated with the risk of CIN2+. No haplotype was associated with CIN2+ risk. According to the results, FOKI and TaqI polymorphisms are associated with CIN2+ risk.

1. Introduction

Cervical cancer is one of the common malignant tumors in gynecology, with high morbidity and mortality [1]. According to statistics, 604,000 new cervical cancer cases and 342,000 deaths were reported worldwide in 2020 [2], and about 85% of cervical cancers occur in developing countries. As the largest developing country in the world, the incidence of cervical cancer in China cannot be underestimated [3]. High-risk human papillomaviruses (HR-HPV) infection is the leading cause of cervical cancer, 70% of which are caused by HPV16/18, but only about 1% of persistent HR-HPV infection causes the occurrence of cervical cancer [4]. In addition, studies have shown that factors such as smoking, prolificity, and long-term oral contraceptives are associated with the occurrence of cervical cancer. Some scholars believe that the lack of nutrients such as vitamin D (VD) and folic acid in the diet can lead to the occurrence of cervical cancer [5].

Current studies have shown that VD deficiency is associated with many diseases, such as disorders of the immune,

Table 1: Basic data for all of the study subjects.

Variable	Cases ($n = 188$)	Controls ($n = 188$)	$t/z/\chi 2$Value	p value
Age	47.77 ± 7.65	45.07 ± 7.93	3.357	<0.001∗
Degree of education				
Illiteracy	10 (5.32)	7 (3.72)	6.021	0.304
Primary school	30 (15.96)	21 (11.17)		
Junior middle school	66 (35.11)	60 (31.91)		
Senior middle school	28 (14.89)	35 (18.62)		
Junior college	30 (15.96)	28 (14.89)		
Bachelor's degree or above	24 (12.77)	37 (19.68)		
Occupation				
Medical personnel	7 (3.72)	7 (3.72)	12.414	0.134
Science and education workers	14 (7.45)	7 (3.72)		
Administration staff	5 (2.66)	16 (8.51)		
Worker	15 (7.98)	7 (3.72)		
Farmer	24 (12.77)	27 (14.36)		
Commerce	14 (7.45)	10 (5.32)		
Service	14 (7.45)	15 (7.98)		
Others	56 (29.79)	63 (33.51)		
Unemployed	39 (20.74)	36 (19.15)		
Smoking				
Yes	49 (26.06)	26 (13.83)	8.811	0.003*
No	139 (73.94)	162 (86.17)		
Husband smoking				
Yes	130 (69.15)	126 (67.02)	0.196	0.658
No	58 (30.85)	62 (32.98)		
Age of menarche	14.38 ± 1.82	14.41 ± 1.69	-0.176	0.860
Menopause				
Yes	111 (59.04)	72 (38.30)	16.192	<0.001∗
No	77 (40.96)	116 (61.70)		
Marriageable age	22.76 ± 2.91	22.83 ± 3.09	-0.240	0.810
Age at first sex life	21.85 ± 2.84	22.49 ± 3.07	-2.113	0.035∗

(∗$p < 0.05$, the difference was statistically significant).

cardiovascular, respiratory, reproductive, and endocrine systems as well as malignancies such as prostate, colorectal, and breast cancers [6, 7]. Increasing evidence shows that VD can regulate the whole process from cancer development to metastasis and the related role of cells with the microenvironment. The specific mechanisms include regulating cell proliferation, differentiation, apoptosis, and autophagy as well as the regulation of angiogenesis, antioxidants, inflammation, and the immune system [8, 9]. VD is a lipid-soluble vitamin derived by sunlight or diet and is first delivered to the liver where it is metabolized into 25(OH)D and circulates in the serum. In the kidney it is further metabolized to the biologically most active form of VD—calcitriol (1α,25(OH)2D). It plays various roles in the body by binding to vitamin D receptors (VDR) [8]. VDR belongs to the nuclear receptor family with a typical nuclear receptor domain, including DNA-binding domain, a hinge region, a ligand-binding domain, and a carboxy-terminal activation function 2 domain that interacts with coregulators [10]. The VDR gene is located on chromosome 12q12-14, which contains two promoters and 8 exons [11]. So far, more than 60 VDR single nucleotide polymorphisms (single-nucleotide polymorphisms, SNPs) have been identified, but the most studied are FOKI (exon 2), BsmI, ApaI (intron 8), and TaqI (exon 9). The four SNP are located in different regions of the VDR gene and are potentially linked to many diseases [11, 12]. Many studies have analyzed the association of VDR SNP with cancer, such as breast cancer (FokI, BsmI, and ApaI), prostate cancer (FokI, BsmI, and TaqI), colorectal cancer (FokI, BsmI, and TaqI), and skin cancer (FokI, BsmI, and TaqI) [13], but there are still controversies. However, there are few studies on VDR SNPs, cervical precancerous lesions and carcinoma in China. The animal experimental results of Shim [14] showed that the treatment regimen of in situ immunization (CPG+OX40) combined

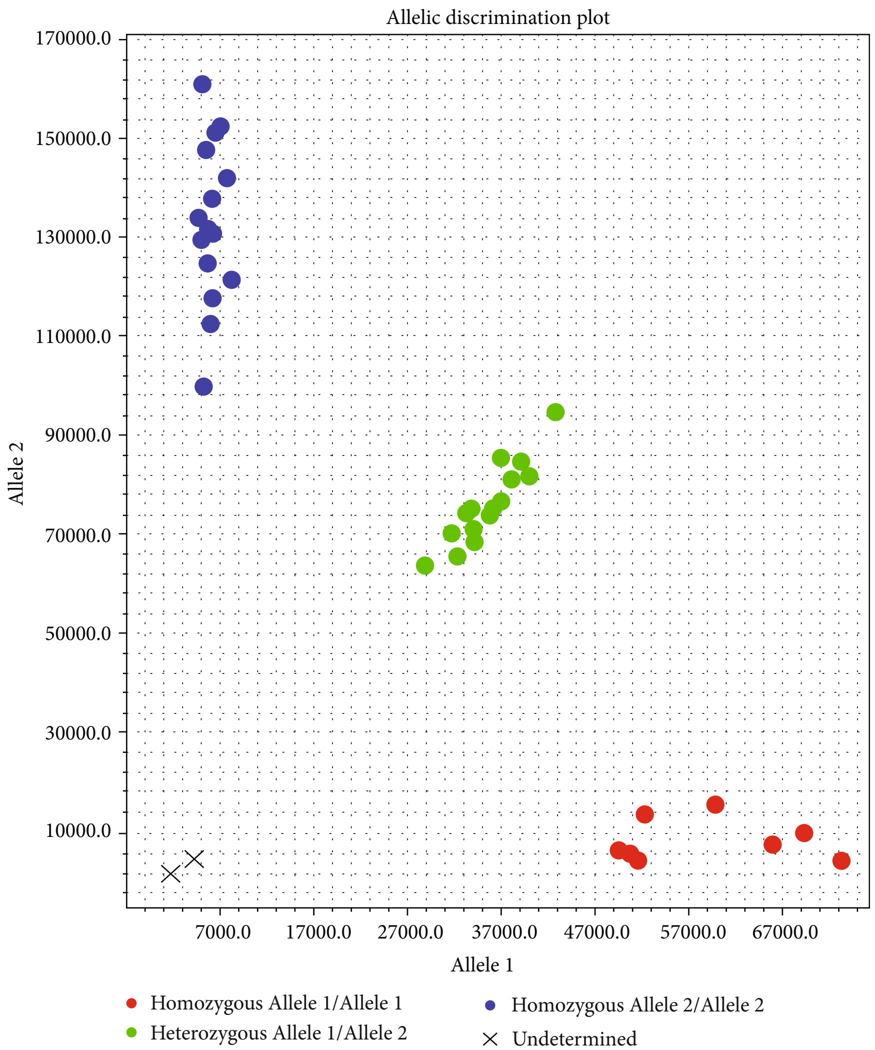


Figure 1: TaqMan genotyping results; X represents blank control.

with antiangiogenesis (anlotinib) increased the infiltration of CD4+ and CD8+ T cells in mice with cervical cancer, inhibited the growth of tumor volume, and prolonged the survival time of mice. These results indicate that immune dysfunction is an important mechanism leading to the development of cervical cancer. Chu found in his study that VD level was positively correlated with CD4 and negatively correlated with CD8 [15].

The occurrence of cervical cancer follows a gradual process from normal cervical to cervical intraepithelial neoplasia grade 1 (CIN)1 to CIN2/3 and finally develops into cervical cancer [16]. Only 1% of CIN1 cases progress to cervical cancer per year, while the risk of CIN2/3 progression to cervical cancer is 16% within 2 years and 25% within 5 years [17]. Therefore, the timely diagnosis and treatment of CIN2/3 is crucial to prevent the occurrence of cervical cancer. This project aimed to investigate the relationship between VDR SNPs (FOKI, BsmI, ApaI, and TaqI) and the risk of CIN2/3 and cervical cancer in Shanxi women. Additionally, serum concentration of 25-(OH)-D3 levels were detected in the VDR genotype difference group.

2. Materials and Methods

2.1. Subjects. The research samples were collected according to the HPV and/or Thinprep cytologic test (TCT) abnormalities from the subjects who visited the hospital from September 1, 2020 to October 1, 2021. The patients signed the informed consent, completed the questionnaire, and took fasting blood samples. Patients undergoing biopsy in the Colposcopy Clinic of the Obstetrics and Gynecology Department, the Second Hospital of Shanxi Medical University, Shanxi Provincial Cancer Hospital, Shuozhou Central Hospital, and Hequ County Hospital, Shanxi Province were selected as HPV16 positive patients, and the pathological results were used as the basis for grouping. The pathological results showed chronic inflammation as the control group and the CIN2+ (CIN2 and CIN3 and cervical cancer) as the case group. After screening according to the inclusion and exclusion criteria, 188 patients from the case group and the control group were separately grouped, among which 20 cases were included in Shanxi Cancer Hospital, and 6 cases were separately included in Shuozhou Central

Table 2: Distribution of VDR SNP genotypes versus alleles in the case and control groups.

Genotype/allele	Case n (%)	Control n (%)	Crude OR (95% CI)	p value	Adjusted OR[b] (95% CI)	p value
FOKI[a]						
FF	28 (14.89)	42 (22.34)	—	—	—	—
Ff	77 (41.96)	84 (44.68)	1.38 (0.78-2.43)	0.273	1.47 (0.81-2.67)	0.205
ff	83 (44.15)	62 (32.98)	2.01 (1.12-3.59)	0.019	2.18 (1.19-3.99)	0.012
Ff+ff	160 (85.11)	146 (77.66)	1.64 (0.97-2.79)	0.065	1.77 (1.02-3.08)	0.041
F	133 (35.37)	168 (44.68)	—	—	—	—
f	243 (64.63)	208 (55.32)	1.48 (1.10-1.98)	0.009	—	—
BsmI[a]						
BB	15 (7.98)	18 (9.57)	—	—	—	—
Bb	61 (32.45)	69 (36.70)	1.06 (0.49-2.28)	0.880	0.92 (0.41-2.08)	0.845
bb	112 (59.57)	101 (53.73)	1.33 (0.64-2.78)	0.447	1.25 (0.58-2.71)	0.571
Bb+bb	173 (92.02)	170 (90.43)	1.22 (0.60-2.50)	0.585	1.12 (0.53-2.39)	0.762
B	91 (24.20)	105 (27.93)	—	—	—	—
b	285 (75.80)	271 (72.07)	1.21 (0.88-1.68)	0.245	—	—
ApaI[a]						
AA	22 (11.70)	31 (16.49)	—	—	—	—
Aa	92 (48.94)	85 (45.21)	1.53 (0.82-2.84)	0.183	1.38 (0.72-2.66)	0.227
aa	74 (39.36)	72 (38.30)	1.45 (0.77-2.74)	0.253	1.34 (0.68-2.63)	0.394
Aa+aa	166 (88.30)	157 (83.51)	1.49 (0.83-2.68)	0.184	1.36 (0.73-2.54)	0.332
A	136 (36.17)	148 (39.36)	—	—	—	—
a	240 (63.83)	228 (60.64)	1.15 (0.85-1.54)	0.367	—	—
TaqI[a]						
TT	29 (15.43)	51 (27.13)	—	—	—	—
Tt	121 (64.36)	105 (55.85)	2.03 (1.20-3.43)	0.008	1.94 (1.12-3.35)	0.018
tt	38 (20.21)	32 (17.02)	2.09 (1.09-4.02)	0.028	2.14 (1.07-4.28)	0.031
Tt+tt	159 (84.57)	137 (72.87)	2.04 (1.23-3.40)	0.006	1.98 (1.17-3.37)	0.012
T	179 (47.61)	207 (55.05)	—	—	—	—
t	197 (52.39)	169 (44.95)	1.35 (1.01-1.80)	0.041	—	—

[a] All genotypes in the control group followed the Hardy-Weinberg equilibrium law. [b] Multivariate logistic regression adjusting for age, smoking, menopause, and age at first sex).

Hospital of Shanxi Province and Hequ County Hospital of Shanxi Province. The remaining cases were obtained from the Second Hospital of Shanxi Medical University.

2.2. Inclusion Criteria. All study subjects were of Han nationality, had lived in Shanxi for more than 5 years, age range from 22 to 65, were married or unmarried, and had at least two years of sexual life history. The exclusion criteria are as follows: patients with multiple systemic diseases such as immune, digestive, blood, and other malignancies; medication affecting vitamin D metabolism within 3 months before the study; pregnant women; patients with cervical adenocarcinoma. All study subjects signed informed consent and drew peripheral blood for analysis of VDR polymorphisms. Serum concentration of 25-(OH)-D3 levels were detected in the VDR genotype difference group. This study was performed with approval from the Ethics Committee of the Second Clinical Medical College of Shanxi Medical University.

2.3. Peripheral Blood DNA Extraction. Genomic DNA was extracted from peripheral blood according to the instructions of the QIAamp DNA Blood Mini (250) kit (Qiagen, Valencia, CA, USA). The concentration and purity of the extracted DNA samples were determined via NanoDrop one (Thermo Fisher Scientific, Waltham, MA, USA), and all the DNA samples that meet the standard were repackaged and stored at -80°C.

2.4. Detection of Serum 25-(OH)-D3. For the detection of serum 25-(OH)-D3, ELISA assay was used. The detection principle is as follows: the reagent used double antibody sandwich enzyme-linked immunosorbent assay. The procedure is as follows: (1) standard dilution, set the sample concentration gradient as follows: 25-(OH)-D3:200 ng/mL, 100 ng/mL, 50 ng/mL, 25 ng/mL, 12.5 ng/mL, 6.25 ng/mL, 3.13 ng/mL, 0 ng/mL; multiple wells were set for each concentration. (2) The same amount of standard/sample was added to be tested and biotinylated detection Ab (configured

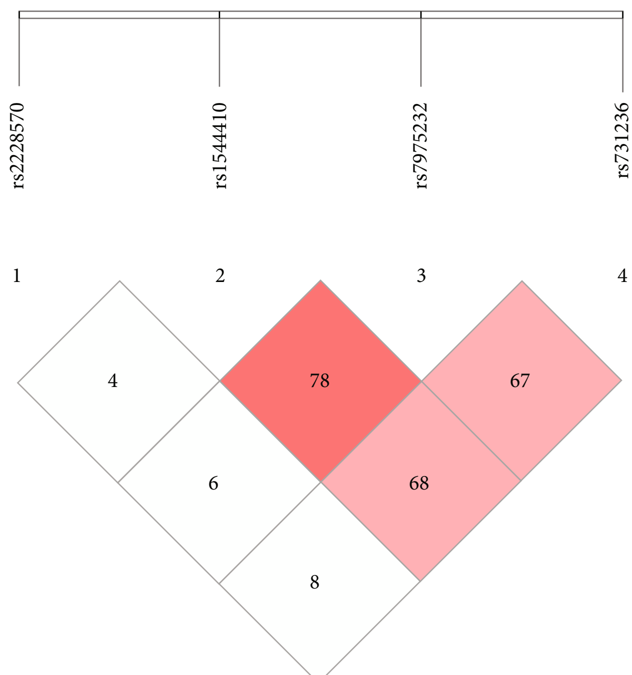


Figure 2: Linkage disequilibrium map of the four SNP species for all the study subjects. The percentage of D' is indicated by the strength of numbers and color.

with the kit) and mixed well. (3) Plate was sealed and incubated at 37°C for 45 minutes. (4) It was mixed with washing liquid. (5) The plate was washed and pat dried 3 times. (6) HRP conjugate to each well was added and incubated at 37°C for 30 minutes. (7) The plate was washed and pat dried 5 times. (8) Substrate reagent was added to each well and incubated at 37°C for 15 min in dark. (9) The stop solution was added to stop the reaction. (10) The parameters of BioTekEPOCH2 microplate reader were set at 450 nm and. (11) The standard curve equation was calculated to obtain the sample concentrations.

2.5. Detection of the Vitamin D-Receptor Genetic Polymorphisms. All of the genomic DNA samples were typed for the following four SNP loci: FOKI (rs2228570), BsmI (rs1544410), ApaI (rs7975232), and TaqI (rs731236). SNP genotyping was performed using the TaqMan fluorescence probe method and ABI real-time PCR (Thermo Fisher Scientific, Waltham, MA, USA) instrument. Forty cycles were performed with the following operating conditions: denaturation step at 95°C for 10 min, denaturation step at 95°C for 15 sec, and annealing extension step at 60°C for 60 sec.

2.6. Statistical Analyses. All of the statistical analyses were performed using the SPSS 26.0 software (IBM). Counting data were expressed as the frequency and percentage, and comparisons between groups were performed using the chi-square ($\chi 2$) test. If the measurement data conform to the normal distribution, it is expressed by t, and the comparison between groups is expressed by the t-test. If not, it is expressed by M50 (25, 75) and the Mann–Whitney U-test is used for intergroup comparison. If the control genotype distribution complied with the Hardy-Weinberg equilibrium law $\chi 2$ test was used. The SHEsis software was used to perform linkage disequilibrium (LD) and haplotype analysis. The logistic regression analysis was applied to identify the association of the VDR SNPs or haplotypes and the risk of cervical lesions and to calculate the OR value and the 95% confidence interval (CI). $p < 0.05$ was defined as statistically significant.

Table 3: The distribution of three SNP haplotypes: BsmI, ApaI, and TaqI.

Haplotype	Case frequency (%)	Control frequency (%)	p value
baT	45.5	42.4	—
BAT	1.0	4.1	0.339
Bat	21.1	19.1	0.939
BaT	0	2.5	0.331
Bat	2.1	2.2	0.906
bAT	1.1	6.0	0.142
bAt	13.0	10.2	0.714
bat	16.2	13.5	0.782
BbAaTt	44 (23.40)	46 (24.47)	—
bbaaTt	38 (20.21)	31 (16.49)	0.440
bbAaTt	35 (18.62)	22 (11.70)	0.139
BBAAtt	12 (6.38)	9 (4.79)	0.497
bbaaTT	28 (14.89)	31 (16.49)	0.864
BbAATT	1 (0.53)	3 (1.60)	0.369
BbAatt	10 (5.32)	8 (4.26)	0.606
BBAATT	0	2 (1.06)	—
BBAATt	1 (0.53)	1 (0.53)	—
Bbaatt	3 (1.60)	3 (1.60)	—
BBAaTT	0	2 (1.06)	—
bbAatt	3 (1.60)	3 (1.60)	—
BBaatt	2 (1.06)	2 (1.06)	—
BBaaTT	0	2 (1.06)	—
BbAATt	3 (1.60)	3 (1.60)	—
BbAAtt	0	2 (1.06)	—
BbAaTT	0	2 (1.06)	—
bbAAtt	5 (2.66)	5 (2.66)	—
BbaaTT	0	2 (1.06)	—
bbAaTT	0	3 (1.60)	—
bbAATT	0	4 (2.13)	—
bbAATt	0	2 (1.06)	—
bbaatt	3 (1.60)	0	—

3. Results

The basic data of all the study subjects are presented in Table 1. The age of the case and control groups was, respectively, 47.77 ± 7.65 and 45.07 ± 7.93 years, which was statistically significant ($p < 0.05$). In addition, the age at first sex,

Table 4: Main effect analysis of serum concentration of 25-(OH)-D3.

Effect class	III square sum mean square of	Degrees of freedom	The mean square	F	p
Correction model	31330.029	8	3916.254	28.558	<0.001
Intercept	171976.908	1	171976.908	1254.101	<0.001
Group	21483.815	2	10741.907	78.333	<0.001
Genotype	61.221	2	30.61	0.223	0.800
Group * genotype (interaction)	1667.494	4	416.874	3.040	0.017
Error	50327.3	367	137.132		

Table 5: Separate effect analysis of serum concentration of 25-(OH)-D3.

Genotype	Control	HSIL group	SCC group	F	p
FF	39.93 ± 14.92	27.74 ± 6.92&	24.00 ± 9.61&	9.003	<0.001
Ff	46.96 ± 12.86 *	26.00 ± 8.98&	20.66 ± 8.22&%	81.000	<0.001
ff	40.13 ± 14.05#	27.09 ± 10.09&	23.13 ± 9.85&	24.855	<0.001
F	5.883	0.377	0.432		
P	0.003	0.686	0.652		

* indicates statistical difference compared with FF, # indicates statistical difference compared with FF, & indicates statistical significance compared with the control group, and % indicates statistical significance compared with the HSIL group.

menopause, and smoking were statistically significant in the case group when compared with the controls ($p < 0.05$).

The results of the partial TaqMan genotyping analysis are given in Figure 1. The distribution of the VDR SNP genotypes and alleles in both groups and the relationship with the risk of developing CIN2+ are listed in Table 2.

For the FOKI polymorphisms, the allele f was more common in the CIN2+ group (64.63%) than in the control group (55.32%). Comparing the Ff genotype with the FF genotype showed that the Ff genotype was not associated with CIN2+ risk (crude OR = 1.38; 95% CI = 0.78 − 2.43; $p = 0.273$ and adjusted OR = 1.47; 95% CI = 0.81 − 2.67; $p = 0.205$). Comparing the ff genotype with the FF genotype showed that the ff genotype significantly increased the risk of CIN2+ occurrence (crude OR = 2.01; 95% CI = 1.12 − 3.59; $p =$ 0.019 and adjusted OR = 2.18; 95% CI = 1.19 − 3.99; $p =$ 0.012). After adjusting for the confounders, f allele carriers were not associated with CIN2+ risk (adjusted OR = 1.77; 95% CI = 1.02 − 3.08; $p = 0.041$). Finally, by comparing the f allele to the F allele, the f allele was associated with an increased CIN2+ risk (OR = 1.48; 95% CI = 1.10 − 1.98; $p = 0.009$).

For the TaqI polymorphisms, the allele t was more common in the CIN2+ group (52.39%) than in the control group (44.95%). The Tt, tt genotypes were separately compared to the TT genotype, the results showed that the Tt and tt genotypes significantly increased the risk of CIN2+ (Tt VS TT: crude OR = 2.03; 95% CI = 1.20 − 3.43; $p = 0.008$ and adjusted OR = 1.94; 95% CI = 1.12 − 3.35; $p = 0.018$. tt VS TT: crude OR = 2.09; 95% CI = 1.09 − 4.02; $p = 0.028$ and adjusted OR = 1.98; 95% CI = 1.17 − 3.37; $p = 0.012$). Comparison of the Tt + tt genotype with the TT genotype showed that the t allele carriers are associated with CIN2+ risk (crude OR = 2.04; 95% CI = 1.23 − 3.40; $p = 0.006$ and adjusted OR = 1.98; 95% CI = 1.17 − 3.37; $p = 0.012$). Finally, comparing the t allele to the T allele revealed that the t allele is a CIN2+ susceptibility gene (OR = 1.35; 95% CI = 1.01 − 1.80; $p = 0.041$). The BsmI and ApaI polymorphisms were not associated with CIN2+ risk.

The SHEsis software was applied to create an LD diagram (Figure 2). As the LD between the FOKI polymorphism and the other SNP was very low, it was excluded, and the haplotypes of the other three SNPs were analyzed. The results of this study showed that the baT haplotypes were the most common in both patients and controls, and that the other haplotypes were not significantly different when compared to the baT. To further observe the genotype distribution across all study subjects, the frequency of the combined BsmI, ApaI, and TaqI genotypes in the CIN2+ and control groups were analyzed (Table 3). As the distribution of BbAaTt was the most common in controls, it was selected as the reference genotype. However, further analysis in this study showed that none of the combined genotypes was associated with the risk of CIN2 +.

Serum concentrations of 25-(OH)-D3 were detected in the VDR FOKI polymorphisms.

The results are different, 25-(OH)-D3 concentration was different among different genotypes in the control group, and the relative 25-(OH)-D3 concentration of F gene was higher among different groups ($p < 0.05$). The relative 25-(OH)-D3 concentration decreased in both F and F genome in HSIL group and SCC group, which was significantly different from that in the control group ($p < 0.05$). There was no significant difference among the groups (Table 4).

The main effect analysis showed that there were interaction effects between groups and genotypes (F interaction = 3.040, $p = 0.017$), so further separate effect analysis was needed (Table 5).

4. Discussion

Due to the increase of new cases and deaths caused by cervical cancer, this cancer may become one of the greatest burdens of oncology, thus, further identification of the factors affecting the development of cervical cancer is crucial. This study analyzed the relationship between VDR polymorphism and HPV16-positive CIN2+ patients in Shanxi women, aiming to provide a theoretical basis for reducing the occurrence of cervical cancer.

VDR is a member of the steroid/thyroid hormone receptor family that regulates biological processes including cell proliferation, differentiation, apoptosis, tumor invasion, and angiogenesis in vivo [18, 19]. VDR polymorphisms themselves may not be genetic loci affecting disease progression, but rather play a role by affecting VDR expression levels [20]. Studies of the VDR polymorphisms suggest that the VDR polymorphism may be an important factor affecting the VDR mRNA and protein numbers and affecting the downstream VD-mediated effects [20]. More than 60 VDR SNPs, located in the promoter regions of exons 2–9, have been linked to the occurrence and prognosis of cancer [12]. However, only a few of them, including FOKI, BsmI, ApaI, and TaqI, have potential functions to affect the expression of VDR genes and are associated with cancer risk [12].

Regarding the FOKI polymorphism, since the ff genotype and the f allele increase the incidence of CIN2+, the present results indicate that the f allele is a susceptibility allele affecting the occurrence of cervical lesions, in line with previous studies in the Thai population [21]. In addition, the f allele is also a susceptibility gene for T cell lymphoma [22], colorectal cancer [12], and ovarian cancer [23]. The FOKI f allele is associated with an increased risk of cervical cancer and most other cancers, possibly due to its reduced VDR activity [22]. However, the meta-analysis of Pu et al. [9] showed that the incidence of head and neck cancer in patients with ff genotype was significantly lower than that in patients with Ff+FF or FF genotype. The FOKI SNP is located in the second exon, and involves the translation initiation point, forming an additional start codon by changing the ACG codon located ten base pairs upstream of the translation start codon [22, 24]. Therefore, two variants of the VDR protein may occur during translation: a longer form of the protein (f allele, containing 427 amino acids) and a shorter form (F allele, containing 424 amino acids) [22, 25]. Studies have shown that the longer form of the VDR protein has lower transcriptional activity, reducing the anticancer properties of calcitriol, and leading to increased cancer susceptibility [21, 22]. Therefore, this study supports the role of the FOKI f allele in increasing susceptibility to most cancers.

Regarding the TaqI polymorphism, the results of this study showed that the t allele affects the risk of the CIN2+ occurrence, consistent with the findings of Phuthong et al. [21]. However, the present study demonstrated that the ApaI and BsmI polymorphisms were not associated with CIN2+ risk. Previous studies have shown that the BsmI polymorphism increases the risk of gastric cancer [19], colorectal cancer [12], melanoma [26], and multiple myeloma [18]. Moreover, Qadir et al. [19] proposed that the BsmI polymorphism follows the "law of dominant inheritance", in which people with the BB genotypes have a lower risk of cancer than those with both the Bb and bb genotypes. In addition, the A allele carriers of the ApaI polymorphism increase the risk of colorectal cancer [12] and multiple myeloma [18], and instead reduce the risk of hepatocellular carcinoma caused by HCV infection [27]. Conversely, the t allele of the TaqI polymorphism is a susceptibility gene for colorectal cancer [12], and it is also an important factor in reducing the incidence of head and neck cancer as well as tobacco-related respiratory and oesophageal cancer [28].

The BsmI, ApaI, and TaqI polymorphisms are located in the $3'$-noncoding region of the VDR genes ($3'$-untranslated, $3'$UTR), and the three SNP species are in strong linkage disequilibrium with each other [19]. These three SNPs do not change the amino acid sequence of proteins but can regulate gene expression, especially mRNA stability [22, 29]. BsmI and ApaI polymorphisms are located in intron 8 of the VDR gene, resulting in silent mutations [30]. One of the mechanisms by which these two SNPs affect VDR expression is that the splicing site of VDR mRNA transcription is disrupted, resulting in truncation or alternate splicing of the protein product; another explanation is the altered polyadenylation of VDR mRNA and thus altered mRNA stability [19]. TaqI SNP, located in exon 9 of the VDR gene, is a synonymous SNP that results in a silent mutation in codon 352 from ATT to ATC, both of which encode isoleucine [29, 30]. In addition, the $3'$ TaqI SNP has been found to affect CpG (where the TaqI SNP is located) methylation and CGI 1060 methylation [19]. CGI 1060 is located at the $3'$ end of the VDR, and its methylation may affect the regulation of the $3'$ promoter, driving lncRNA transcription in this region, and may regulate the expression of the VDR posttranscriptionally [19].

Studies of haplotypes and combined genotypes can provide more conclusive information about genetic variation, however, in this study, no single haplotype or combined genotype was found to be associated with CIN2+ risk. In a meta-analysis by Khan et al. [24] it was shown that bAT haplotypes are more common in African populations (59%), whereas baT and BAt haplotypes are more common in Asian (75%) and Caucasian populations (39%). In the current study, the baT haplotype was the most frequent in both patients and controls, which is consistent with the results of Khan et al. [24]. In another study, the baT haplotype was found to be the most common in colorectal cancer [31]. Gleba et al. [22] suggested that the baT haplotype increases the sensitivity of leukemia and lymphoma cells to calcitriol, and Gleba believes that the baT haplotype is associated with increased VDR mRNA transcriptional activity, which can lead to the formation of more many VDR proteins. Latacz et al. [31] found that tAb and Bat haplotypes increased the risk of colorectal cancer (tAb: OR = 3.84; 95% CI 1.29-11.38; $p = 0.01$, Bat: OR = 30.22; 95% CI 2.81 -325.31; $p = 0.01$). However, Qadir et al. [19] suggested that the ATC (bat) haplotype increases the risk of gastric cancer, while the GTT (BaT) haplotype plays a protective role. Different haplotypes are expressed differently in different cancers, possibly due to differences in gene-environment interactions and lifestyles. Large differences in VDR

genotypes by ethnicity have been demonstrated [32]. The results of this study demonstrated the following: 25-(OH)-D3 in the control group indicates that the F-gene population requires relatively high 25-(OH)-D3 concentrations under normal conditions. 25-(OH)-D3 concentration decreases and the disease progresses. Combined with FOKI polymorphisms analysis, F gene is a risk factor for CIN2+ disease, and the decrease of 25-(OH)-D3 concentration in F gene population suggests an increased risk of CIN2+ disease. In addition, geographic differences and a limited number of studies may also contribute to this disparity. To our knowledge, this study is the first to report the relationship between VDT polymorphisms and the risk of HPV 16-positive CIN2+ disease in the Shanxi Province population. In this study, only HPV 16-positive population was included while the other high-risk HPV-infected populations were not included, which cannot represent all high-risk HPV-infected populations. This is the limitation of the study and the addition of other high-risk HPV-infected populations to verify the results in the subsequent studies are warranted.

5. Conclusions

This study is the first to demonstrate the role of the VDR polymorphism in HPV16-positive cervical lesions in Shanxi women, and the FOKI and TaqI genotypes may be associated with a high risk of CIN2+. However, no single haplotype was found to increase the CIN2+ risk in our study. The binding ability of the VDR to its target sequence may be influenced by specific DNA sequences, VDR isoforms, cell-specific phosphorylation, and changes in core regulators in different tissues [9]. Therefore, the underlying mechanisms of different VDR gene polymorphisms in cervical cancer remain to be further investigated.

Data Availability

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

Dongyan Li and Yan Liu contributed equally to this work and are co-first authors.

Acknowledgments

This research was funded by The Central Government Guides Local Science and Technology Development Fund Projects, grant No. YDZJSX2021B016 and Province Key and Plan Project, grant No. 201903D321158.

References

[1] J. D. Watson and F. H. C. Crick, "Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid," *Nature*, vol. 171, no. 4356, pp. 737-738, 1953.

[2] S. Cheng, S. Liu, J. Yu et al., "Robust whole slide image analysis for cervical cancer screening using deep learning," *Nature Communications*, vol. 12, no. 1, p. 5639, 2021.

[3] N. Li, X. Guo, L. Liu, L. Wang, and R. Cheng, "Molecular mechanism of miR-204 regulates proliferation, apoptosis and autophagy of cervical cancer cells by targeting ATF2," *Artif Cells Nanomed Biotechnol*, vol. 47, no. 1, pp. 2529–2535, 2019.

[4] M. Chen, L. Li, and P. S. Zheng, "SALL4 promotes the tumorigenicity of cervical cancer cells through activation of the Wnt/β-catenin pathway via CTNNB1," *Cancer Science*, vol. 110, no. 9, pp. 2794–2805, 2019.

[5] G. Abuduxikuer, M. Kasimu, G. Abulizi, and L. Hua, "Relationship of human papilloma virus infection, high-grade cervical lesions including cervical cancer and serum vitamins of Uyghur women in Bachu County, Xinjiang," *Journal of Xinjiang Medical University*, vol. 40, no. 1, pp. 6–10, 2017.

[6] T. Gromowski, P. Gapska, R. J. Scott et al., "Serum 25(OH)D concentration, common variants of the VDR gene and lung cancer occurrence," *International Journal of Cancer*, vol. 141, no. 2, pp. 336–341, 2017.

[7] L. Vuolo, C. Di Somma, A. Faggiano, and A. Colao, "Vitamin D and cancer," *Front Endocrinol (Lausanne)*, vol. 3, article 58, 2012.

[8] S. M. Jeon and E. A. Shin, "Exploring vitamin D metabolism and function in cancer," *Experimental & Molecular Medicine*, vol. 50, no. 4, pp. 1–14, 2018.

[9] Y. Pu, G. Zhu, Y. Xu et al., "Association between vitamin D exposure and head and neck cancer: a systematic review with meta-analysis," *Frontiers in Immunology*, vol. 12, article 627226, 2021.

[10] I. A. Voutsadakis, "Vitamin D baseline levels at diagnosis of breast cancer: a systematic review and meta-analysis," *Hematology/Oncology and Stem Cell Therapy*, vol. 14, no. 1, pp. 16–26, 2021.

[11] A. B. Al-Ghafari, K. S. Balamash, and H. A. Al Doghaither, "TaqI and ApaI variants of vitamin D receptor gene increase the risk of colorectal cancer in a Saudi population," *Saudi Journal of Medicine & Medical Sciences*, vol. 8, pp. 188–195, 2020.

[12] I. Messaritakis, A. Koulouridi, M. Sfakianaki et al., "The role of vitamin D receptor gene polymorphisms in colorectal cancer risk," *Cancers (Basel)*, vol. 12, no. 6, p. 1379, 2020.

[13] V. Rai, J. Abdo, S. Agrawal, and D. K. Agrawal, "Vitamin D receptor polymorphism and cancer: an update," *Anticancer Research*, vol. 37, no. 8, pp. 3991–4003, 2017.

[14] J. Shim, A. Pérez, E. Symanski, and A. Nyitray, "Association between serum 25-hydroxyvitamin D level and human papillomavirus cervicovaginal infection in women in the United States," *The Journal of Infectious Diseases*, vol. 213, no. 12, pp. 1886–1892, 2016.

[15] T. Chu, J. Jhao, T. Lin et al., "Vitamin D in gynecological diseases," *Journal of the Chinese Medical Association: JCMA*, vol. 84, no. 11, pp. 1054–1059, 2021.

[16] H. Jia, L. Ding, Y. Han et al., "Genotype-specific distribution and change of high-risk human papillomavirus infection and

the association with cervical progression risk in women with normal pathology and abnormal cytology in a population-based cohort study in China," *Journal of Cancer*, vol. 12, no. 14, pp. 4379–4388, 2021.

[17] E. M. Burd, "Human papillomavirus and cervical cancer," *Clinical Microbiology Reviews*, vol. 16, no. 1, pp. 1–17, 2003.

[18] R. Kumar, N. G. Himani, N. Gupta et al., "Unveiling molecular associations of polymorphic variants of VDR gene (FokI, BsmI and ApaI) in multiple myeloma patients of Indian population," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 199, article 105588, 2020.

[19] J. Qadir, S. Majid, M. S. Khan, and M. D. Wani, "Association of vitamin D receptor gene variations with gastric cancer risk in Kashmiri population," *Molecular Biology Reports*, vol. 48, no. 4, pp. 3313–3325, 2021.

[20] N. Divanoglou, D. Komninou, E. A. Stea et al., "Association of vitamin D receptor gene polymorphisms with serum vitamin D levels in a Greek rural population (Velestino study)," *Lifestyle Genom*, vol. 14, no. 3, pp. 81–90, 2021.

[21] S. Phuthong, W. Settheetham-Ishida, S. Natphopsuk, and T. Ishida, "Genetic polymorphisms of vitamin D receptor gene are associated with cervical cancer risk in northeastern Thailand," *Asian Pacific Journal of Cancer Prevention*, vol. 21, no. 10, pp. 2935–2939, 2020.

[22] J. J. Gleba, D. Kłopotowska, J. Banach et al., "Polymorphism of VDR gene and the sensitivity of human leukemia and lymphoma cells to active forms of vitamin D," *Cancers (Basel)*, vol. 14, no. 2, p. 387, 2022.

[23] E. Deuster, U. Jeschke, Y. Ye, S. Mahner, and B. Czogalla, "Vitamin D and VDR in gynecological cancers-a systematic review," *International Journal of Molecular Sciences*, vol. 18, no. 11, p. 2328, 2017.

[24] M. I. Khan, Z. F. Bielecka, M. Z. Najm et al., "Vitamin D receptor gene polymorphisms in breast and renal cancer: current state and future approaches (review)," *International Journal of Oncology*, vol. 44, no. 2, pp. 349–363, 2014.

[25] A. G. Uitterlinden, Y. Fang, J. B. Van Meurs, H. A. Pols, and J. P. Van Leeuwen, "Genetics and biology of vitamin D receptor polymorphisms," *Gene*, vol. 338, no. 2, pp. 143–156, 2004.

[26] M. Birke, J. Schope, S. Wagenpfeil, T. Vogt, and J. Reichrath, "Association of vitamin D receptor gene polymorphisms with melanoma risk: a meta-analysis and systematic review," *Anticancer Research*, vol. 40, no. 2, pp. 583–595, 2020.

[27] P. Barooah, S. Saikia, R. Bharadwaj et al., "Role ofVDR,GC, andCYP2R1polymorphisms in the development of hepatocellular carcinoma in hepatitis C virus-infected patients," *Genetic Testing and Molecular Biomarkers*, vol. 23, no. 5, pp. 325–331, 2019.

[28] L. Laczmanski, I. Laczmanska, and F. Lwow, "Association of select vitamin D receptor gene polymorphisms with the risk of tobacco-related cancers - a meta-analysis," *Scientific Reports*, vol. 9, no. 1, p. 16026, 2019.

[29] V. Aristarco, H. Johansson, S. Gandini et al., "Association of vitamin D receptor and vitamin D-binding protein polymorphisms with familial breast cancer prognosis in a mono-institutional cohort," *Nutrients*, vol. 13, no. 4, p. 1208, 2021.

[30] A. L. Becker, E. L. Carpenter, A. T. Slominski, and A. K. Indra, "The role of the vitamin D receptor in the pathogenesis, prognosis, and treatment of cutaneous melanoma," *Frontiers in Oncology*, vol. 11, article 743667, 2021.

[31] M. Latacz, D. Rozmus, E. Fiedorowicz et al., "Vitamin D receptor (VDR) gene polymorphism in patients diagnosed with colorectal cancer," *Nutrients*, vol. 13, no. 1, p. 200, 2021.

[32] S. Gandini, P. Gnagnarella, D. Serrano, E. Pasquali, and S. Raimondi, "Vitamin D receptor polymorphisms and cancer," *Advances in Experimental Medicine and Biology*, vol. 810, pp. 69–105, 2014.

Hindawi
BioMed Research International
Volume 2022, Article ID 2268818, 10 pages
https://doi.org/10.1155/2022/2268818

Research Article

Bioactive Vitamin D Attenuates MED28-Mediated Cell Growth and Epithelial–Mesenchymal Transition in Human Colorectal Cancer Cells

Chun-Yin Huang,[1] Yu-Ting Weng,[1,2] Nien-Tsu Hsieh,[1] Po-Chen Li,[1] Tzu-Yi Lee,[1] Chun-I Li,[3] Hsiao-Sheng Liu,[3,4] and Ming-Fen Lee[1,2]

[1]*Department of Nutrition, China Medical University, Taichung, Taiwan*
[2]*Department of Nutrition and Health Sciences, Chang Jung Christian University, Tainan, Taiwan*
[3]*Department of Microbiology and Immunology, National Cheng Kung University, Tainan, Taiwan*
[4]*Center for Cancer Research, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*

Correspondence should be addressed to Ming-Fen Lee; leemf@mail.cmu.edu.tw

Received 6 April 2022; Revised 20 July 2022; Accepted 30 July 2022; Published 29 August 2022

Academic Editor: Jessy Abraham

Copyright © 2022 Chun-Yin Huang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Inadequate vitamin D status may increase the risk of developing multiple types of cancer. Epidemiological studies suggest an inverse association between 25-hydroxyvitamin D_3 (25(OH)D_3) and malignancy, including colorectal cancer. Previous studies have suggested that MED28, a Mediator subunit involved in transcriptional regulation, is associated with the growth of colorectal cancer cells; however, its role in the progression of metastasis such as epithelial–mesenchymal transition (EMT) and cell migration of colorectal cancer is unclear at present. The aim of this study was to investigate a potentially suppressive effect of calcitriol, 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$), a bioactive form of vitamin D, and the role of MED28 in the progression of EMT in human colorectal cancer cells. Suppression of MED28 increased the expression of E-cadherin and reduced the expression of several mesenchymal and migration biomarkers and Wnt/β-catenin signaling molecules, whereas overexpression of MED28 enhanced the EMT features. Calcitriol suppressed the expression of MED28, and the effect of calcitriol mirrored that of MED28 silencing. Our data indicate that calcitriol attenuated MED28-mediated cell growth and EMT in human colorectal cancer cells, underlining the significance of MED28 in the progression of colorectal cancer and supporting the potential translational application of calcitriol.

1. Introduction

Biological features of malignant tumor cells include unchecked cell growth, epithelial-mesenchymal transition (EMT), invasion to neighboring tissues, and metastasis at distant sites [1]. During the development of invasive cancer, cells gradually lose epithelial features and acquire mesenchymal and invasive characteristics. E-cadherin is an epithelial junction protein responsible for the maintenance of the epithelial status. Three groups of transcription factors, Snail zinc finger family, zinc finger E-box-binding homeobox family proteins, and basic helix-loop-helix family can repress the expression of E-cadherin [2]. As in the case of colorectal cancer (CRC), cancer cells may display loss of polarity, epithelial markers such as E-cadherin, and upregulation of mesenchymal markers such as vimentin as well as metalloproteinases MMP2 and MMP9 [3]. Intermediate filament vimentin contributes to the change of cell shape, motility, and adhesion during the progression of EMT [4]. Loss of E-cadherin, together with dysregulation in the Wnt/β-catenin pathway, a common theme in colorectal cancer, is implicated in the progression of cancer presumably by promoting EMT, invasion, and metastasis [5].

Skin cells produce vitamin D_3 (cholecalciferol), a natural form of vitamin D, through a series of reactions upon sun exposure. Dietary sources of vitamin D are limited to mainly

fortified foods and fish oils. After two hydroxylation events at C-25 and C-1 of cholecalciferol in the liver and kidneys, respectively, our body can produce 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$), *i.e.*, calcitriol, the biologically active form of vitamin D. The genomic action of calcitriol is mediated through binding to a heterodimer, vitamin D receptor (VDR) and retinoid X receptor (RXR), two steroid receptor family members [6]. The complex then recognizes and binds to vitamin D response element (VDRE) of the target genes to activate or repress gene expression [7]. Calcitriol can be also involved in nongenomic, transcription-independent signaling in certain contexts [8]. In terms of physiological roles, in addition to the maintenance of calcium and bone homeostasis, the initially identified function, calcitriol also exhibits other important biological effects on normal cell differentiation, immune function, and cancer, among others. *In vitro* studies have shown anticancer effects of calcitriol, including antiproliferation, prodifferentiation, and apoptosis [7, 9]. Vitamin D deficiency may increase the risk of developing multiple types of malignancies [9]. Most epidemiological studies report an inverse tendency between 25-hydroxyvitamin D_3 (25$(OH)D_3$) and cancer, including colorectal cancer [9–11]. Therefore, maintenance of adequate vitamin D status appears to reduce the incidence and mortality of colorectal cancer [12].

Mediator subunit MED28 exhibits multiple cellular roles, including facilitating transcriptional activation [13] and interacting with signaling molecules such as Grb2 and Src family proteins [14–16]. Recently, we have reported that MED28 appears involved in Wnt/β-catenin signaling in human colorectal cancer cells [17] as well as EMT and migration in human breast cancer cells [18, 19]. However, whether MED28 is also involved in the EMT event in colorectal cancer is unclear at present.

The aim of this study was to investigate the role of MED28 in the progression of EMT and a potentially suppressive effect of calcitriol, the bioactive form of vitamin D, on MED28 and EMT in human colorectal cancer cells. Suppression of MED28 by RNA interference upregulated E-cadherin, an epithelial marker, but downregulated several mesenchymal biomarkers as well as Wnt/β-catenin signaling molecules, whereas overexpression of MED28 affected EMT in an opposite manner. Calcitriol suppressed the expression of MED28, and the effect of calcitriol mirrored that of MED28 silencing. Moreover, calcitriol could reverse the stimulatory effect of MED28 on EMT. Our data indicate that calcitriol attenuated MED28-mediated cell growth and EMT in colorectal cancer cells, reinforcing the implication of MED28 in the progression of colorectal cancer and supporting a promising, clinical application of calcitriol.

2. Materials and Methods

2.1. Cell Culture. The chemicals and cell culture supplies stated in the current study were purchased from MilliporeSigma (Burlington, MA) and Invitrogen (Carlsbad, CA), respectively, unless stated otherwise. Human colorectal cancer cell lines, HT29 and SW480 (American Type Culture Collection, Manassas, VA), were cultured in 10% fetal bovine serum-containing Dulbecco's modified Eagle's medium and Leibovitz's L-15 medium without CO_2, respectively. For RNA interference experiments, cells were transfected with MED28-specific small interfering RNA pools (Thermo Fisher Scientific, Lafayette, CO) using Lipofectamine™ transfection RNAiMAX Reagent. For overexpression experiments, cells were transfected with Flag-MED28 cDNA (OriGene Technologies, Inc., Rockville, MD) using Lipofectamine™ 3000 Transfection Reagent.

2.2. Preparation of Total Cell Lysates and Western Blotting. After the addition of 100 nM of calcitriol (Cayman Chemical, Ann Arbor, Michigan) for 48 h or transfection for 24 h (overexpression) or 72 h (RNAi), HT29 or SW480 cells were washed with PBS briefly, and the preparation of total cell lysates was as stated before [20]. Cellular proteins of the whole cell lysates were separated and resolved by SDS–PAGE and electrophoretically transferred onto polyvinylidene fluoride membranes (Perkin Elmer Life Sciences, Inc., Waltham, MA). Western blotting was performed as previously described [20] with the following primary and secondary antibodies: MED28, β-catenin, and p-GSK3β (Ser9) antibodies from MilliporeSigma (Burlington, MA); fibronectin, E-cadherin, MMP2, and MMP9 antibodies from Abcam (Waltham, MA); anti-Flag-tag and anti-p21 from Proteintech Group, Inc. (Rosemont, IL); antibodies for vimentin, Snail, Slug, Twist, ZEB2, proliferating cell nuclear antigen (PCNA), α-tubulin, β-actin, vinculin, and GAPDH from Santa Cruz Biotechnology (Santa Cruz, CA); and anti-c-Myc, anticyclin D1, and goat antirabbit or antimouse-conjugated horseradish peroxidase secondary antibodies from GeneTex, Inc. (Irvine, CA).

2.3. RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). Following the addition of 100 nM of calcitriol for 48 h, HT29 or SW480 cells were washed with PBS briefly, and the protocols of the RNA extraction and cDNA synthesis using Total RNA Mini Kit (NovelGene, Taipei, Taiwan) and iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA), respectively, were as described before [21, 22]. We employed the Bio-Rad products, including iQ™ SYBR® Green Supermix, CFX Connect™ Real-Time PCR Detection System, and CFX Manager™ 3.0 Software, for quantification, detection, and analysis, respectively. The following PCR primers were used: MED28 (forward, 5′-TTCGAACCGGTGTTGATCAG-3′; reverse, 5′-GCCAAT GCCTCAGCTTTGTC-3′); GAPDH (forward, 5′-CGAC CACTTTGTCAAGCTCA-3′; reverse, 5-AGGGGAGATTC AGTGTGGTG-3′).

2.4. Xenograft Mice. The animal study with the approved protocol (CJCU-104-001) issued by the Institutional Animal Care and Use Committee, Chang Jung Christian University (Tainan, Taiwan), was previously described [20]. Briefly, three-week-old male NOD/SCID mice were allowed for one week of acclimation after transferred from the National Cheng Kung University Animal Center (Tainan, Taiwan). These mice were subcutaneously implanted with human colorectal cancer HT29 cells (5×10^6) to their flank regions, followed by random allocation into three groups, with four

mice in each group. Two weeks later, each mouse was intraperitoneally administered with the, respectively, assigned dose, 0 μg (vehicle), 0.5 μg, or 1 μg of calcitriol every other day. After 4 weeks, these mice were sacrificed, and their tissue sections and proteins were subjected to immunohistochemical or Western blotting analysis.

2.5. Immunohistochemical Analysis. Xenograft tumors were fixed in 4% paraformaldehyde, cut with a thickness of 5 μm, and deparaffinized with xylene. Endogenous peroxidase was quenched with 3% H_2O_2 followed by dehydration with ethanol and antigen retrieval in a microwave for 5 min. After blocking with 5% bovine serum albumin solution for 1 h, tissue sections were incubated with anti-MED28 antibodies (1 : 50) for 2 h followed by secondary antibodies for 1 h and 3,3′-diaminobenzidine tetrahydrochloride. Immunostaining was observed under Nikon (Eclipse 50i) light microscope at 400× and photographed.

2.6. Statistical Analysis. Data are presented as means ± standard deviation which was analyzed by Student's t test or the analysis of variance (ANOVA) test followed by Tukey's post hoc comparison to analyze the differences between groups as appropriate. The results were considered significantly different at $p < 0.05$.

3. Results

3.1. In Vivo Effect of Calcitriol on the Expression of E-Cadherin, MED28, β-Catenin Signaling Molecules, and PCNA. Previously, we employed a subcutaneous HT29 xenograft NOD/SCID mouse model to examine the *in vivo* effect of calcitriol (bioactive vitamin D), and we found a dose-dependent tendency of calcitriol in reducing the tumor volumes and weights of the xenografts [20]. Loss of cadherin-mediated adhesion is implicated in the progression of cancer [5], and calcitriol upregulates the expression of E-cadherin in HT29 and SW480 human colorectal cancer cells [20]. In the current study, we further identified that calcitriol increased the expression of E-cadherin in the subcutaneous HT29 xenografts (Figure 1(a)). In an earlier study, we reported that MED28 appears to regulate Wnt/β-catenin signaling and cell growth in human colorectal cancer cells [17]. Therefore, we asked whether calcitriol may affect the expression of MED28, Wnt/β-catenin signaling molecules, and cell growth *in vivo*. As shown in Figure 1, both Western blot (Figure 1(a)) and immunohistochemical (Figure 1(b)) analyses revealed that calcitriol reduced the expression of MED28 in a dose-responsive manner in the HT29 xenografts. Moreover, calcitriol reduced the expression of PCNA, a proliferation marker, and Wnt/β-catenin signaling molecules, including β-catenin, c-Myc, and cyclin D1, in the HT29 xenografts (Figure 1(a)). These data indicated an *in vivo* effect of calcitriol on E-cadherin, MED28, Wnt/β-catenin signaling, and cell growth in human colorectal cancer.

3.2. Effect of Calcitriol on MED28, Epithelial and Mesenchymal Markers, and Wnt/β-Catenin Target Genes in Human Colorectal Cancer Cells. Wnt/β-catenin signaling is associated with EMT and migration during the development of malignancy [2]. Previously we found a link of MED28 with Wnt/β-catenin signaling in human colorectal cancer cells and a connection of MED28 and EMT in human breast cancer cells [17, 19]; we therefore asked whether calcitriol may also affect MED28 and EMT in colorectal cancer. In agreement with our previous findings [20], the addition of calcitriol at 100 nM for 48 h in HT29 cells reduced the expression of multiple proteins, including p-GSK3β, β-catenin, cyclin D1, and c-Myc, involved in Wnt/β-catenin signaling, and PCNA, but upregulated p21 (Figure 2(a)). Also, calcitriol suppressed the protein levels of fibronectin, a mesenchymal marker, and MED28, but upregulated the expression of E-cadherin (Figure 2(a)). Next, we employed RNAi to examine the role of MED28, and we found that suppression of MED28 mimicked the effect of calcitriol on E-cadherin, fibronectin, and Wnt/β-catenin signaling molecules in HT29 human colorectal cancer cells (Figure 2(b)). These data indicated that calcitriol may suppress the Wnt/β-catenin signaling pathway partially through MED28 in human colorectal cancer cells.

Next we investigated the effect of calcitriol on SW480, another human colorectal cancer cell line. The addition of calcitriol upregulated the expression of E-cadherin and p21 but suppressed the expression of PCNA, β-catenin, cyclin D1, c-Myc, and MMP9, a metalloproteinase involved in migration and invasion, and MED28 in SW480 cells (Figure 3(a)). Suppression of MED28 led to downregulation of MMP9, vimentin, and Slug and Twist, two transcription factors involved in EMT, in addition to Wnt/β-catenin signaling molecules such as p-GSK3β, β-catenin, and c-Myc, but upregulation of E-cadherin (Figure 3(b)). In contrast, overexpression of MED28 increased the expression of mesenchymal markers, including vimentin and fibronectin, and transcription factors Snail and ZEB2, but decreased the expression of E-cadherin and p21 (Figures 3(c) and 3(d)). However, calcitriol could reverse the effect of MED28, thereby upregulating E-cadherin and p21 and downregulating fibronectin (Figure 3(d)). The suppressive effect of calcitriol on MED28 appeared at the level of transcription because calcitriol reduced the mRNA expression of MED28 in both HT29 and SW480 cells (Figure 4(a)). Taken together, our data suggested that MED28 was involved in Wnt/β-catenin signaling and EMT, and calcitriol could inhibit the effect of MED28 on cell growth and EMT in colorectal cancer (Figure 4(b)).

4. Discussion

In the current study, we employed two human colorectal cancer cell lines, HT29 and SW480; these cells carry wild-type *CDH1* (E-cadherin) and *CTNNB1* (β-catenin), but mutant *APC* [23], and exhibit active Wnt/β-catenin signaling. We identified a role of MED28 in cell growth and epithelial-mesenchymal transition in these human colorectal cancer cells, where the effect of MED28 knockdown mimicked that of calcitriol, partially by upregulating E-cadherin as well as attenuating Wnt/β-catenin signaling and epithelial-mesenchymal transition (Figure 4(b)).

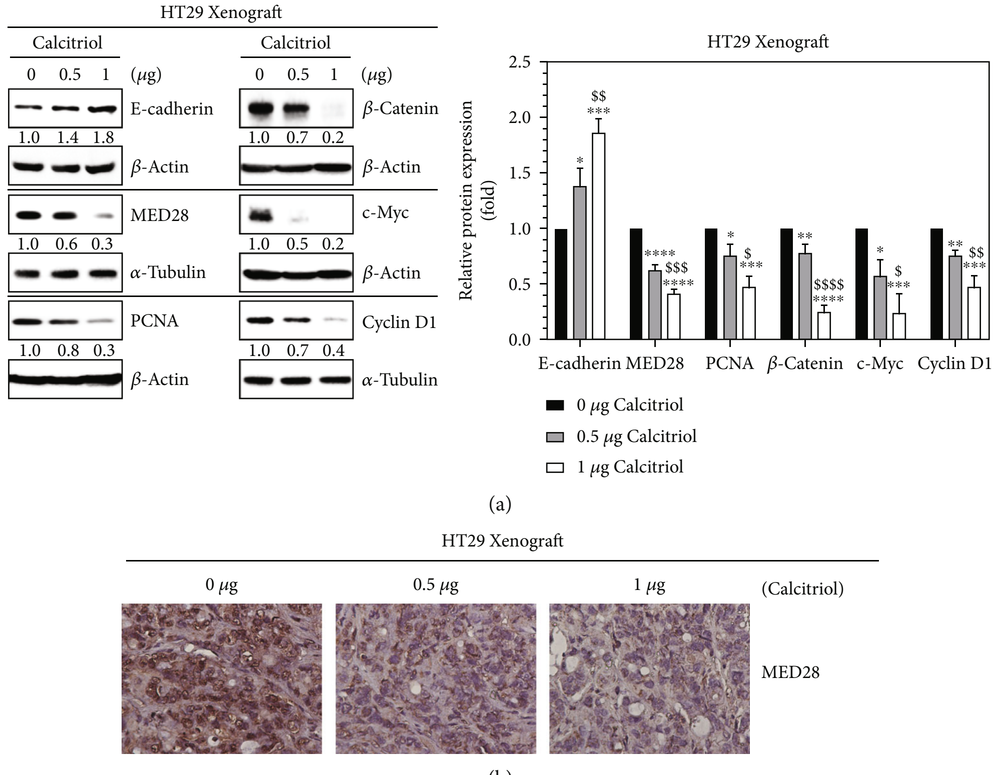


Figure 1: Calcitriol reduced the expression of MED28, PCNA, β-catenin, c-Myc, and cyclin D1 but upregulated the expression of E-cadherin in the subcutaneous HT29 xenografts. Four-week-old male NOD/SCID mice were subcutaneously implanted with human colorectal cancer HT29 cells to their flank regions, and these animals were then randomly allotted into three groups, vehicle control (0 μg) or calcitriol (0.5 μg or 1 μg), with four mice in each group. Two weeks later, the animals were intraperitoneally administered with the assigned dose, 0 μg (vehicle), 0.5 μg, or 1 μg of calcitriol every other day. The animals were sacrificed after 4 weeks. (a) Representative images and densitometric quantification for the relative protein expression of the xenografts. The expression levels of E-cadherin, MED28, PCNA, β-catenin, c-Myc, and cyclin D1 in the xenografts, along with loading controls, were detected by Western blotting. The ratios below the images (left panel) indicate the relative expression of the specific proteins with respect to those of 0 μg after normalization with the expression of the corresponding loading controls, β-actin or α-tubulin. Densitometric data (right panel) are expressed as means ± standard deviation, $n = 3$; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ as compared with 0 μg; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$, and $^{\$\$\$\$}p < 0.0001$ as compared with 0.5 μg. (b) Tissue sections of the xenografts were subjected to immunohistochemical analysis with anti-MED28 antibodies followed by secondary antibodies and diaminobenzidine staining. Brown-colored immunostaining was observed under Nikon (Eclipse 50i) light microscope at 400× and photographed.

Cellular β-catenin may be present in multiple subcellular compartments including cell membrane, cytoplasm, and nucleus, and in normal cells, β-catenin is mainly found in cell–cell junctions associated with E-cadherin [24]. The distribution of cellular β-catenin is controlled by a multiprotein disruption complex, mainly consisting of axin, adenomatosis polyposis coli (APC), and glycogen synthase kinase 3β (GSK3β) [25]. In the absence of upstream stimulation, axin and APC hold β-catenin in place, and casein kinase 1 and GSK3β phosphorylate β-catenin at the designated amino acids residues, which ultimately lead to ubiquitination and proteasome-mediated degradation of cytosolic β-catenin. Upon activation, the interaction of Wnt and receptors rescues β-catenin from disruption, stabilizes β-catenin, and allows the entry of β-catenin into the nucleus [26]. β-catenin then forms a transcriptionally active complex with T cell factor (TCF) and lymphoid enhancer factor (LEF) and transactivates various downstream targets involved in cell growth such as cyclin D1 and c-Myc, as well as epithelial-mesenchymal transition (EMT) including fibronectin for mesenchymal adhesion and transcription factors Slug and Twist for transcriptional downregulation of E-cadherin. Therefore, E-cadherin and Wnt/β-catenin signaling are implicated in cancer development in the aspect of EMT, invasion, and metastasis [5].

The E-cadherin/β-catenin complex is involved in the stabilization of cell–cell contact for epithelial cells [27]. Damage of cadherin-mediated cell adhesion disrupts regular assembly of the epithelial structures, which could increase the likelihood of free cytosolic β-catenin translocating into the nucleus to transactivate the Wnt/β-catenin target genes [5], indicating a connection between loss of cadherin-mediated cell adhesion and activation of Wnt/β-catenin signaling. Ultimately EMT could ensue if accompanied by the induction of the mesenchymal markers. Calcitriol upregulated the expression of E-cadherin (Figures 1(a), 2(a), and 3(a)), which could presumably increase the likelihood of E-cadherin–β-catenin assembly at the adherens

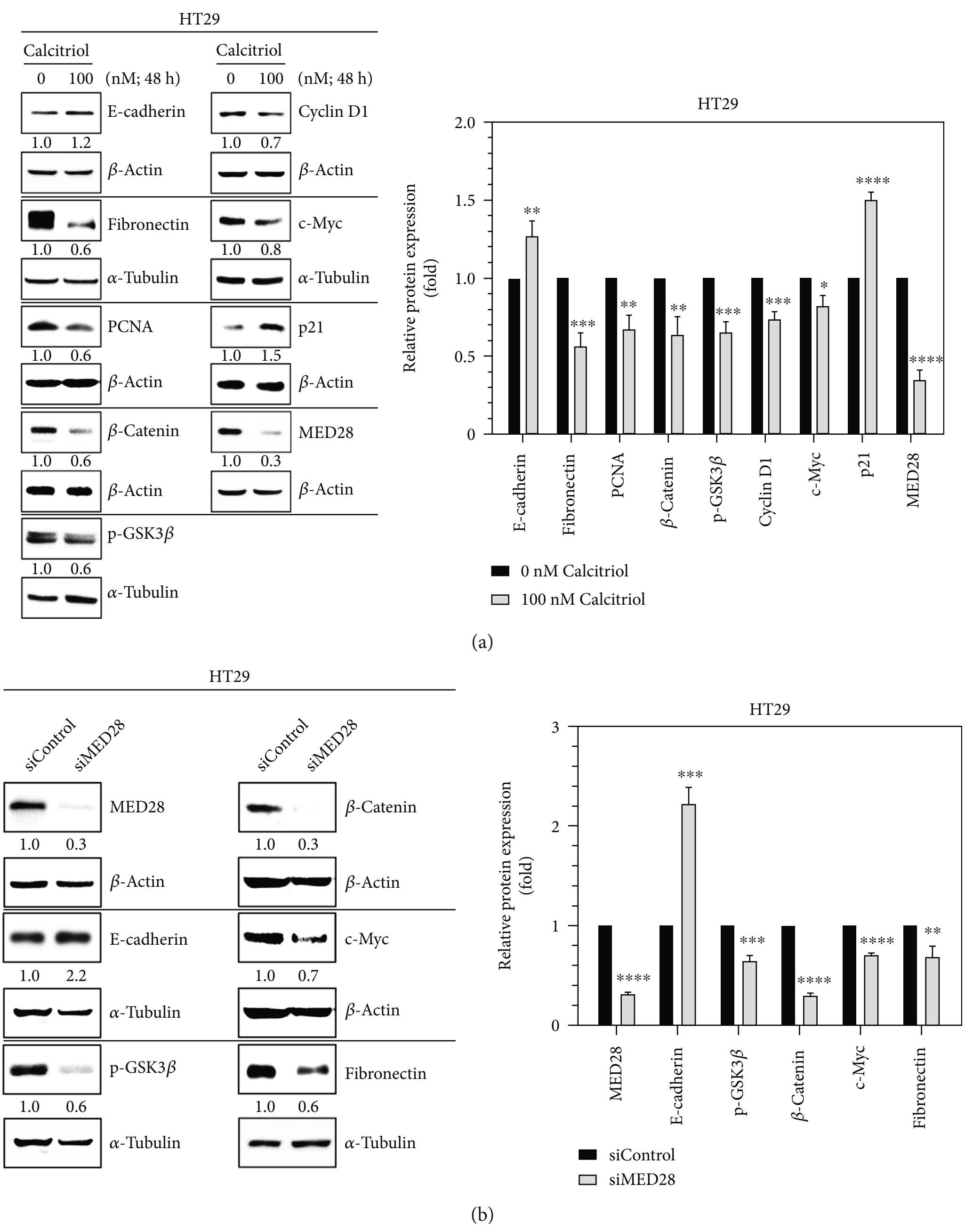


Figure 2: MED28 knockdown mimicked the effect of calcitriol on E-cadherin and Wnt/β-catenin signaling in HT29 human colorectal cancer cells. HT29 cells were treated with calcitriol (100 nM) for 48 h (a) or undergone MED28-specific siRNA (siMED28) for 72 h (b) with respective controls and subjected to Western blotting with the antibodies indicated. The ratios below the representative images (left panels) indicate the relative expression of the specific proteins with respect to those of 0 nM (a) or siControl (b) after normalization with the expression of the corresponding loading controls, β-actin or α-tubulin. Densitometric data (right panels) are expressed as means ± standard deviation, $n = 3$; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ as compared with 0 nM (a) or siControl (b).

junction to maintain the integrity of the epithelial structure. In addition, MED28 downregulated the expression of E-cadherin, whereas calcitriol could not only reduce the expression of MED28 but also relieve the inhibitory restraint of MED28 for E-cadherin (Figures 1, 2, and 3). Both HT29 and SW480 cells express fibronectin without external induction. However, these cells exhibit differential protein expression of vimentin and E-cadherin. HT29 cells exhibit considerable, endogenous E-cadherin, but do not express vimentin by default. In contrast, SW480 cells express vimentin, but their E-cadherin expression is barely detectable unless subjected to ectopic induction such as calcitriol or MED28 silencing. Nevertheless, both MED28 knockdown and calcitriol addition could downregulate the expression of fibronectin and upregulate that of E-cadherin in either HT29 or SW480 cells (Figures 2 and 3), and their control over EMT appeared partially through regulating transcriptional repressors of E-cadherin (Figures 3(b) and 3(c)). In

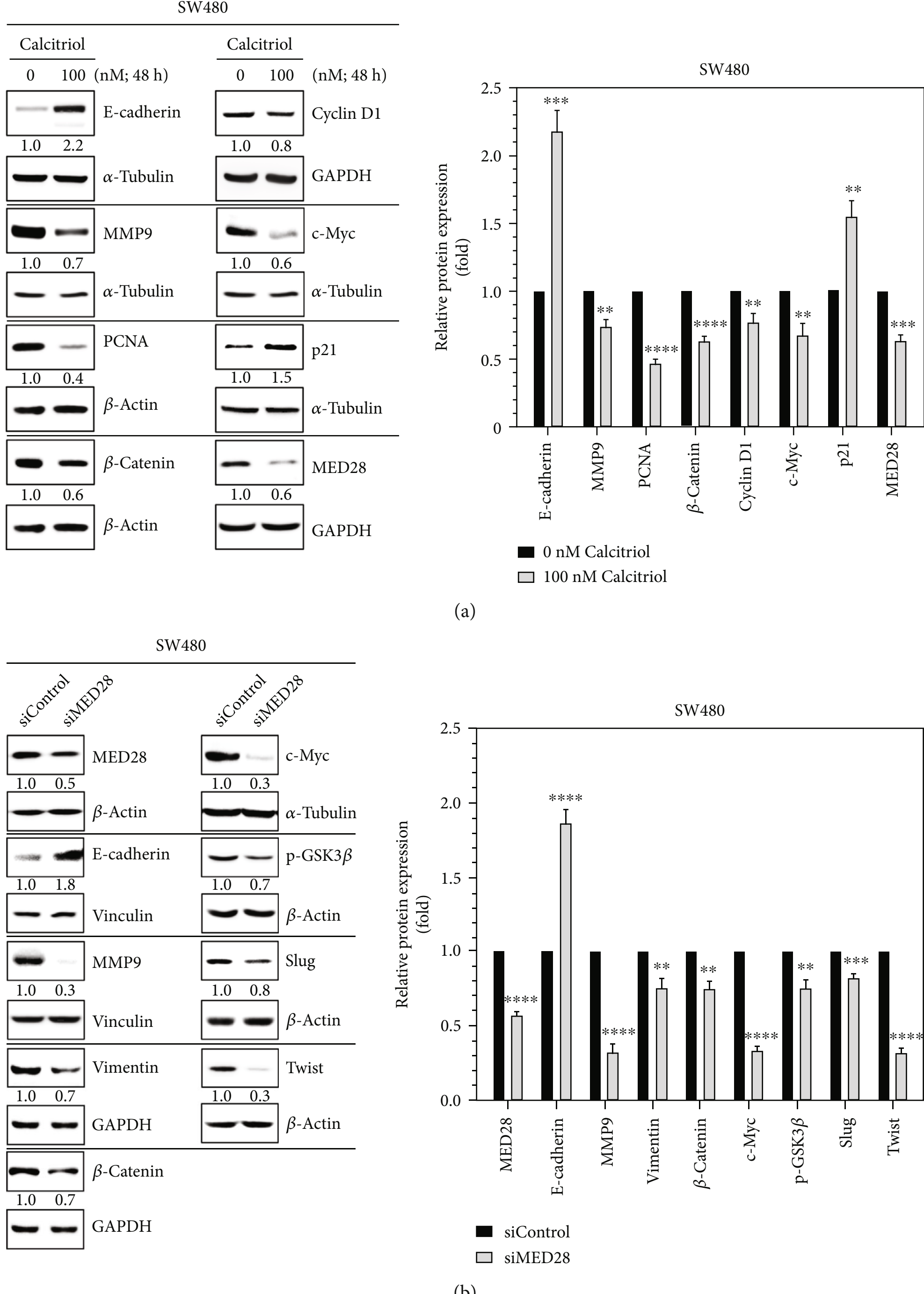


Figure 3: Continued.

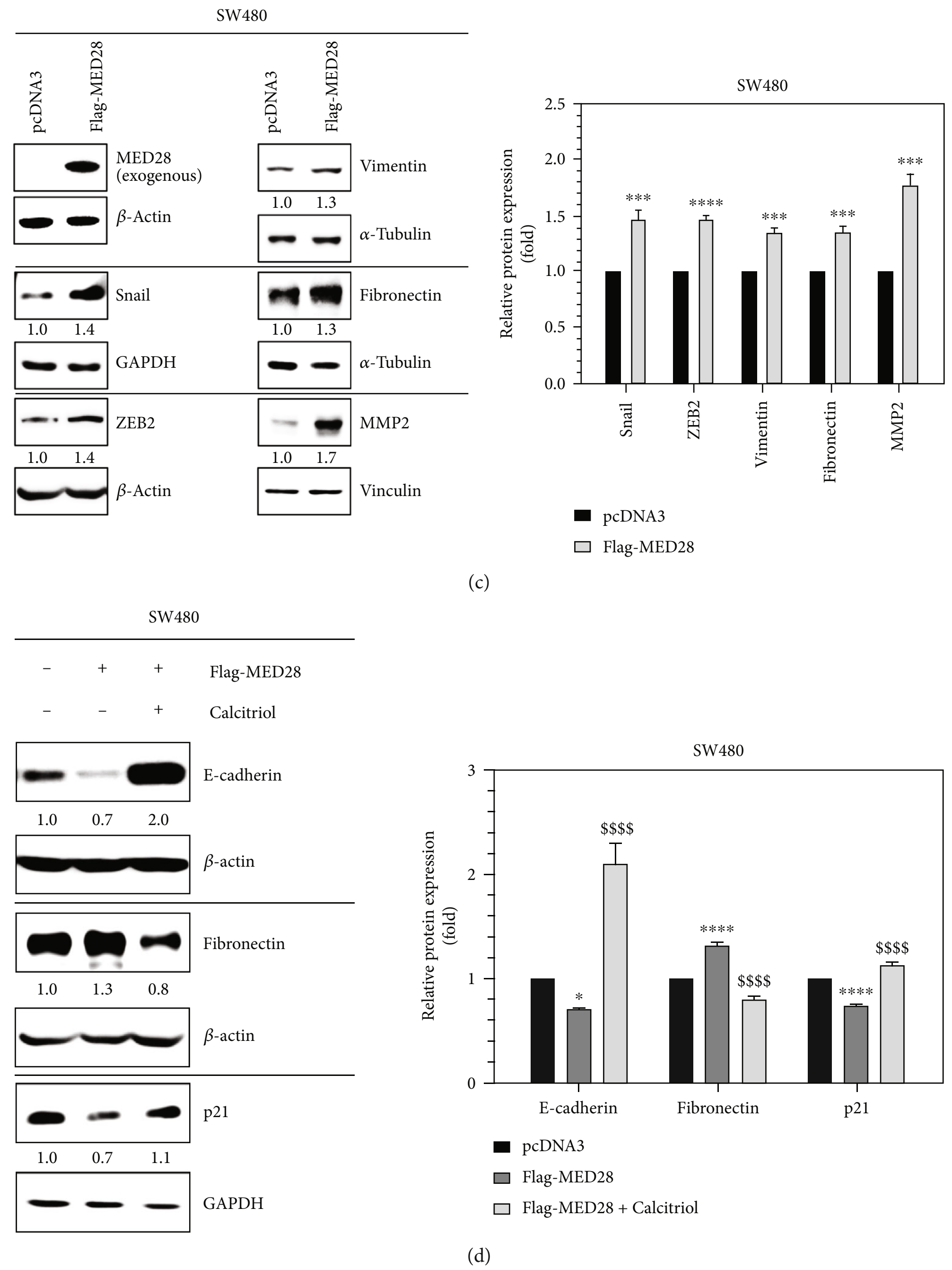


Figure 3: Calcitriol suppressed the upregulatory effect of MED28 on cell growth and epithelial-mesenchymal transition in SW480 human colorectal cancer cells. SW480 cells were treated with calcitriol (100 nM) for 48 h (a), undergone MED28-specific siRNA (siMED28) for 72 h (b), or MED28 overexpression plasmid (Flag-MED28) for 48 h (c), with respective controls, and subjected to Western blotting. The ratios below the representative images (left panels) indicate the relative expression of the specific proteins with respect to those of 0 nM (a), siControl (b), or pcDNA3 (c) after normalization with the expression of the corresponding loading controls, β-actin, α-tubulin, GAPDH, or vinculin. Densitometric data (right panels) are expressed as means ± standard deviation, $n = 3$; $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ as compared with 0 nM (a), siControl (b), or pcDNA3 (c). (d) SW480 cells were transfected with MED28 overexpression plasmid (Flag-MED28), followed by the addition of calcitriol for 48 h, and then subjected to Western blotting. "–" indicates vehicle control (0 nM of calcitriol) or pcDNA3 transfection. The ratios below the representative images (left panel) indicate the relative expression of the specific proteins with respect to those of lane 1 (0 nM and pcDNA3) after normalization with the expression of the corresponding loading controls, β-actin, or GAPDH. Densitometric data (right panel) are expressed as means ± standard deviation, $n = 3$; $^{*}p < 0.05$ and $^{****}p < 0.0001$ as compared with lane 1 (0 nM and pcDNA3); $^{\$\$\$\$}p < 0.0001$ as compared with lane 2 (0 nM and Flag-MED28).

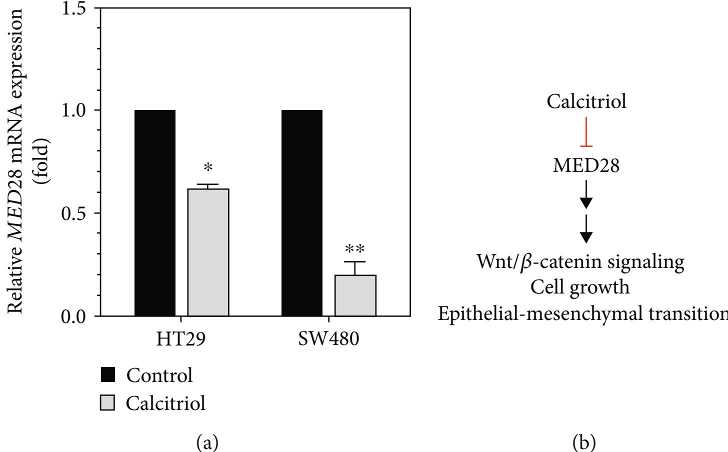


Figure 4: Calcitriol suppresses Wnt/β-catenin signaling, cell growth, and epithelial-mesenchymal transition, partially through downregulating MED28 expression, in human colorectal cancer cells. (a) SW480 and HT29 cells were treated with calcitriol (100 nM) for 48 h and subjected to RNA extraction and quantitative real-time polymerase chain reaction as described in Materials and Methods. Data are expressed as means ± standard deviation, $n = 3$; $^{*}p < 0.05$ and $^{**}p < 0.01$ as compared with the respective control (Control; 0 nM of calcitriol) after normalization with the expression of GAPDH. (b) MED28 upregulates Wnt/β-catenin signaling, cell growth, and epithelial-mesenchymal transition, which can be suppressed by calcitriol.

addition to EMT, calcitriol could also exert its effect on cell growth. Calcitriol reduced the expression of PCNA, a marker for cell proliferation, in HT29 xenografts as well as HT29 cells and SW480 cells (Figures 1(a), 2(a), and 3(a)). Furthermore, calcitriol or MED28 knockdown reduced the expression of Wnt/β-catenin signaling molecules, including c-Myc, cyclin D1, β-catenin, and p-GSK3β, but upregulated p21 (Figures 1(a), 2, and 3). Therefore, either MED28 silencing or calcitriol intervention could probably suppress cell proliferation and rescue the loss of epithelial polarity to reverse malignancy.

Calcitriol appears to exert its genomic and nongenomic effects to modulate the development of colorectal cancer through multiple avenues [8]. For example, calcitriol upregulates E-cadherin, which presumably increases the likelihood of β-catenin staying at adherens junctions. In the current study, we showed that calcitriol upregulated the expression of E-cadherin (Figures 1(a), 2(a), and 3(a)). Vaughan-Shaw et al. identified that calcitriol upregulates the expression of E-cadherin at the stages of both transcription and translation using *ex vivo* (patient-derived epithelial organoids) and *in vitro* (multiple CRC cell lines) approaches [28]. Xin et al. reported that calcitriol increases the binding between E-cadherin and β-catenin in SW480 cells using immunoprecipitation assay [29]. The formation of the calcitriol/VDR complex sequesters β-catenin from interacting with TCF/LEF1, which suppresses the transcriptional activation of the Wnt/β-catenin target genes [8]. Previously using the TOPFlash/FOPFlash reporter system, our laboratory has shown that both calcitriol addition and MED28 suppression downregulate the transcriptional activation of the Wnt/β-catenin signaling in SW480 cells [17, 20]. It is noteworthy that the genomic action of vitamin D exerts its effect on transcription through VDR, by interacting with other transcription factors and Mediator complex [7]. Therefore, through VDR-Mediator association, vitamin D could presumably regulate tumorigenesis. Interestingly, we found that calcitriol suppressed the mRNA expression of MED28 (Figure 4(a)), and MED28 is a Mediator subunit involved in the transcriptional activation of the RNA polymerase II-encoding genes [13]. Therefore, our data indicated that calcitriol could also exert its inhibitory effect on its downstream targets such as Wnt/β-catenin signaling molecules through suppressing the gene expression of MED28. Considering MED28 as a subunit of the Mediator complex and a link with Wnt/β-catenin signaling, it is intuitive to propose MED28 as a central player involved in the effect of calcitriol on the development of colorectal cancer.

In this study we identified a connection of MED28 with EMT and cell growth in human colorectal cancer cells such that MED28 regulates Wnt/β-catenin signaling and controls transcription factors involved in downregulating E-cadherin and upregulating mesenchymal markers such as MMP9 and fibronectin. The growth-promoting and EMT-upregulating modes of MED28 could be repressed by the addition of calcitriol (Figures 3(d) and 4(b)). Calcitriol may suppress the development of colorectal cancer through multiple pathways [8]. The reasons that we focused on Wnt/β-catenin signaling include a threefold explanation: (1) Wnt/β-catenin signaling, one of the major players in the progression of CRC, regulates cell growth and epithelial-mesenchymal transition in CRC. (2) MED28 regulates Wnt/β-catenin signaling and the expression of its downstream targets. (3) Calcitriol suppressed the expression of MED28 and Wnt/β-catenin signaling molecules. Therefore, we propose that one suppressive effect of calcitriol on CRC may work through MED28-mediated Wnt/β-catenin signaling. Calcitriol exhibits promising clinical application. For example, Yu et al. reported that calcitriol confers radiosensitivity in colorectal cancer, suggesting a synergistic effect between ionizing radiation and calcitriol [30]. Together, our data support that MED28 plays an important role in the development of colorectal cancer and indicate that calcitriol may be translationally applicable as an adjuvant in fighting this malignancy.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

Conceptualization was contributed by M.-F.L. and H.-S.L.; methodology was contributed by C.-Y.H., Y.-T.W., N.-T.H., P.-C.L., T.-Y.L., C.-I.L., H.-S.L., and M.-F.L.; software was contributed by C.-Y.H. and M.-F.L.; validation was performed by C.-Y.H. and M.-F.L.; formal analysis was performed by C.-Y.H., Y.-T.W., and N.-T.H.; investigation was performed by C.-Y.H., Y.-T.W., N.-T.H., P.-C.L., T.-Y.L., and C.-I.L.; resources was contributed by C.-Y.H., H.-S.L., and M.-F.L.; data curation was contributed by C.-Y.H. and M.-F.L.; writing—original draft preparation was performed by C.-Y.H. and M.-F.L.; writing—review and editing was performed by C.-Y.H., H.-S.L., and M.-F.L.; visualization was contributed by C.-Y.H. and M.-F.L.; supervision was performed by H.-S.L. and M.-F.L.; project administration was contributed by H.-S.L. and M.-F.L.; funding acquisition was contributed by Y.-T.W. and M.-F.L. All authors have read and agreed to the published version of the manuscript. Yu-Ting Weng and Nien-Tsu Hsieh are the two authors that contributed equally.

Acknowledgments

This work was supported by the Ministry of Science and Technology, Taiwan (MOST111-2320-B-039-019-MY3, MOST110-2320-B-039-052, MOST106-2320-B-039-062-MY3, and MOST105-2320-B-309-001 to M.-F.L. and 105-2815-C-309-006-B to Y.-T.W.) and China Medical University, Taiwan (CMU107-S-29, CMU108-MF-13, and CMU109-S-61 to M.-F.L.). Experiments and data analysis were performed in part through the use of the Medical Research Core Facilities Center, Office of Research & Development at China Medical University, Taichung, Taiwan.

References

[1] D. Hanahan and R. A. Weinberg, "Hallmarks of cancer: the next generation," *Cell*, vol. 144, no. 5, pp. 646–674, 2011.

[2] J. H. Tsai and J. Yang, "Epithelial-mesenchymal plasticity in carcinoma metastasis," *Genes & Development*, vol. 27, no. 20, pp. 2192–2206, 2013.

[3] N. Gavert and A. Ben-Ze'ev, "Epithelial-mesenchymal transition and the invasive potential of tumors," *Trends in Molecular Medicine*, vol. 14, no. 5, pp. 199–209, 2008.

[4] M. G. Mendez, S. Kojima, and R. D. Goldman, "Vimentin induces changes in cell shape, motility, and adhesion during the epithelial to mesenchymal transition," *The FASEB Journal*, vol. 24, no. 6, pp. 1838–1851, 2010.

[5] J. Heuberger and W. Birchmeier, "Interplay of cadherin-mediated cell adhesion and canonical Wnt signaling," *Cold Spring Harbor Perspectives in Biology*, vol. 2, no. 2, article a002915, 2010.

[6] R. Sanchez-Martinez, A. I. Castillo, A. Steinmeyer, and A. Aranda, "The retinoid X receptor ligand restores defective signalling by the vitamin D receptor," *EMBO Reports*, vol. 7, no. 10, pp. 1030–1034, 2006.

[7] S. Christakos, P. Dhawan, A. Verstuyf, L. Verlinden, and G. Carmeliet, "Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects," *Physiological Reviews*, vol. 96, no. 1, pp. 365–408, 2016.

[8] G. Ferrer-Mayorga, M. J. Larriba, P. Crespo, and A. Munoz, "Mechanisms of action of vitamin D in colon cancer," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 185, pp. 1–6, 2019.

[9] M. F. Holick, "Vitamin D deficiency," *The New England Journal of Medicine*, vol. 357, no. 3, pp. 266–281, 2007.

[10] R. Dou, K. Ng, E. L. Giovannucci, J. E. Manson, Z. R. Qian, and S. Ogino, "Vitamin D and colorectal cancer: molecular, epidemiological and clinical evidence," *The British Journal of Nutrition*, vol. 115, no. 9, pp. 1643–1660, 2016.

[11] J. Zhou, X. Ge, X. Fan, J. Wang, L. Miao, and D. Hang, "Associations of vitamin D status with colorectal cancer risk and survival," *International Journal of Cancer*, vol. 149, no. 3, pp. 606–614, 2021.

[12] W. B. Grant, "Review of recent advances in understanding the role of vitamin D in reducing cancer risk: breast, colorectal, prostate, and overall cancer," *Anticancer Research*, vol. 40, no. 1, pp. 491–499, 2020.

[13] S. Sato, C. Tomomori-Sato, T. J. Parmely et al., "A set of consensus mammalian mediator subunits identified by multidimensional protein identification technology," *Molecular Cell*, vol. 14, no. 5, pp. 685–691, 2004.

[14] M. F. Lee, R. L. Beauchamp, K. S. Beyer, J. F. Gusella, and V. Ramesh, "Magicin associates with the Src-family kinases and is phosphorylated upon CD3 stimulation," *Biochemical and Biophysical Research Communications*, vol. 348, no. 3, pp. 826–831, 2006.

[15] M. Lu, L. Zhang, M. R. Sartippour, A. J. Norris, and M. N. Brooks, "EG-1 interacts with c-Src and activates its signaling pathway," *International Journal of Oncology*, vol. 29, no. 4, pp. 1013–1018, 2006.

[16] T. Wiederhold, M. F. Lee, M. James et al., "Magicin, a novel cytoskeletal protein associates with the NF2 tumor suppressor merlin and Grb2," *Oncogene*, vol. 23, no. 54, pp. 8815–8825, 2004.

[17] M. F. Lee, N. T. Hsieh, C. Y. Huang, and C. I. Li, "All *trans*-retinoic acid mediates MED28/HMG box-containing protein 1 (HBP1)/β-catenin signaling in human colorectal cancer cells," *Journal of Cellular Physiology*, vol. 231, no. 8, pp. 1796–1803, 2016.

[18] C. Y. Huang, Y. H. Chou, N. T. Hsieh, H. H. Chen, and M. F. Lee, "MED28 regulates MEK1-dependent cellular migration in human breast cancer cells," *Journal of Cellular Physiology*, vol. 227, no. 12, pp. 3820–3827, 2012.

[19] C. Y. Huang, N. T. Hsieh, C. I. Li, Y. T. Weng, H. S. Liu, and M. F. Lee, "MED28 regulates epithelial–mesenchymal transition through NFκB in human breast cancer cells," *Journal of Cellular Physiology*, vol. 232, no. 6, pp. 1337–1345, 2017.

[20] C. Y. Huang, Y. T. Weng, P. C. Li et al., "Calcitriol suppresses Warburg effect and cell growth in human colorectal cancer cells," *Life*, vol. 11, no. 9, p. 963, 2021.

[21] N. T. Hsieh, C. Y. Huang, C. C. Li, I. C. Wang, and M. F. Lee, "MED28 and forkhead box M1 (FOXM1) mediate matrix metalloproteinase 2 (MMP2)-dependent cellular migration in human nonsmall cell lung cancer (NSCLC) cells," *Journal of Cellular Physiology*, vol. 234, no. 7, pp. 11265–11275, 2019.

[22] C. Y. Chan, C. H. Lien, M. F. Lee, and C. Y. Huang, "Quercetin suppresses cellular migration and invasion in human head and neck squamous cell carcinoma (HNSCC)," *Biomedicine*, vol. 6, no. 3, p. 15, 2016.

[23] M. El-Bahrawy, R. Poulsom, A. J. Rowan, I. T. Tomlinson, and M. R. Alison, "Characterization of the E-cadherin/catenin complex in colorectal carcinoma cell lines," *International Journal of Experimental Pathology*, vol. 85, no. 2, pp. 65–74, 2004.

[24] N. A. Wong and M. Pignatelli, "Beta-catenin–a linchpin in colorectal carcinogenesis?," *The American Journal of Pathology*, vol. 160, no. 2, pp. 389–401, 2002.

[25] J. L. Stamos and W. I. Weis, "The beta-catenin destruction complex," *Cold Spring Harbor Perspectives in Biology*, vol. 5, no. 1, article a007898, 2013.

[26] B. T. MacDonald, K. Tamai, and X. He, "Wnt/β-catenin signaling: components, mechanisms, and diseases," *Developmental Cell*, vol. 17, no. 1, pp. 9–26, 2009.

[27] X. Tian, Z. Liu, B. Niu et al., "E-cadherin/beta-catenin complex and the epithelial barrier," *Journal of Biomedicine & Biotechnology*, vol. 2011, Article ID 567305, 6 pages, 2011.

[28] P. G. Vaughan-Shaw, J. P. Blackmur, G. Grimes et al., "Vitamin D treatment induces in vitro and ex vivo transcriptomic changes indicating anti-tumor effects," *The FASEB Journal*, vol. 36, no. 1, article e22082, 2022.

[29] Y. Xin, L. He, Z. Luan et al., "E-cadherin mediates the preventive effect of vitamin D_3 in colitis-associated carcinogenesis," *Inflammatory Bowel Diseases*, vol. 23, no. 9, pp. 1535–1543, 2017.

[30] X. Yu, Q. Wang, B. Liu, N. Zhang, and G. Cheng, "Vitamin D enhances radiosensitivity of colorectal cancer by reversing epithelial-mesenchymal transition," *Frontiers in Cell and Development Biology*, vol. 9, article 684855, 2021.