Cancer Biomarkers 41 (2024) 133–143
DOI 10.3233/CBM-230566
IOS Press

Vitamin D receptor polymorphisms associate with the efficacy and toxicity of radioiodine-131 therapy in patients with differentiated thyroid cancer

Yuanhong Deng[a,1], Ying Fu[b,1], Ganghua Feng[c] and Yi Zhang[c,*]
[a]*Department of Nuclear Medicine, The Seventh Affiliated Hospital, Sun Yat-Sen University, Shenzhen, China*
[b]*Department of Nuclear Medicine, The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University/Hunan Cancer Hospital, Changsha, China*
[c]*Department of Neurology, Chenzhou First People's Hospital, Chenzhou, China*

Received 2 February 2024
Accepted 25 August 2024

Abstract.
BACKGROUND: Radioiodine-131 (I-131) therapy is the common postoperative adjuvant therapy for differentiated thyroid cancer (DTC) However, methods to evaluate the efficacy and toxicity of I-131 on DTC are still lacking.
OBJECTIVE: To evaluate the association between vitamin D receptor (VDR) gene polymorphisms and the efficacy and toxicity of I-131 in DTC patients.
METHODS: A total of 256 DTC patients who received I-131 therapy were enrolled. The patients were divided into effective group and ineffective group. 4 single nucleotide polymorphisms (SNPs) (rs7975232, rs731236, rs1544410 and rs10735810) of VDR were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) Cell counting kit-8 (CCK-8) and flow cytometry were used to detect the proliferation and apoptosis of thyroid cancer cells.
RESULTS: Patients in effective group had more CC genotype of rs7975232 and GG genotype of rs10735810 compared with patients in ineffective group They were also independent factors for influencing the efficacy of I-131. PTC-1 and FTC-133 cells transfected with CC genotype of rs7975232 showed lower proliferative activity and higher apoptosis rate after being treated with I-131 In addition, patients with CC genotype at rs7975232 had fewer adverse reactions after I-131 treatment.
CONCLUSIONS: VDR gene polymorphisms may be associated with the efficacy and toxicity of I-131 in DTC patients, which will help to personalize the treatment for patients.

Keywords: Differentiated thyroid cancer, Vitamin D receptor, polymorphism, radioiodine-131, efficacy

1. Introduction

Thyroid cancer is a common malignant tumor of the neck, which mainly originates from thyroid follicular epithelial cells. Thyroid cancer is divided into differentiated thyroid cancer (DTC), squamous carcinoma, undifferentiated carcinoma, medullary carcinoma, of which the incidence of DTC accounts for more than 90% of the thyroid cancer [1,2]. In the past decades, the mortality rate of thyroid cancer in China has remained the highest in the world [3]. Radioiodine-131 (I-131) can be absorbed by thyroid cancer tissues with high specificity The high-energy β-ray generated by the physical decay of I-131 is often used clinically to

[1]Yuanhong Deng and Ying Fu equally contributed.
*Corresponding author: Yi Zhang, Department of Neurology, Chenzhou First People's Hospital, No. 102 Luojiajing, Beihu District, Chenzhou, Hunan 423000, China. Tel.: +86 15573551185; E-mail: zhangyi_0628@126.com.

destroy residual thyroid cancer tissues for therapeutic purpose [4]. Although the clinical value of I-131 in removing residual thyroid tissue has been widely recognized, the sensitivity and effective half-life of I-131 vary among different patients, the therapeutic effect can be affected by clinical and pathological factors [5]. Therefore, it is important to identify the factors that affect the efficacy of I-131 ablation for improving the efficacy of remnant ablation.

At present, the pathogenesis and progression of thyroid cancer are still under discussion. Some scholars believed that chemical toxins, insulin resistance, metabolic syndrome, and vitamin D deficiency were potential risk factors for thyroid cancer [6,7,8]. The active form of vitamin D, 1,25-(OH)2D3, mainly binds to the nuclear vitamin D receptor (VDR) and controls the expression of more than 200 genes to exert physiological functions [9]. Previous studies have shown that VDR was closely related to thyroid cancer. The expression of VDR was increased in DTC and benign thyroid tumors compared with normal thyroid [10]. Some researchers also found that VDR knockdown could attenuate the anti-proliferation, pro-apoptosis and anti-invasion effects of vitamin D in papillary thyroid carcinoma (PTC) by activating the Wnt/β-catenin signaling pathway [11]. In recent years, a large number of studies have found the correlation between VDR gene polymorphism and various types of cancer. As the first VDR gene single nucleotide polymorphisms (SNPs) discovered in DTC patients, rs7975232, rs731236 rs1544410 and rs10735810 were reported to be closely related to the risk and pathogenesis of thyroid cancer [12,13]. Among them, the Ff genotype of rs731236 was shown to account for a higher proportion of DTC patients [14]. The genotypes AA of rs7975232 and FF of rs10735810 showed less frequent in patients with follicular thyroid cancer (FTC) [12]. In addition, rs7975232 and rs1544410 have been proved that they were susceptible for development of breast cancer [15]. The association between the two SNPs with risk of thyroid cancer has also been explored by many researchers [13]. The above studies have shown that the expression and polymorphism of VDR were closely related to the risk and prognosis of DTC. In addition, rs7975232, rs731236, rs1544410 and rs10735810 SNPs were also strongly associated with the risk or development of thyroid cancer. While I-131 is the most common treatment after DTC, few studies reported the relationship between key SNPs of VDR and the efficacy of I-131 in DTC patients.

In this study, 4 key SNPs, rs7975232, rs731236, rs1544410 and rs10735810 were selected to analyze the effects of these SNPs on the clinical efficacy and side effects of I-131 in patients with DTC. The aim of this study is to provide more theoretical reference for I-131 therapy.

2. Materials and methods

2.1. Patient enrollment

All participants in this study were from the Chinese Han population. A total of 256 patients with DTC were diagnosed by pathological examination in Chenzhou first people's Hospital. These patients underwent residual ablation of I-131 at hospital (dose of 100 mci), including 251 patients with PTC and 5 patients with FTC, 53 males and 203 females. Informed consent was obtained from hospitalized patients or their guardians, and the ethics committee of Chenzhou first people's Hospital approved this study.

Exclusion criteria for this study were: pregnancy, hepatic or renal insufficiency, or concurrent malignancy Patients with distant metastasis. A history of radiotherapy or chemotherapy. No history of clinical examination [16].

2.2. Evaluation of postoperative I-131 efficacy

Efficacy evaluation criteria [16]: Approximately 6 months after treatment with I-131, patients discontinued thyroid hormone and a low-iodine diet for 2–4 weeks and thyroid uptake of I-131 was measured. If no spots were found in the thyroid bed (I-131 uptake), the thyroid uptake rate of I-131 after 24 hours of I-131 treatment was $< 1\%$, serum thyroid stimulating hormone (TSH) level > 10 mIU/l, thyroglobulin (TG) level < 10 ng/ml, the treatment was considered successful and patients with these symptoms were divided into the effective group. Conversely, treatment failure was considered and patients with these symptoms were classified as ineffective.

2.3. Genotyping

In the morning, 2 ml of fasting elbow venous blood was collected into anticoagulant tubes containing ethylene diamine tetraacetic acid (EDTA). According to the DNA extraction kit (cat.no. 69504; Qiagen, Hilden, Germany) to extract genomic DNA. The genotypes of 4 SNPs (rs7975232, rs731236, rs1544410 and rs10735810) in VDR gene were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) [16].

2.4. Cell culture

TPC-1 and FTC-133 human thyroid cancer cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). The Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; cat. no. 11965092; Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; cat. no. 16140089; Gibco) and 100 mg/ml streptomycin (cat. no. ST487; Beyotime, Shanghai, China) and 100 IU/ml penicillin (cat. no. ST486; Beyotime) at 37°C in a 5% CO_2 incubator.

2.5. Plasmid construction and transfection

Referring to the methods described by previous [17]. A VDR promoter region containing the polymorphisms rs7975232 and rs10735810 were obtained by PCR. PCR products containing the VDR polymorphism were digested with Bgl II (cat. no. ER0081; Thermo Fisher Scientific, Waltham, MA, USA) and Kpn I (cat. no. ER0521; Thermo Fisher Scientific) and ligated into the vector pGL3-basic (cat. no. HG-VQP0121; HonorGene, Changsha, Hunan Province, China) to construct the rs7975232 recombinant plasmids (vector-AA and vector-CC) and rs10735810 recombinant plasmids (vector-AA and vector-GG). Lipofectamine 2000 (cat. no. 11668019; Thermo Fisher Scientific) were transfected into TPC-1 and FTC-133 cells with vectors.

2.6. Cell counting kit-8 (CCK-8) assay

CCK-8 solution (cat. no. ab228554, Abcam, Burlingame, CA, USA) was recommended to assess cell viability of TPC-1 and FTC-133 cells. The cells were inoculated into 96-well plates at a density of 1×10^4 cells/ml for 24 h. The cells of I-131 group were exposed to 1 mCi I-131 for 24 h, 48 h and 72 h. Add 10 μl CCK-8 reagent to the well of each group after time and mix, then incubate in an incubator for 2 h. The optical density (OD) at 450 nm was measured using a microplate reader (Bio-Rad, Hercules, CA, USA). Each experiment was repeated three times.

2.7. Flow cytometry

Flow cytometry was performed by using Alexa Fluor 488 Annexin V/Dead Cell Apoptosis Kit (cat. no. V13241, Thermo Fisher Scientific) following manufacturer's instructions. TPC-1 and FTC-133 cells were exposed to 1 mCi I-131 for 12 h. For cell apoptosis analysis, cells were harvested and fixed in pre-cold 70% ethanol at 4°C overnight. The cells were stained with Annexin V-FITC and propidium iodide (PI) and subsequently the ratio of apoptotic cells was tested by flow cytometry (BD FACS Calibur, Becton Dickinson, Franklin Lake, New Jersey, USA). Each experiment was repeated three times.

2.8. Statistical analysis

Visualization and analysis of the data were performed using GraphPad Prism 6.0. An F-test was performed for normality of the measurement data, and a t-test was used for differences between the two groups. The Chi-square test was used to compare the count data between the two groups. Hardy-Weinberg equilibrium (HWE) tests were performed on the SHEsis online platform (http://analysis.bio-x.cn/myAnalysis.php) to verify the representative of the samples. Bonferroni correction was performed for data for multiple comparisons, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Clinical baseline information

The basic characteristics of all patients are summarized in Table 1, including 153 patients in the effective group and 103 patients in the ineffective group. There were no significant differences in gender, age, body mass index (BMI) and pathological type between the two groups ($P > 0.05$). The effective group had a significantly higher serum TSH level ($P = 0.003$) and a significantly lower serum TG level ($P = 0.038$) before I-131 therapy than the ineffective group. In addition, there was a significant difference in tumor node metastasis (TNM) stage between the two groups ($P = 0.006$).

3.2. HWE test

The distribution of the genotypes of the 4 polymorphism sites of VDR in the study samples was tested by HWE test. The P values of HWE test were greater than 0.05 in the effective and ineffective groups. In other words, the gene frequencies of the selected study population can represent the gene distribution of the general population, and the sample is population representative.

Table 1
Clinical baseline information of DTC patients

Characteristics	Effective group ($n = 153$)	Ineffective group ($n = 103$)	P value
Gender (male, %)	33 (21.57)	20 (19.42)	0.677
Age (mean ± SD)	43.64 ± 12.12	42.17 ± 11.76	0.335
BMI			
18.5	11 (7.19)	8 (7.77)	0.084
18.5–24	88 (57.51)	45 (43.69)	
24	54 (35.30)	50 (48.54)	
Serum TSH levels before therapy (mean ± SD)	79.38 ± 28.59	68.76 ± 28.72	0.004
Serum TG levels before therapy (mean ± SD)	16.11 ± 3.67	17.03 ± 3.09	0.038
Types of pathology			
PTC	150 (98.04)	101 (98.06)	0.991
FTC	3 (1.96)	2 (1.94)	
TNM			
Stage I	125 (81.70)	66 (64.08)	0.006
Stage II	11 (7.19)	13 (12.62)	
Stage III	17 (11.11)	24 (23.30)	

BMI, Body mass index; TSH, Thyroid stimulating hormone; TG, Thyroglobulin; PTC, Papillary thyroid carcinoma; FTC, Follicular thyroid carcinoma; TNM, Tumor node metastasis.

Table 2
Association between polymorphisms of VDR gene and I-131 efficacy

SNPs	Effective group ($n = 153$)	Ineffective group ($n = 103$)	P value	P value*	OR	95% CI
rs7975232						
AA	7 (4.58)	12 (11.65)				
AC	50 (32.68)	46 (44.66)	0.225	0.899	0.537	0.195–1.480
CC	96 (62.74)	45 (43.69)	0.008	0.030	0.273	0.101–0.741
CC+AC	146 (95.42)	91 (88.35)	0.034	0.136	0.364	0.138–0.957
A	64 (20.92)	70 (33.98)				
C	242 (79.08)	136 (66.02)	0.001	0.004	0.514	0.345–0.766
rs731236						
GG	1 (0.65)	1 (0.97)				
AG	17 (11.11)	18 (17.48)	0.968	3.874	1.059	0.061–18.30
AA	135 (88.24)	84 (81.55)	0.736	2.945	0.622	0.038–10.08
AA+AG	152 (99.35)	102 (99.03)	0.777	3.110	0.671	0.042–10.85
G	19 (6.21)	20 (9.71)				
A	287 (93.79)	186 (90.29)	0.143	0.573	0.616	0.320–1.185
rs1544410						
TT	2 (1.31)	1 (0.97)				
CT	30 (19.61)	20 (19.42)	0.817	3.274	1.333	0.113–15.70
CC	121 (79.08)	82 (79.61)	0.805	3.218	1.355	0.121-15.19
CC+CT	151 (98.69)	102 (99.03)	0.806	3.225	1.351	0.121–15.10
T	34 (11.11)	22 (10.68)				
C	272 (88.89)	184 (89.32)	0.878	3.512	1.045	0.592–1.845
rs10735810						
AA	22 (14.38)	26 (25.24)				
AG	72 (47.06)	51 (49.52)	0.134	0.534	0.599	0.306–1.173
GG	59 (38.56)	26 (25.24)	0.007	0.30	0.371	0.179–0.775
GG+AG	131 (85.62)	77 (74.76)	0.029	0.116	0.497	0.264–0.937
A	116 (37.91)	103 (50.00)				
G	190 (62.09)	103 (50.00)	0.007	0.027	0.611	0.427–0.873

*Represents the P value after Bonferroni correction. SNPs, Single nucleotide polymorphisms; OR, Odd ratio; CI, Confidence interval.

3.3. Association between polymorphisms of VDR gene and I-131 efficacy

The results of the analyses between VDR gene polymorphisms and I-131 efficacy were shown in Table 2. The distribution of CC genotype at rs7975232 was significantly increased in the effective group (CC vs AA, $P = 0.030$). In the recessive model of this locus, the P value of CC+AC vs AA was significantly different before correction, but not after correction ($P = 0.136$). In addition, patients carrying the GG genotype of rs10735810 had a higher response rate to I-131 treat-

Table 3
Logistic regression analysis of factors associated with efficacy of I-131 in DTC patients

Factor	B	SE	OR	95%CI	P value
Serum TSH levels before therapy	0.013	0.005	0.987	0.978–0.997	0.009
Serum TG levels before therapy	0.082	0.041	1.086	1.003–1.175	0.042
TNM stage	0.547	0.181	1.728	1.211–2.464	0.003
rs7975232	0.567	0.221	0.567	0.367–0.875	0.010
rs10735810	0.388	0.197	0.678	0.461–0.998	0.049

B, Bias; SE, Standard error; OR, Odds ratio; CI, Confidence interval; TSH, Thyroid-stimulating hormone; TG, Thyroglobulin; TNM, Tumor node metastasis.

ment (GG vs. AA, $P = 0.030$). These results suggest that CC genotype of rs7975232 and GG genotype of rs10735810 were significantly correlated with the efficacy of I-131 treatment.

3.4. Logistic regression analysis of factors associated with the efficacy of I-131 treatment of DTC patients

To further clarify the independent factors influencing the efficacy of I-131, we performed multifactorial regression analyses with characteristics that differed in univariate analyses (patients' pre-therapy serum TG and TSH levels, TNM stage, rs7975232 polymorphisms, and rs10735810) as independent variables and treatment outcome (effective or ineffective) as the dependent variable. The results showed that all of the above dependent variables were significantly associated with the efficacy of I-131 treatment (all $P < 0.05$), suggesting that all of these factors may independently influence the efficacy of I-131 treatment, and the results were shown in Table 3.

3.5. Proliferation and apoptotic ability of transfected TPC-1 and FTC-133 cells

In order to further clarify the effect of rs7975232 and rs10735810 on the biological function of thyroid cancer cells, functional tests were performed on transfected TPC-1 and FTC-133 cells. The results of CCK-8 assay showed that with the extension of cell culture time, the proliferation activity of TPC-1 and FTC-133 cells in CC genotype group (rs7975232) was significantly reduced compared with that in AA genotype group (Fig. 1A–B). At the same time, no matter the mock group, AA genotype group or CC genotype group, the cell proliferation activity was significantly down-regulated after I-131 treatment (Fig. 1A–B). On the other hand, AA and GG genotypes of rs10735810 could not regulate the proliferation of TPC-1 and FTC-133 cells (Fig. 2A–B). The effect of rs7975232 and rs10735810 on the apoptosis level of TPC-1 and FTC-133 cells was further explored. Apoptosis experiment also showed that the apoptosis rate of AA genotype in TPC-1 and FTC-133 cells was not significantly different from that of control cells (Fig. 1C–D). Compared with AA genotype, CC genotype could enhance the apoptosis rate of TPC-1 and FTC-133 cells (Fig. 1C–D). After exposure to I-131, apoptosis rate of thyroid cancer cells in 3 groups was significantly up-regulated (Fig. 1C–D). On the other hand, AA and GG genotypes of rs10735810 could not regulate the apoptosis rate of TPC-1 and FTC-133 cells (Fig. 2C–D). These results suggest that CC genotype of rs7975232 can inhibit the proliferation of thyroid cancer cell, which may promote the therapeutic effect of I-131.

3.6. Relationship between polymorphisms of VDR gene and I-1311 toxicity

In order to further explore whether the toxic side effects after I-131 treatment were related to VDR gene polymorphism. We collected different adverse reactions of patients, which mainly manifested as neck discomfort, gastrointestinal side effects, and salivary gland side effects. The results showed (Table 4) that patients with rs7975232 CC genotype had less adverse events ($P < 0.05$).

4. Discussion

A total of 256 DTC patients who received postoperative I-131 ablation therapy were enrolled in this study. The patients were divided into effective group and ineffective group according to serum TG, TSH and imaging results. The results showed that patients with CC genotype at rs7975232 and GG genotype at rs10735810 were more sensitive to I-131 treatment. Biological experiments also verified the above conclusions, that the proliferation activity of cancer cells transfected with rs7975232 CC genotype was significantly decreased after I-131 treatment, while the level of apoptosis was significantly increased. In terms of adverse reactions,

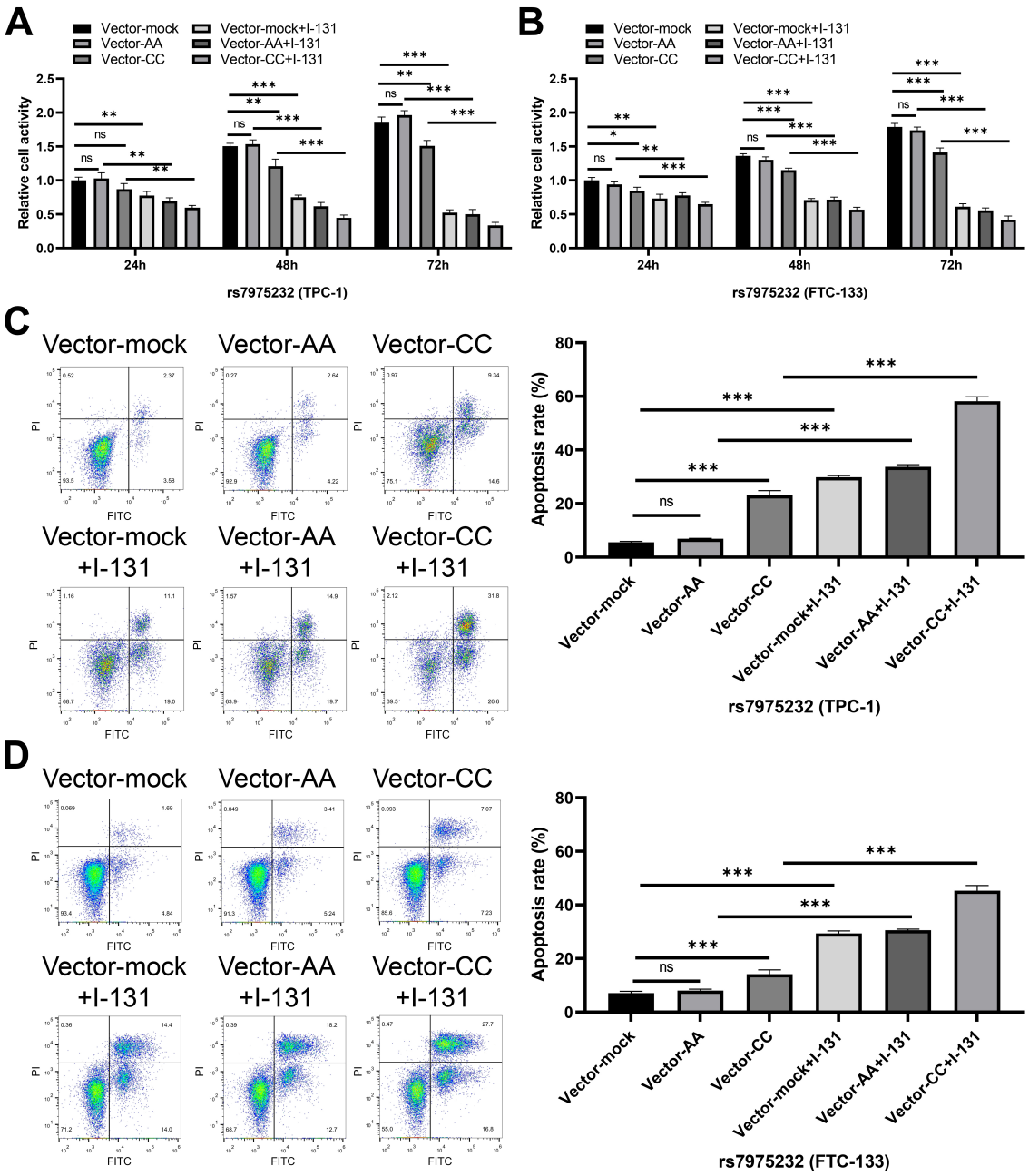

Fig. 1. The effect of rs7975232 on proliferation and apoptosis of thyroid cancer cells. (A–B) The proliferation activity of TPC-1 (A) and FTC-133 (B) cells in different treatment groups (rs7975232) was analyzed via CCK-8 assay. Data are represented as mean ± SD from three independent experiments. (ns represents no significant difference, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). (C–D) The apoptosis level of TPC-1 (C) and FTC-133 (D) cells in different treatment groups (rs7975232) was analyzed via flow cytometry. Data are represented as mean ± SD from three independent experiments. (ns represents no significant difference, $^{***}P < 0.001$).

patients with CC genotype at rs7975232 locus have fewer adverse reactions after I-131 treatment. The results of this study for clinical screening potential future patients benefit from the I-131 provides a certain theoretical basis.

I-131 therapy is a commonly used adjuvant treatment for patients with DTC after resection, which can effectively remove residual thyroid tissue and play a crucial role in improving the clinical treatment effect and prognosis of patients [18]. However, some patients

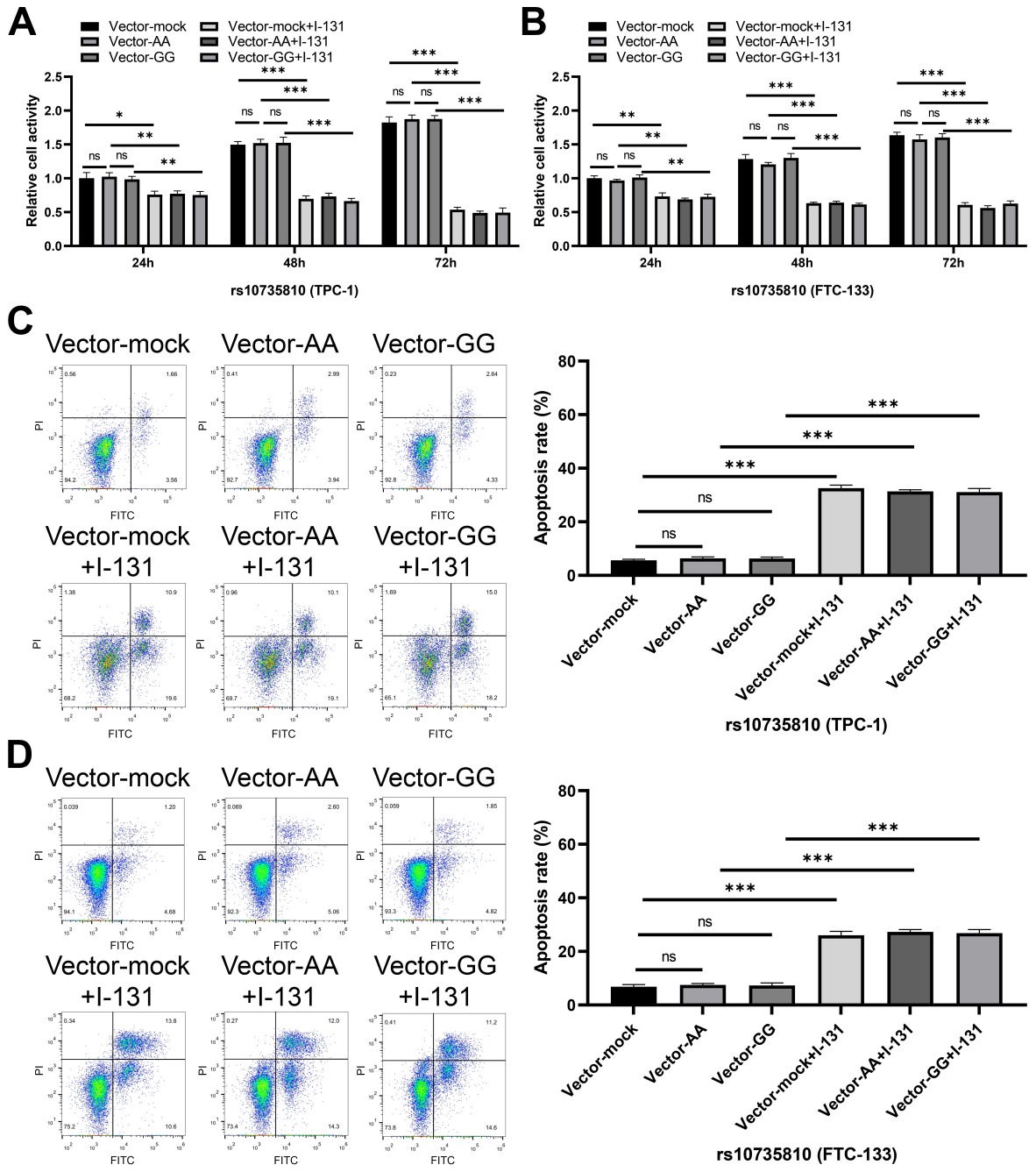

Fig. 2. The effect of rs10735810 on proliferation and apoptosis of thyroid cancer cells. (A–B) The proliferation activity of TPC-1 (A) and FTC-133 (B) cells in different treatment groups (rs10735810) was analyzed via CCK-8 assay. Data are represented as mean ± SD from three independent experiments. (ns represents no significant difference, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). (C–D) The apoptosis level of TPC-1 (C) and FTC-133 (D) cells in different treatment groups (rs10735810) was analyzed via flow cytometry. Data are represented as mean ± SD from three independent experiments. (ns represents no significant difference, $^{***}P < 0.001$).

do not respond to I-131 therapy in clinical practice. Some reports have suggested that the efficacy of I-131 is associated with SNPs in some genes. Zhang and his partners found that cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) gene polymorphisms were associated with the efficacy of I-131 in DTC patients, with a significantly greater distribution of AG + AA genotypes of +49A > G and A alleles of CT60A > G in

Table 4
Relationship between polymorphisms of VDR gene and I-131 toxicity

SNPs	Effective group					P value	Ineffective group					P value
	n	Neck discomfort	SG side effects	GI symptoms	Total adverse reaction		n	Neck discomfort	SG side effects	GI symptoms	Total adverse reaction	
rs7975232												
AA	7	2	0	1	3	0.005	12	4	2	2	8	$P < 0.001$
AC	50	4	0	1	5		46	2	1	1	4	
CC	96	3	1	2	6		45	1	1	1	3	
rs731236												
GG	1	0	0	0	0	0.419	1	0	0	0	0	0.205
AG	17	2	0	1	3		18	3	1	1	5	
AA	135	7	1	3	11		84	4	3	3	10	
rs1544410												
TT	2	1	0	0	1	0.079	1	0	0	0	0	0.917
CT	30	2	1	1	4		20	2	0	1	3	
CC	121	6	0	3	9		82	5	4	3	12	
rs10735810												
AA	22	1	0	1	2	0.694	26	1	1	1	3	0.680
AG	72	5	1	2	8		51	4	3	2	9	
GG	59	3	0	1	4		26	2	0	1	3	

SNPs, Single nucleotide polymorphisms; SG, Salivary gland; GI, Gastrointestinal.

the effective group [19]. In graves' disease, the efficacy of I-131 was shown to be significantly associated with the CTLA-4 exon-1 49 A/G polymorphism [20]. In addition, many studies have shown that VDR polymorphisms are closely related to risk and efficacy of cancers. Researchers have found that the BB genotype of rs7975232 is associated with the risk of lung cancer in Asians [21]. TT genotype of rs1544410 has also been found to be associated with the risk of colorectal cancer [22]. Patients with breast cancer homozygous for the rare allele of rs731236 showed a tendency toward an increased risk for breast cancer-specific mortality [23]. For rs10735810, which was found to be associated with an increased risk of breast cancer in patients with early onset [24]. Beysel and his colleagues also investigated the association of rs1544410, rs7975232 and rs731236 with the risk of developing PTC. However, no association was found between these three SNPs and PTC risk [25]. In terms of treatment, changes in rs1544410 genotype frequency were thought to predict androgen-deprivation therapy (ADT) effects of prostate cancer [26]. In addition to rs1544410, the relationship between the other three SNPs and cancer therapy has been poorly reported. In the present study, we first proved that patients with CC genotype of rs7975232 and GG genotype of rs10735810 were more sensitive to I-131 treatment. This suggests that VDR rs7975232 and rs10735810 polymorphisms may be potential markers for judging the clinical efficacy of I-131 in DTC patients, and may have a positive impact on the success rate of ablation after DTC surgery.

Because of the ligand-dependent nature of VDR, its biological effects are affected by gene expression, so some VDR gene polymorphisms may affect the binding and biological activity of vitamin D and VDR [27,28]. Previous studies have indicated that the system on low VDR expression, can activate the Wnt/β-catenin signaling pathway, and further weaken the vitamin D in the PTC resistance proliferation, promote apoptosis, and resistance to attack action [11]. Similarly, VDR inhibited proliferation and promoted differentiation of DTC cells by regulating E-cadherin/β-catenin complex [29]. In addition, some researchers have demonstrated that VDR/protein tyrosine phosphatase non-receptor type 2 (PTPN2)/phospho-signal transducer and activator of transcription 3 (p-STAT3) signaling pathway is related to the sensitivity of PTC cells to doxorubicin [30]. In this study, to verify the effect of rs7975232 polymorphism on the phenotype of DTC cancer cells, CCK-8 and flow cytometry experiments were performed. The results showed that the proliferation activity of the CC genotype transfected cells was significantly decreased and the apoptosis level was significantly increased after I-131 treatment, this implies that DTC cells carrying the CC genotype are more sensitive to I-131 therapy. This may be because rs7975232 is located in the 3' untranslated region, which may affect the biological function of cancer cells by affecting the stability and protein efficiency of VDR gene mRNA.

In addition, although I-131 can eliminate residual thyroid tissue, its β-rays are not selective, so it may also damage other parts of the body (salivary glands, liver, etc.), and then cause a series of side effects [31]. For example, in DTC patients, carriers of the T allele have a higher risk of I-131 radiation-induced GI reactions compared with carriers of the C allele of rs620815, and the GA genotype may increase the incidence of neck pain compared with the GG genotype of rs1800629 [32]. Chang and his colleagues showed that polymorphisms in the CTLA-4 gene were significantly associated with the frequency of adverse reactions to I-131 therapy in DTC patients [33]. In this study, we also conducted a study on the related side effects, and we found that patients with CC genotype at rs7975232 locus had fewer adverse reactions after I-131 treatment. These results may be useful for clinical screening of DTC patients who are more suitable for I-131 therapy after surgery.

Despite the above findings, this study has some shortcomings. Although we found through biological experiments that VDR polymorphism does affect the proliferation and apoptosis of thyroid cancer cells, the specific mechanism of the relationship between VDR polymorphism and DTC needs to be further explored In addition, this study demonstrated that CC genotype of rs7975232 could inhibit the proliferation of thyroid cancer cell *in vitro*. However, this conclusion has not been confirmed *in vivo*. Finally, the conclusions of this study are based on a small sample size and have not been validated in other sample sets or data sets. Therefore, it is necessary to verify the conclusions of this study in other sample sets in future studies.

5. Conclusion

In conclusion, CC genotype of rs7975232 and GG genotype of rs10735810 were significantly associated with better I-131 efficacy in DTC patients after surgery, and cancer cells transfected with CC genotype of rs7975232 were more sensitive to I-131 treatment. This may be of great value in evaluating the efficacy of I-131. In addition, in terms of adverse reactions, pa-

tients with CC genotype at rs7975232 locus had fewer adverse reactions after I-131 treatment. It also suggests that VDR polymorphism can be used as a potential target to predict the efficacy and toxicity of I-131 in DTC patients, which can be applied to clinical practice in the future to improve the clinical efficacy and reduce the toxicity of patients with thyroid cancer.

Acknowledgments

Not applicable.

Author contributions

Conception: Yi Zhang. Interpretation or analysis of data: Yuanhong Deng and Ying Fu. Preparation of the manuscript: Yuanhong Deng, Ying Fu and Ganghua Feng. Revision for important intellectual content: Yuanhong Deng and Yi Zhang. Supervision: Yi Zhang.

Data availability

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

Ethical approval and consent to participate

The experiments were approved by the ethics committee of Chenzhou first people's Hospital (Approval No. 2023014).

Consent for publication

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

References

[1] J.A. Fagin and S.A. Wells, Jr., Biologic and Clinical Perspectives on Thyroid Cancer, *N Engl J Med* **375** (2016), 1054–67.
[2] K.A. Araque, S. Gubbi and J. Klubo-Gwiezdzinska, Updates on the Management of Thyroid Cancer, *Horm Metab Res* **52** (2020), 562–577.
[3] Y. Deng, H. Li, M. Wang, N. Li, T. Tian, Y. Wu, P. Xu, S. Yang, Z. Zhai, L. Zhou, Q. Hao, D. Song, T. Jin, J. Lyu and Z. Dai, Global Burden of Thyroid Cancer From 1990 to 2017, *JAMA Netw Open* **3** (2020), e208759.
[4] B.R. Haugen, E.K. Alexander, K.C. Bible, G.M. Doherty, S.J. Mandel, Y.E. Nikiforov, F. Pacini, G.W. Randolph, A.M. Sawka, M. Schlumberger, K.G. Schuff, S.I. Sherman, J.A. Sosa, D.L. Steward, R.M. Tuttle and L. Wartofsky, 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer, *Thyroid* **26** (2016), 1–133.
[5] A. Ciarallo and J. Rivera, Radioactive Iodine Therapy in Differentiated Thyroid Cancer: 2020 Update, *AJR Am J Roentgenol* **215** (2020), 285–291.
[6] B. Biondi, D. Arpaia, P. Montuori, G. Ciancia, S. Ippolito, G. Pettinato and M. Triassi, Under the shadow of vesuvius: a risk for thyroid cancer, *Thyroid* **22** (2012), 1296–7.
[7] M. Roskies, Y. Dolev, D. Caglar, M.P. Hier, A. Mlynarek, A. Majdan and R.J. Payne, Vitamin D deficiency as a potentially modifiable risk factor for thyroid cancer, *J Otolaryngol Head Neck Surg* **41** (2012), 160–3.
[8] W. Borena, T. Stocks, H. Jonsson, S. Strohmaier, G. Nagel, T. Bjørge, J. Manjer, G. Hallmans, R. Selmer, M. Almquist, C. Häggström, A. Engeland, S. Tretli, H. Concin, A. Strasak, P. Stattin and H. Ulmer, Serum triglycerides and cancer risk in the metabolic syndrome and cancer (Me-Can) collaborative study, *Cancer Causes Control* **22** (2011), 291–9.
[9] P. Kmieć and K. Sworczak, Vitamin D in thyroid disorders, *Exp Clin Endocrinol Diabetes* **123** (2015), 386–93.
[10] I. Clinckspoor, E. Hauben, L. Verlinden, A. Van den Bruel, L. Vanwalleghem, V. Vander Poorten, P. Delaere, C. Mathieu, A. Verstuyf and B. Decallonne, Altered expression of key players in vitamin D metabolism and signaling in malignant and benign thyroid tumors, *J Histochem Cytochem* **60** (2012), 502–11.
[11] R. Pang, Y. Xu, X. Hu, B. Liu and J. Yu, Vitamin D receptor knockdown attenuates the antiproliferative, pro-apoptotic and anti-invasive effect of vitamin D by activating the Wnt/β-catenin signaling pathway in papillary thyroid cancer, *Mol Med Rep* **22** (2020), 4135–4142.
[12] M. Penna-Martinez, E. Ramos-Lopez, J. Stern, N. Hinsch, M.L. Hansmann, I. Selkinski, F. Grünwald, C. Vorländer, R.A. Wahl, W.O. Bechstein, S. Zeuzem, K. Holzer and K. Badenhoop, Vitamin D receptor polymorphisms in differentiated thyroid carcinoma, *Thyroid* **19** (2009), 623–8.
[13] A. Maciejewski and K. Lacka, Vitamin D-Related Genes and Thyroid Cancer-A Systematic Review, *Int J Mol Sci* **23** (2022).
[14] A.M. Cocolos, A. Muresan, A. Caragheorgheopol, M. Ghemigian, D. Ioachim and C. Poiana, Vitamin D Status and VDR Polymorphisms as Prognostic Factors in Differentiated Thyroid Carcinoma, *In Vivo* **36** (2022), 2434–2441.
[15] M.U.N. Iqbal and T.A. Khan, Association between Vitamin D receptor (Cdx2, Fok1, Bsm1, Apa1, Bgl1, Taq1, and Poly (A)) gene polymorphism and breast cancer: A systematic review and meta-analysis, *Tumour Biol* **39** (2017), 1010428317731280.
[16] D.F. Chang, X.H. Chen, J. Huang, Y.M. Sun, D.Y. Zhu and Z.Q. Xu, CTLA-4 gene polymorphisms associate with efficacy of postoperative radioiodine-131 for differentiated thyroid carcinoma, *Future Oncol* **13** (2017), 1057–1068.
[17] Y. Chen, R. Lu, H. Zheng, R. Xiao, J. Feng, H. Wang, X. Gao and L. Guo, The NFKB1 polymorphism (rs4648068) is associated with the cell proliferation and motility in gastric cancer, *BMC Gastroenterol* **15** (2015), 21.

[18] P. Kumar, N.A. Damle, S. Agarwala, S.N. Dwivedi and C. Bal, Individualized dosimetry in children and young adults with differentiated thyroid cancer undergoing iodine-131 therapy, *J Pediatr Endocrinol Metab* (2020).
[19] Retraction, *Future Oncol* **16** (2020), 613.
[20] X.R. Han, X. Wen, S. Wang, S.H. Fan, J. Zhuang, Y.J. Wang, Z.F. Zhang, M.Q. Li, B. Hu, Q. Shan, C.H. Sun, Y.X. Bao, D.M. Wu, J. Lu and Y.L. Zheng, Correlations of CTLA-4 exon-1 49 A/G and promoter region 318C/T polymorphisms with the therapeutic efficacy of (131) I radionuclide in graves' disease in Chinese Han population, *J Cell Biochem* **119** (2018), 6383–6390.
[21] H. Zhong, R. Zhou, Y. Feng, G.X. Zheng, Y. Liang, J.Y. Zhang, X.Q. Qin, W. Chen, J.Q. Wu and Y.H. Zhong, Association of vitamin D receptor gene polymorphism with the risk of lung cancer: a meta-analysis, *J Recept Signal Transduct Res* **34** (2014), 500–5.
[22] J. Li, S. Qin, S. Zhang, Y. Lu, Q. Shen, L. Cheng and R. Zhong, Serum vitamin D concentration, vitamin D-related polymorphisms, and colorectal cancer risk, *Int J Cancer* **153** (2023), 278–289.
[23] L. Perna, K. Butterbach, U. Haug, B. Schöttker, H. Müller, V. Arndt, B. Holleczek, B. Burwinkel and H. Brenner, Vitamin D receptor genotype rs731236 (Taq1) and breast cancer prognosis, *Cancer Epidemiol Biomarkers Prev* **22** (2013), 437–42.
[24] P. Gapska, R.J. Scott, P. Serrano-Fernandez, T. Huzarski, T. Byrski, J. Kładny, J. Gronwald, B. Górski, C. Cybulski, J. Lubinski and T. Debniak, Vitamin D receptor variants and breast cancer risk in the Polish population, *Breast Cancer Res Treat* **115** (2009), 629–33.
[25] S. Beysel, N. Eyerci, F.A. Pinarli, M. Apaydin, M. Kizilgul, M. Caliskan, O. Ozcelik, S. Kan and E. Cakal, VDR gene FokI polymorphism as a poor prognostic factor for papillary thyroid cancer, *Tumour Biol* **40** (2018), 1010428318811766.
[26] J.B. Pao, Y.P. Yang, C.N. Huang, S.P. Huang, T.C. Hour, T.Y. Chang, Y.H. Lan, T.L. Lu, H.Z. Lee, S.H. Juang, C.Y. Huang and B.Y. Bao, Vitamin D receptor gene variants and clinical outcomes after androgen-deprivation therapy for prostate cancer, *World J Urol* **31** (2013), 281–7.
[27] H. Sun, S.R. Long, X. Li, H. Ge, X. Liu, T. Wang, F. Yu, Y. Wang, Y. Xue and W. Li, Serum vitamin D deficiency and vitamin D receptor gene polymorphism are associated with increased risk of cardiovascular disease in a Chinese rural population, *Nutr Res* **61** (2019), 13–21.
[28] 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* **338** (2004), 143–56.
[29] Y. Ling, F. Xu, X. Xia, D. Dai, R. Sun and Z. Xie, Vitamin D receptor regulates proliferation and differentiation of thyroid carcinoma via the E-cadherin-β-catenin complex, *J Mol Endocrinol* **68** (2022), 137–151.
[30] T. Zhang, L. He, Z. Wang, W. Dong, W. Sun, Y. Qin, P. Zhang and H. Zhang, Calcitriol enhances Doxorubicin-induced apoptosis in papillary thyroid carcinoma cells via regulating VDR/PTPN2/p-STAT3 pathway, *J Cell Mol Med* **24** (2020), 5629–5639.
[31] L. Yang, J. Ma, P. Lei, J. Yi, Y. Ma, Z. Huang, T. Wang, H. Ping, D. Ruan, D. Sun and H. Pan, Advances in Antioxidant Applications for Combating (131)I Side Effects in Thyroid Cancer Treatment, *Toxics* **11** (2023).
[32] J. Liu, X. Tang, F. Shi, C. Li, K. Zhang, J. Liu, G. Wang, J. Yin and Z. Li, Genetic polymorphism contributes to (131)I radiotherapy-induced toxicities in patients with differentiated thyroid cancer, *Pharmacogenomics* **19** (2018), 1335–1344.
[33] I.S. Carvalho, C.I. Gonçalves, J.T. Almeida, T. Azevedo, T. Martins, F.J. Rodrigues and M.C. Lemos, Association of Vitamin D Pathway Genetic Variation and Thyroid Cancer, *Genes (Basel)* **10** (2019).