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Association of *RTN4* indel polymorphisms with the risk of tumorigenesis in the Chinese Han population

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Meta-Analysis

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ABSTRACT

Although studies have reported the association of two insertion/deletion (indel) polymorphisms in the 3'-untranslated region (UTR) of the *RTN4* gene with the risk of tumorigenesis, the findings are inconsistent and require further explanation. Comprehensive literature searches were undertaken in Pubmed, Embase, Web of Science, China National Knowledge Infrastructure, and WangFang database. The risk of tumorigenesis was determined using odds ratios (ORs) and 95% confidence intervals (CIs) based on STATA 12.0 software. A total of four case-control studies with 1214 patients and 1850 controls focused on the *RTN4* gene TATC/- polymorphism and five case-control studies with 1625 patients and 2321 controls on the *RTN4* gene CAA/- polymorph. They were all conducted in the Chinese Han population. Pooled analysis showed that the TATC/- polymorphism was not associated with the risk of tumorigenesis under all genetic models and the CAA/- polymorphism was significantly associated with the risk of tumorigenesis under the homozygote genetic model (Del/Del vs. Ins/Ins: OR=1.32, 95%CI=1.04-1.68, P=0.02). In conclusion, the current findings suggested that the CAA/- polymorphism in the 3'-UTR of the *RTN4* gene was significantly associated with the risk of tumorigenesis and may serve as a valuable marker for predicting tumor risk in the Chinese Han population.

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Introduction

Cancer is the second leading cause of death worldwide, accounting for 9.6 million deaths in 2018 (1). Smoking, drinking, a poor diet, and a lack of exercise are all known to be important cancer risk factors around the world (2). Some biological factors, such as Helicobacter pylori, human papillomavirus, hepatitis B virus, and hepatitis C virus, can, of course, significantly raise the risk of cancer. The occurrence of different cancers varies greatly across different ethnic groups, which may be related in part to lifestyle and genetic background (3). Identifying risk factors that lead to cancer, especially genetic risk factors, will contribute to the precise prevention of cancer.

The human reticulon 4 (*RTN4*) gene is located on chromosome 2p16 and can encode three Nogo protein isoforms including Nogo-A, B, and C through different splicing and the use of different promoters (4-6). The Nogo proteins belong to myelin-associated endoplasmic reticulum proteins that share a conserved reticulum homology domain (RHD) containing a 66 amino-acid loop domain called Nogo-66 (7,8). Studies have shown that Nogo proteins play an important role in the development of cancer (9-13). Nogo-A is an axon regeneration inhibitor that has been linked to the malignancy of the oligodendroglial tumor (9). In addition, the expression of Nogo-A was shown to be increased in cancer stem-like cells originating from glioblastoma and played a crucial role in encouraging malignant tendencies (10). In hepatocellular carcinoma, Nogo-B stimulated tumor angiogenesis and offered a possible therapeutic target (11). Furthermore, through its interaction with c-FLIP in colorectal cancer cells, Nogo-B performed as a negative regulator of the apoptotic pathway (12). The overexpression of Nogo-C in hepatocellular carcinoma cells could inhibit cell proliferation (13). Therefore, factors affecting Nogo proteins expression can be associated with cancer development. Studies have shown that genetic variants in human gene regulatory regions such as the 3'-untranslated region (UTR) and 5'-UTR can affect gene expression and thus correlate with the risk of tumor development (14,15). Two insertion/deletion (indel) polymorphisms located in the 3'-UTR of the *RTN4* gene have the potential to regulate the expression of RTN4, and they are associated with the risk of tumors such as hepatocellular carcinoma, non-small cell lung cancer, and gastric cancer (16-21). However, it has also been suggested that these two indel polymorphisms are not associated with the risk of tumorigenesis (17,20,21). The current study will use meta-analysis to clarify the relationship between these two indel polymorphisms and the risk of tumor development.

Materials and Methods

Literature search

Comprehensive literature searches were undertaken for all publications published in Pubmed, Embase, Web of Science, China National Knowledge Infrastructure, and WangFang Database up to November 10, 2022. These studies reported the association between two indel polymorphisms (TATC/- and CAA/-) in the 3'-UTR of the *RTN4*

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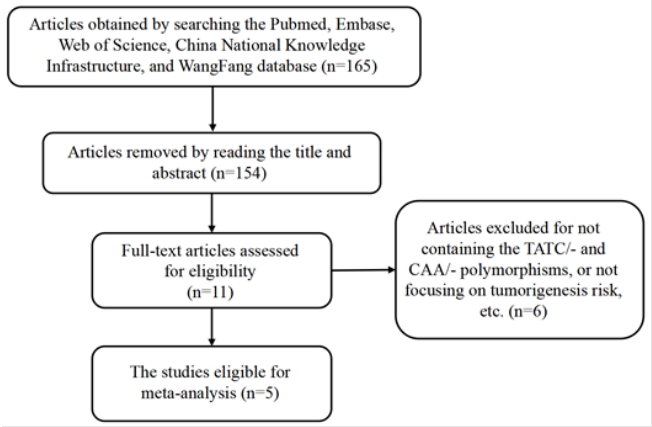

Figure 1. The flow diagram of literature selection for this meta-analysis.

gene and the risk of tumorigenesis. "RTN4" and ("polymorphism" or "mutation" or "variant") and ("cancer" or "carcinoma" or "tumor" or "neoplasms") were the search terms. Figure 1 depicted the process of identifying qualified studies.

Inclusion and exclusion criteria

The qualified studies must meet the following requirements: (a) case-control studies on the association between the *RTN4* polymorphism (TATC/- and CAA/-) and the risk of tumorigenesis; (b) studies that had sufficient information for calculating the odds ratios (ORs) with their 95% confidence intervals (CIs); (c) The genotype frequency distribution of the control group was consistent with Hardy-Weinberg equilibrium (HWE). The exclusion criteria were as follows: (a) insufficient genotype information provided; (b) family-based studies and case-only studies.

Data extraction

Two authors separately searched the literature and extracted important data, and any disagreements were resolved through discussion by the third author. For each study, the first author's name, year of publication, country, tumor type, genotyping method, number of different genotypes in case-control groups, and HWE test results of the control group were collected (Table 1).

Statistical analysis

STATA 12.0 software was used for the meta-analysis. The chi-square test was used to assess HWE for each study to ensure that the control population was representative. The genotypes of the control group were discordant with HWE if $P_{HWE}<0.05$. ORs and 95% CIs were used to estimate the association between the *RTN4* polymorphism (TATC/- and CAA/-) and the risk of tumorigenesis. Pooled ORs and 95% CIs were calculated under homozygote, heterozygote, dominant, recessive, and allelic genetic models. The pooled OR was evaluated using the Z test, and $P<0.05$ was declared statistically significant. The heterogeneity test results were used to decide whether the fixed effect model or the random effect model should be utilized. The Q test and I^2 statistics were used to assess heterogeneity. If the test result is $I^2>50\%$ or $P<0.1$, suggesting heterogeneity, the random effect model is chosen. If not, the fixed-effects model is used. Sensitivity analysis was used to assess the impact of each study on the merged OR. Begg's and Egger's tests were used to examine publication bias in all genetic models, and $P<0.05$ was considered a significant publication bias.

Results

Main characteristics of included studies

The meta-analysis included four case-control studies with 1214 patients and 1850 controls for the TATC/- polymorphism and five case-control studies with 1625 patients and 2321 controls for the CAA/- polymorphism (Table 1). These case-control studies were published between 2012 and 2018, and they were all conducted in the Chinese Han population. Tumor types included in the study were clear cell renal cell carcinoma, hepatocellular carcinoma, non-small cell lung cancer, uterine leiomyomas, and cervical squamous cell carcinoma. All studies were genotyped using PCR-PAGE. Genotype distribution of the control population was consistent with HWE.

Meta-analysis results

The association between the TATC/- and CAA/- polymorphisms in the 3'-UTR of the *RTN4* gene and the risk of tumorigenesis was shown in Table 2 and Table 3, respectively. For the TATC/- polymorphism, no significant association was found under all genetic models. However, a significant association was found between the CAA/- polymorphism and the risk of tumorigenesis under the homozygote genetic model (Del/Del vs. Ins/Ins: OR=1.32, 95%CI=1.04-1.68, P=0.02).

Sensitivity analysis and publication bias

Sensitivity analysis revealed that in the studies regarding the TATC/- polymorphism, the merged OR did not change significantly after deleting each study in turn under homozygote, heterozygote, dominant, and allelic genetic models (Figure 2). However, under the recessive genetic model, the merged OR was found to be significantly altered after removing the study by Zhang et al (Figure 2). In the studies regarding the CAA/- polymorphism, the merged OR did not change significantly after deleting each study in turn under heterozygote and dominant genetic models (Figure 3). However, under the homozygote, recessive, and allelic genetic model, the merged OR was found to be significantly altered after removing the individual studies (Figure 3).

The P values of the Begg's and Egger's test were >0.05

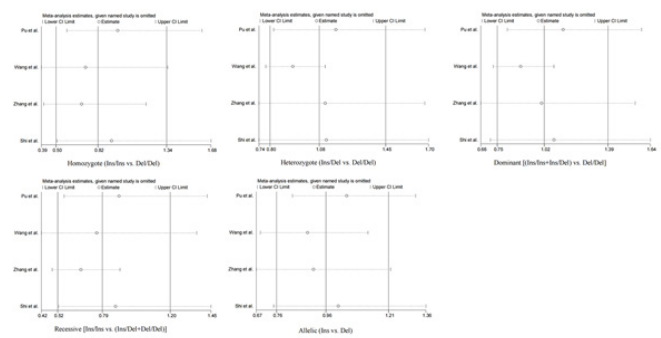

Figure 2. Sensitivity analysis of the association between the RTN4 TATC/- polymorphism and the risk of tumorigenesis.

Table 1. Characteristics of the studies eligible for meta-analysis.

Authors	Year	Country	Tumor type	Genotyping method	Polymorphism	Cases				Controls				P_{HWE}
						Del/Del	Del/Ins	Ins/Ins	total	Del/Del	Del/Ins	Ins/Ins	total	
Pu et al.(17)	2018	China	Clear cell renal cell carcinoma	PCR-PAGE	TATC/-	145	135	28	308	187	206	73	466	0.20
					CAA/-	32	128	148	308	45	200	221	466	0.98
Wang et al.(18)	2018	China	Hepatocellular carcinoma	PCR-PAGE	TATC/-	96	152	36	284	215	201	68	484	0.06
					CAA/-	29	148	107	284	32	217	235	484	0.05
Lu et al.(19)	2014	China	Non-small cell lung cancer	PCR-PAGE	CAA/-	21	185	205	411	18	172	281	471	0.18
Zhang et al.(20)	2013	China	Uterine leiomyomas	PCR-PAGE	TATC/-	111	121	54	286	182	201	67	450	0.35
					CAA/-	40	126	120	286	42	205	203	450	0.34
Shi et al.(21)	2012	China	Cervical squamous cell carcinoma	PCR-PAGE	TATC/-	147	158	31	336	182	201	67	450	0.35
					CAA/-	27	154	155	336	42	205	203	450	0.34

PCR-PAGE: polymerase chain reaction-polyacrylamide gel electrophoresis.

Table 2. Meta-analysis of the association between the *RTN4* TATC/- polymorphism and the risk of tumorigenesis.

Genetic model	Cases/Controls	Heterogeneity			Association		
		I^2	P	Model	OR	95%CI	P
Homozygote (Ins/Ins vs. Del/Del)	648/1041	78%	<0.01	Random	0.82	0.50-1.34	0.44
Heterozygote (Ins/Del vs. Del/Del)	1065/1575	72%	0.01	Random	1.08	0.80-1.45	0.61
Dominant [(Ins/Ins+Ins/Del) vs. Del/Del]	1214/1850	77%	0.01	Random	1.02	0.75-1.39	0.89
Recessive [Ins/Ins vs. (Ins/Del+Del/Del)]	1214/1850	74%	0.01	Random	0.79	0.52-1.20	0.27
Allelic (Ins vs. Del)	2428/3700	79%	<0.01	Random	0.96	0.76-1.21	0.71

Table 3. Meta-analysis of the association between the *RTN4* CAA/- polymorphism and the risk of tumorigenesis.

Genetic model	Case/Control	Heterogeneity			Association		
		I^2	P	Model	OR	95%CI	P
Homozygote (Del/Del vs. Ins/Ins)	884/1322	40%	0.16	Fixed	1.32	1.04-1.68	0.02
Heterozygote (Del/Ins vs. Ins/Ins)	1476/2142	54%	0.07	Random	1.17	0.96-1.43	0.12
Dominant [(Del/Del+Del/Ins) vs. Ins/Ins]	1625/2321	60%	0.04	Random	1.20	0.98-1.47	0.08
Recessive [Del/Del vs. (Del/Ins+Ins/Ins)]	1625/2321	11%	0.34	Fixed	1.25	0.99-1.57	0.06
Allelic (Del vs. Ins)	3250/4642	58%	0.05	Random	1.16	1.00-1.35	0.05

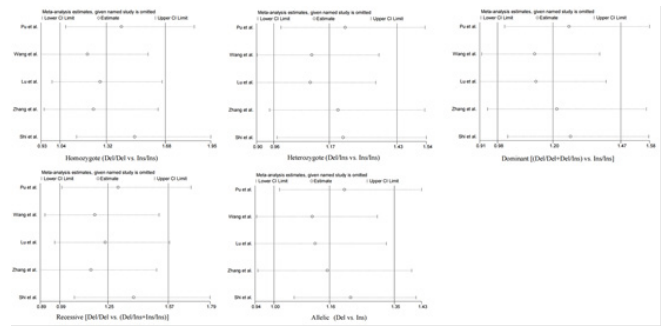

Figure 3. Sensitivity analysis of the association between the RTN4 CAA/- polymorphism and the risk of tumorigenesis.

in all genetic models, which indicated that there was no significant publication bias in the included studies (Table 4 and Table 5).

Discussion

The relationship between indel polymorphisms on tumor-associated genes, including the *RTN4* gene, and the risk of tumor development has been widely reported (17-23). Pu et al. found that the Ins allele of the TATC/- polymorphism in the *RTN4* 3′-UTR was linked to a lower risk of clear cell renal cell carcinoma, while the CAA/- polymorphism was not associated with the risk of clear cell renal cell carcinoma in the Chinese Han population (17). Wang et al. observed that both CAA/- and TATC/- polymorphisms in the *RTN4* 3′-UTR are associated with the risk of hepatocellular carcinoma in the Chinese Han population (18). The CAA/- polymorphism genotypes Ins/Del and Del/Del were strongly related to an elevated risk of hepatocellular carcinoma as compared to the Ins/Ins genotype. Meanwhile, the Del allele of the CAA/- polymorphism was found to be strongly associated with an elevated risk of hepatocellular carcinoma when compared to the Ins allele. When compared to the Del/Del genotype, the Ins/Del genotype of the TATC/- polymorphism was substantially related to an elevated risk of hepatocellular carcinoma. Lu et al. found that the Del allele of the CAA/- polymorphism could significantly increase the risk of non-small cell lung cancer in the Chinese Han population (19). Zhang et al. found that there was no statistically significant link between the TATC/- polymorphism and uterine leiomyomas risk in Chinese Han women. In the recessive and codominant models, however, the CAA/- polymorphism was strongly related to an increased risk of uterine leiomyomas (20). Shi et al. observed that in a recessive model, the TATC/- polymorphism was related to lower cervical squamous cell carcinoma risk in the Chinese Han population, however, no significant association was found between the CAA/- polymorphism and the risk of cervical squamous cell carcinoma in different genetic models (21). We used meta-analysis to combine the above inconsistent results and found that the TATC/- polymorphism was not associated with the risk of tumorigenesis under all genetic models, while the CAA/- polymorphism was associated with the risk of tumorigenesis under the homozygote genetic model. Individuals carrying the Del/Del genotype of the CAA/- polymorphism had a 32% increased risk of developing tumors compared to individuals carrying the Ins/Ins genotype. Therefore, the CAA/- polymorphism had the potential to be used as a biomarker to predict the risk of tumorigenesis.

Although the current study used meta-analysis to clarify the relationship between two indel polymorphisms in the 3'-UTR of the *RTN4* gene and the risk of tumorigenesis and found some meaningful results, there are still some shortcomings. The results of the sensitivity analysis suggested that the included studies were still inadequate and that more case-control studies were needed subsequently to confirm the current findings. In addition, the specific molecular functions of the CAA/- polymorphism contributing to tumorigenesis were not revealed.

In conclusion, the current findings revealed that the CAA/- polymorphism in the 3'-UTR of the *RTN4* gene could contribute to tumorigenesis in the Chinese Han population. However, more and better-designed studies are

Table 4. Publication bias analysis of the association between the *RTN4* TATC/- polymorphism and the risk of tumorigenesis.

Genetic model	P	
	Begg's test	Egger's test
Homozygote (Ins/Ins vs. Del/Del)	0.31	0.34
Heterozygote (Ins/Del vs. Del/Del)	0.73	0.14
Dominant [(Ins/Ins+Ins/Del) vs. Del/Del]	0.31	0.08
Recessive [Ins/Ins vs. (Ins/Del+Del/Del)]	0.31	0.25
Allelic (Ins vs. Del)	0.73	0.87

Table 5. Publication bias analysis of the association between the *RTN4* CAA/- polymorphism and the risk of tumorigenesis.

Genetic model	P	
	Begg's test	Egger's test
Homozygote (Del/Del vs. Ins/Ins)	1	0.64
Heterozygote (Del/Ins vs. Ins/Ins)	0.81	0.88
Dominant [(Del/Del+Del/Ins) vs. Ins/Ins]	0.81	0.76
Recessive [Del/Del vs. (Del/Ins+Ins/Ins)]	1	0.71
Allelic (Del vs. Ins)	0.22	0.26

needed to confirm the current findings.

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Conflict interest
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Author's contribution
XG designed the study. XO and SP collected data. XG and XO performed the statistical analyses. XO, SP, XH, SZ, and LF interpreted and discussed the results. XG wrote the paper. XG, XO, SP, XH, SZ, and LF contributed to the final version of the manuscript.

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