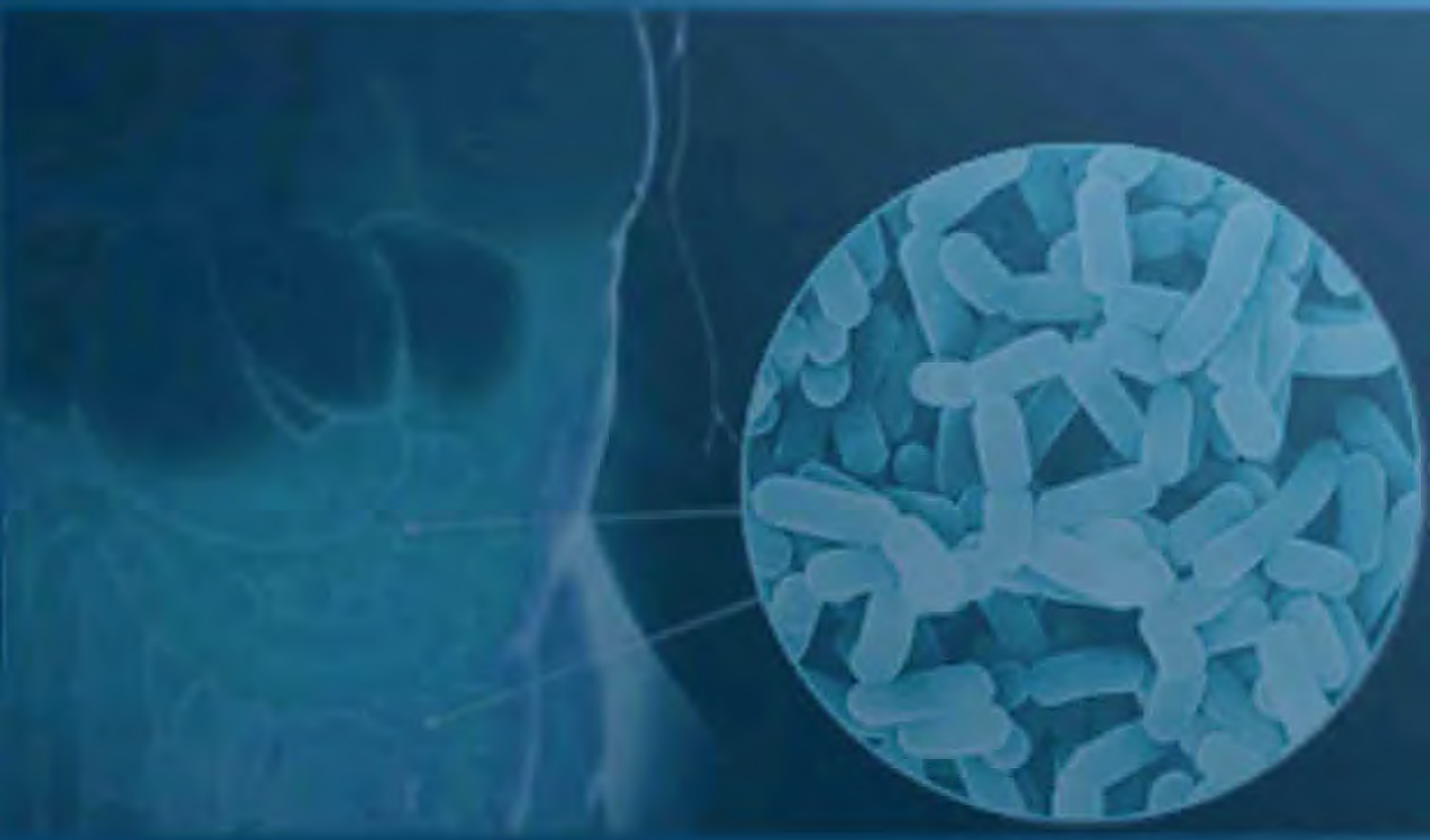
MICROBE AND DISEASE



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Three Polymorphisms of XRCC1 (Arg194Trp, Arg280His, Arg399Gln) and the Risk of Gliomas: a Meta-analysis

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Abstract

Gliomas are one of the most common malignant tumors in the central nervous system. At present, many publications have assessed the association between the polymorphisms of *XRCC1* and glioma susceptibility. However, the results remain inconclusive. In this study, we aimed to exhaustively assess the association between the polymorphisms of *XRCC1* (including Arg194Trp, Arg280His, Arg399Gln) and the incidence risk of gliomas. Firstly, studies which related to the genetic polymorphisms of *XRCC1* and glioma susceptibility were searched for in PubMed, Cochrane, CBM, Wanfang, and CNKI databases. Then the related data was extracted and analyzed by Revman 5.3 software. Finally, 19 case-control studies involving 7,024 glioma patients and 8,425 control subjects were included. The cumulative meta-analysis showed that there is no obvious association between *XRCC1* Arg194Trp or Arg280His polymorphisms and a high risk of gliomas, but the Arg399Gln polymorphism of *XRCC1* may be an important factor in the development of gliomas, especially in an Asian population.

Keywords: XRCC1; Polymorphisms; Glioma; Meta-analysis

Introduction

Gliomas, which originate from the brain or spinal cord, are one of the most common malignant tumors in the central nervous system (CNS) at present, as they account for more than 90% of malignant brain tumors. Although there are many advanced diagnostic tools and therapeutic methods, gliomas are still incurable and have a poor prognosis and low survival rate[1,2]. This is mainly due to a glioma's aggressive growth around brain tissue and how to determine the tumor boundary is difficult, which complicates removing it surgically. Furthermore, glioma is one of the tumors that has a large number of blood vessels in vivo and glioma cells have a strong proliferation capacity[3]. At present, due to the factors of blood-brain barrier and the irreversible toxic effects, the common anti-tumor drugs have a very poor effect in glioma treatment. The glioma pathogenesis is related to multiple processes as affected by dozens of regulatory factors[4], and exact causes are still unclear. According to the analysis of epidemiological investigations, the only certain environmental cause of gliomas is radiation exposure, especially ionizing radiation and X-ray exposure[5,6].

As it is well known, most tumors are caused by the interaction of environmental and genetic factors. However, not all those who are exposed to ionizing radiation and X-rays will suffer from gliomas. Their incidence may be related to other factors, among which genetic polymorphisms may be the important. Environmental carcinogens or metabolites can cause the damage of DNA, if the intracellular DNA repair gene is defective and the damaged gene cannot be repaired in time, this results in an increased mutation rate and gene instability, which causes cell proliferation and differentiation out of control, and increases the susceptibility of developing a tumor.

XRCC1 belongs to the family of nucleotide resection and repair (NER) genes, and is a key gene in the NER pathway. Studies have shown

that the polymorphisms of *XRCC1* may lead to the damage of the NER mechanism and cause the instability of genomes, thereby increasing the likelihood of tumor occurrence[7,8]. Up to now, many scholars have researched the association between *XRCC1* polymorphisms and glioma susceptibility, but the results are inconsistent. To better understand the causes of gliomas and provide an accurate theory for the development of clinical studies, in this study, we sought to make a systematic and objective evaluation of the association between *XRCC1* polymorphisms and glioma susceptibility using the method of Evidence-Based Medicine (EBM).

Materials and Methods

Search Strategy

The PubMed, Cochrane, CBM (Chinese Biomedical Database), Wanfang and CNKI (China National Knowledge Internet) databases were comprehensively searched (the last search was updated on July 1, 2017) using the following terms: ("polymorphism" OR "mutation" OR "variant") AND ("glioma" OR "brain tumor" OR "glioblastoma" OR "glial cell tumors" OR "brain neoplasms") AND ("*XRCC1*" OR "x-ray cross complementing group 1"). During the searching process, the language restrictions were not set, and for the possible references listed in the review, we made a second search and used manual retrieval if necessary. All the studies searched were published.

Eligibility Criteria

The retrieved literature was included according to the following criteria: (1) the study must be involved with *XRCC1* polymorphisms (including the sites of Arg399Gln or Arg194Trp or Arg280His) and gliomas; (2) the design was a case-control study or a cohort study; (3) it must have detailed data from studies that included the distribution of genotypes, sample size, odds ratio (OR) and 95% CIs; (4) the patients in the case group had gliomas diagnosed microscopically; (5) the distribution of genotypes in the control group conformed to Hardy-Weinberg Law (HWE).

Meanwhile, we also made a series of exclusionary criteria as follows: (1) if the experiment designs were obviously different from other studies; (2) if the necessary data described above was not listed; (3) if the studies had repeated reports, were of poor quality, or had incomplete data or reviews.

Data Extraction

All included literature was read by two researchers with appropriate backgrounds, and the relevant data extracted included the first author's name, year of publication, ethnicity of subjects, source of control, genotyping method, sample size of cases and control group, genotype frequency, OR, and its 95% CIs. All disagreements were resolved with a third researcher's intervention and the final conclusion was decided *via* a vote.

Data Analysis

For each study, whether the distribution frequency of genotypes in control group was consistent with Hardy-Weinberg Law was tested using the Chi-square test. Revman 5.3 software was used to perform the meta-analysis. Meanwhile, we assume the mutant allele is a gene susceptible of developing gliomas. Four genetic models were used to analyze the relationship between the polymorphisms of *XRCC1* and the incidence risk glioma, including an allele contrast model (mut *vs* wild), dominant model (mut/mut+mut/wild *vs* wild/wild), recessive model (mut/mut *vs* mut/wild+wild/wild), and homozygote comparison model (mut/mut *vs* wild/wild).

Subsequently, the heterogeneity among included studies was detected using I^2 statistics, and the value of I^2 was used to assess the degree of heterogeneity[9]. If there was no obvious heterogeneity, a fixed effect model was employed; otherwise, a random effects model was used. The OR and its 95% CI were calculated to estimate the association between the polymorphisms of *XRCC1* and the incidence risk of glioma under the aforementioned four genetic

models. Sensitivity analysis was applied by excluding a single study each time to explore the stability of overall results. The publication bias was calculated by a funnel plot analysis and a Egger linear regression test[10].

Results

Characteristics of Included Studies

According to the included and excluded criteria described above, 19 case-control studies involving 7,024 glioma patients and 8,425 control subjects were included in this meta analysis (**Figure.1**)[11-29]. Among the 19 included literatures, 13 studies had reported the polymorphism of Arg194Trp and the genotype distribution of control subjects in 9 studies were consistent with HWE; 8 studies had reported the polymorphism of Arg280His and the genotype distribution of control subjects in 6 studies were consistent with HWE; 17 studies had reported the polymorphism of Arg399Gln and the genotype distribution of control subjects in 14 studies were consistent with HWE. The detailed information of the SNPs studied in this meta-analysis was shown in **Table.1** and **Table.2**.

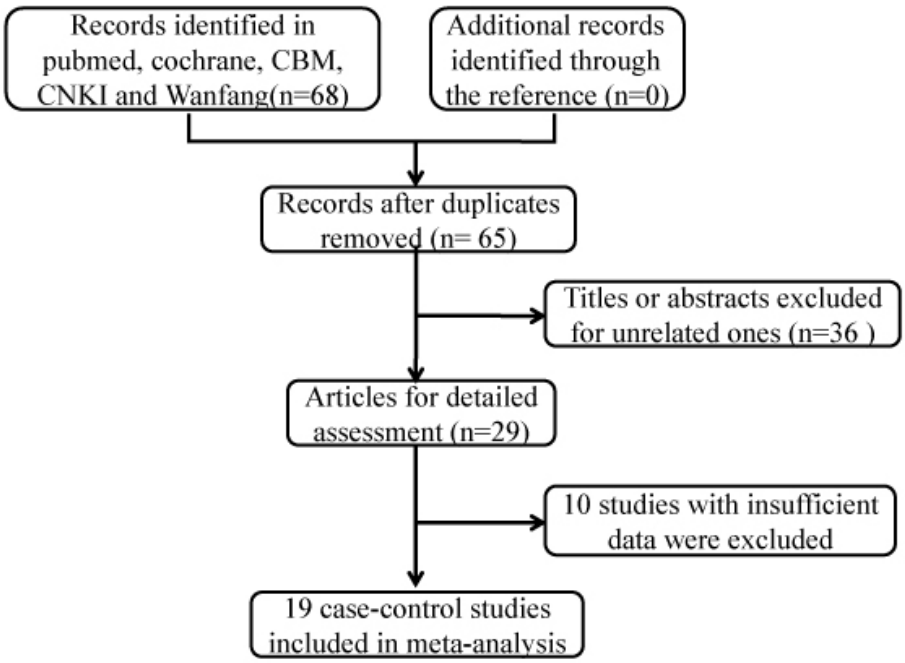

Figure.1 A flow diagram of the study selection process.

Meta-Analysis of *XRCC1* Arg194Trp Polymorphism

As shown in **Table.3**, the results of three genetic models (allele contrast, dominant model, homozygote model) showed there was no obvious association between the *XRCC1* Arg194Trp polymorphism and a high risk of gliomas, and the total odds ratio (OR) and its 95% confidence interval (95% CI) of each genetic model are: for allele comparison model (Trp *vs* Arg), OR=0.95, 95% CI=[0.75, 1.21]; for dominant model (TrpTrp+ArgTrp *vs* ArgArg), OR=1.06, 95% CI=[0.94, 1.20]; for recessive model (TrpTrp *vs* ArgTrp+ArgArg), OR=0.64, 95% CI=[0.44, 0.92]; for homozygote comparison model, OR=1.47, 95% CI=[1.00, 2.16].

Meanwhile, we analyzed the heterogeneity of the data in each genetic model, and the results showed that the dominant model (I^2=35%,

P=0.14), recessive model (I^2=55%, *P*=0.02), and homozygote comparison model (I^2=55%, *P*=0.02) had no obvious heterogeneity, but data analysis of the allele contrast comparison (I^2=88%, *P*<0.00001) had a high heterogeneity. Then we made a subgroup analysis and the results showed no obvious association between the *XRCC1* Arg194Trp polymorphism and a high risk of gliomas both in Asian and Caucasian subgroups.

Table.1 Characters of included studies.

Reference	Country	Research type	Genotyping method	Case (Male/Female)	Control (Male/Female)
Wang, 2016	China Han	case-control	Sequencing	368(189/179)	346(170/176)
Franceschi, 2016	Tuscan	case-control	Sequencing	85(49/36)	168(99/79)
Fan, 2016	China Han	case-control	PCR-RFLP	115(49/66)	228(104/124)
Wang, 2015	China	case-control	PCR-RFLP	387(187/200)	400(199/201)
Xu, 2014	China	case-control	PCR-RFLP	886(487/399)	886(483/403)
Li, 2014	China	case-control	Sequencing	368(189/179)	346(170/176)
Jin, 2014	China Han	case-control	PCR-RFLP	620(387/233)	630(402/228)
Hu, 2013	China Han	case-control	PCR-RFLP	366(216/150)	377(288/139)
Pan, 2013	China Han	case-control	Sequenom MassARRAY	443(257/186)	443(257/186)
Luo, 2013	China	case-control	Sequenom MassARRAY	297(170/127)	415(250/165)
Wang, 2012	China Han	case-control	PCR-RFLP	624(319/305)	580(303/277)
Liu, 2012	China	case-control	Sequenom MassARRAY	312(185/127)	312(171/141)
Zhou,2011	Southern China	case-control	PCR-RFLP	271(168/103)	289(180/109)
Hu, 2011	China	case-control	PCR-CTPP	127(87/40)	249(166/83)
Custodio, 2011	Caucasian	case-control	Sequencing	80(52/28)	100(63/37)
Yosunkaya, 2010	Turkey	case-control	PCR-RFLP	119(~)	180(~)
Rajaraman, 2010	Caucasian	case-control	TaqMan assays	489(~)	489(~)
Felini, 2007	Caucasian	case-control	PCR-RFLP	366(~)	427(~)
Kiuru, 2008	Caucasian	case-control	PCR-RFLP	699(~)	1549(~)

Notes: (1) Abbreviation, PCR-RFLP: cleaved amplification polymorphism sequence-tagged sites; PCR-CTPP: polymerase chain reaction with confronting two-pair primers. (2) "~" represents the numbers of male or female was not reported detailly.

Meta-Analysis of *XRCC1* Arg280His Polymorphism

As shown in **Table.4**, the results of allele contrast and dominant genetic comparisons model suggested there was no obvious association between the Arg280His polymorphism of *XRCC1* and high incidence of gliomas. What follows was the meta-analysis of an allele contrast model (His *vs* Arg), dominant model (HisHis+ArgHis *vs* ArgArg), recessive model (HisHis *vs* ArgArg+ArgHis) and homozygote model (HisHis vs ArgArg). For His *vs* Arg, OR=0.84, 95% CI=[0.64, 1.11]; for HisHis+ArgHis *vs* ArgArg, OR=0.82, 95% CI=[0.64, 1.04]; for HisHis *vs* ArgArg+ArgHis, OR=0.46, 95%

Table.2 The genotype distribution of *XRCC1* in each study.

Reference	Arg194Trp (Case/Control)			Arg280His (Case/Control)			Arg399Gln (Case/Control)		
	Arg/Arg	Arg/Trp	Trp/Trp	Arg/Arg	Arg/His	His/His	Arg/Arg	Arg/Gln	Gln /Gln
Wang, 2016	182/175	171/151	15/20	302/251	61/79	5/16	142/176	167/132	59/38
Franceschi, 2016		~			~		39/81	28/66	16/21
Fan, 2016	31/82	58/109	26/37		~		42/92	51/99	22/37
Wang, 2015		~			~		45/211	164/157	178/32
Xu, 2014	525/540	301/311	60/35	618/621	177/178	91/87	451/469	365/372	70/45
Li, 2014	183/175	171/151	16/20	302/251	61/79	5/16	142/176	167/132	59/38
Jin, 2014		~			~			~	
Hu, 2013		~			~		157/196	165/151	44/30
Pan, 2013	301/327	116/101	27/6		~		226/244	190/178	27/21
Luo, 2013	204/297	63/96	30/22		~		111/189	134/181	51/45
Wang, 2012	376/355	218/205	30/20	506/473	115/98	3/9	270/300	279/232	75/48
Liu, 2012	294/334	105/89	45/19		~			~	
Zhou,2011	145/159	113/117	14/13	218/240	45/44	8/5	121/147	113/118	37/24
Hu, 2011	71/163	38/64	18/22	72/153	28/58	27/38	58/145	48/75	21/29
Custodio, 2011	15/67	31/4	34/29		~		23/29	33/20	24/51
Yosunkaya, 2010		~			~		15/91	67/71	37/18
Rajaraman, 2010	304/394	38/73	0/1	312/417	28/48	0/1	142/205	164/201	44/72
Felini, 2007		~			~		158/180	155/196	53/51
Kiuru, 2008	626/1377	71/177	3/2	633/1399	67/157	1/4	284/645	324/728	91/176

Notes: "~" represents the corespondenting polymorphism was not reported.

Table.3 Summary about the meta-analysis on the association between *XRCC1* Arg194Trp polymorphism and risk of gliomas.

	Allele Contrast			Dominant Model			Recessive Model			Homozygote Model		
	OR (95% CI)	*P* for OR	I^2	OR (95% CI)	*P* for OR	I^2	OR (95% CI)	*P* for OR	I^2	OR (95% CI)	*P* for OR	I^2
Overall	0.95 [0.75, 1.21]	0.69	88%	1.06 [0.94,1.20]	0.33	35%	0.64 [0.44, 0.92]	0.02	55%	1.47 [1.00, 2.16]	0.05	55%
Ethnicity												
Asian	0.99 [0.75, 1.32]	0.96	91%	1.12 [1.01, 1.24]	0.04	0%	0.62 [0.42, 0.92]	0.02	63%	1.45 [0.96, 2.18]	0.07	63%
Caucasian	0.83 [0.60, 1.14]	0.24	44%	0.81 [0.60, 1.09]	0.16	31%	0.97 [0.17,5.54]	0.98	12%	1.8 6 [0.31,11.32]	0.50	16%

CI=[0.23, 0.90]; for HisHis *vs* ArgArg, OR=0.4, 95% CI=[0.22, 0.90]. Moreover, the heterogeneity test showed no obvious heterogeneity was found in the recessive model (I^2=32%, P=0.19) and homozygote model (I^2=38%, P=0.15), but high heterogeneity existed in the allele contrast comparison (I^2=77%, P=0.0006) and dominant model (I^2=63%, P=0.02). Then, we further performed a subgroup analysis and the results indicated there was no obvious association between the Arg280His polymorphism of *XRCC1* and a high risk of gliomas both in Asian and Caucasian populations.

Table.4 Summary about the meta-analysis on the association between *XRCC1* Arg280His polymorphism and risk of gliomas.

	Allele Contrast			**Dominant Model**			**Recessive Model**			**Homozygote Model**		
	OR (95% CI)	*P* for OR	I^2	OR (95% CI)	*P* for OR	I^2	OR (95% CI)	*P* for OR	I^2	OR (95% CI)	*P* for OR	I^2
Overall	0.84 [0.64, 1.11]	0.22	77%	0.82 [0.64,1.04]	0.10	63%	0.46 [0.23, 0.90]	0.02	32%	0.44 [0.22, 0.90]	0.03	38%
Ethnicity												
Asian	0.74 [0.53, 1.04]	0.08	77%	0.80 [0.55, 1.15]	0.22	76%	0.45 [0.19, 1.08]	0.07	59%	0.43 [0.17, 1.08]	0.07	63%
Caucasian	1.08 [0.76, 1.54]	0.67	52%	0.88 [0.69, 1.14]	0.34	0%	0.52 [0.09,3.19]	0.48	0%	0.52 [0.08, 3.15]	0.47	0%

Meta-Analysis of XRCC1 Arg399Gln Polymorphism

As shown in **Figure.2**, the results of four genetic comparison models suggested that the Arg399Gln polymorphisms of *XRCC1* noticeably increased the incidence risk of gliomas. The heterogeneity analysis showed that for an allele contrast comparison (Gln *vs* Arg), OR=1.22, 95% CI=[1.11, 1.33], I^2=48% (P=0.04); for dominant model (GlnGln+ArgGln *vs* ArgArg), OR=1.26, 95% CI=[1.12, 1.41], I^2=44%, (P=0.06); for recessive model (GlnGln *vs* ArgGln+ArgArg), OR=1.32, 95% CI=[1.16, 1.51], I^2=4% (P=0.41); for homozygote model (GlnGln *vs* ArgArg), OR=1.46, 95% CI=[1.24, 1.73], I^2=29% (P=0.17). Meanwhile, the Arg399Gln polymorphisms of *XRCC1* was further analyzed in the subgroup. As shown in **Table.5**, the Arg399Gln polymorphisms of *XRCC1* might bring about higher risk of gliomas in Asian populations, but these results were not found in Caucasians. For *XRCC1* Arg399Gln polymorphisms in Asians, Gln *vs* Arg: OR=1.33, 95% CI=[1.23, 1.45]; GlnGln+ArgGln *vs* ArgArg: OR=1.41, 95% CI=[1.27, 1.58]; GlnGln vs ArgGln+ArgArg: OR=1.51, 95% CI=[1.27, 1.80]; GlnGln *vs* ArgArg: OR=1.76, 95% CI=[1.46, 2.11].

Sensitivity and Publication Bias Analysis

In addition, in order to evaluate the stability of the meta-analysis, we made a sensitivity analysis using a single variable sensitivity analysis to test whether the study had a significant effect on the overall outcome, and the results suggested the pooled ORs were statistically robust and reliable. Meanwhile, as shown in **Figure.3**, the results of funnel plots suggested no obvious publication bias existed, especially in the analysis of *XRCC1* Arg399Gln polymorphism, and the funnel plot of each genetic models all showed symmetry.

Discussion

For a long time, tumors have been considered

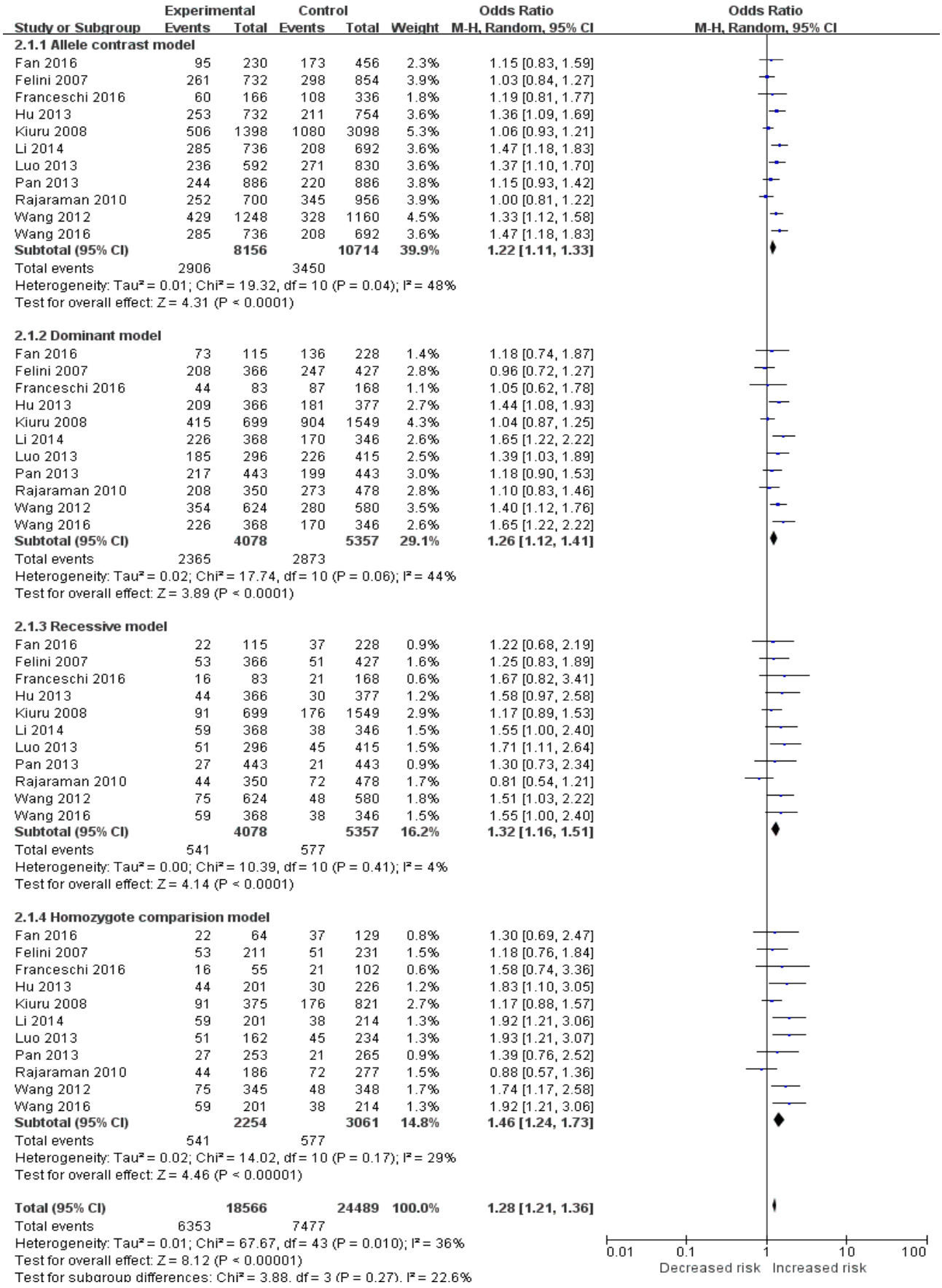

Figure.2 Forest plot of Arg399Gln polymorphism in *XRCC1* and risk of gliomas in four genetic comparison models.

Table.5 Summary about the meta-analysis on the association between *XRCC1* Arg399Gln polymorphism and risk of gliomas.

	Allele Contrast			**Dominant Model**			**Recessive Model**			**Homozygote Model**		
	OR (95% CI)	*P* for OR	I^2	OR (95% CI)	*P* for OR	I^2	OR (95% CI)	*P* for OR	I^2	OR (95% CI)	*P* for OR	I^2
Overall	1.22 [1.11, 1.33]	0.0001	48%	1.26 [1.12,1.41]	0.0001	44%	1.32 [1.16, 1.51]	0.0001	4%	1.46 [1.24, 1.73]	0.00001	29%
Ethnicity												
Asian	1.33 [1.23, 1.45]	0.00001	0%	1.41 [1.27, 1.58]	0.00001	0%	1.51 [1.27, 1.80]	0.0001	0%	1.76 [1.46, 2.11]	0.00001	0%
Caucasian	1.06 [0.96, 1.16]	0.24	0%	1.04 [0.91, 1.18]	0.59	0%	1.12 [0.89, 1.42]	0.33	26%	1.13 [0.92, 1.38]	0.24	0%

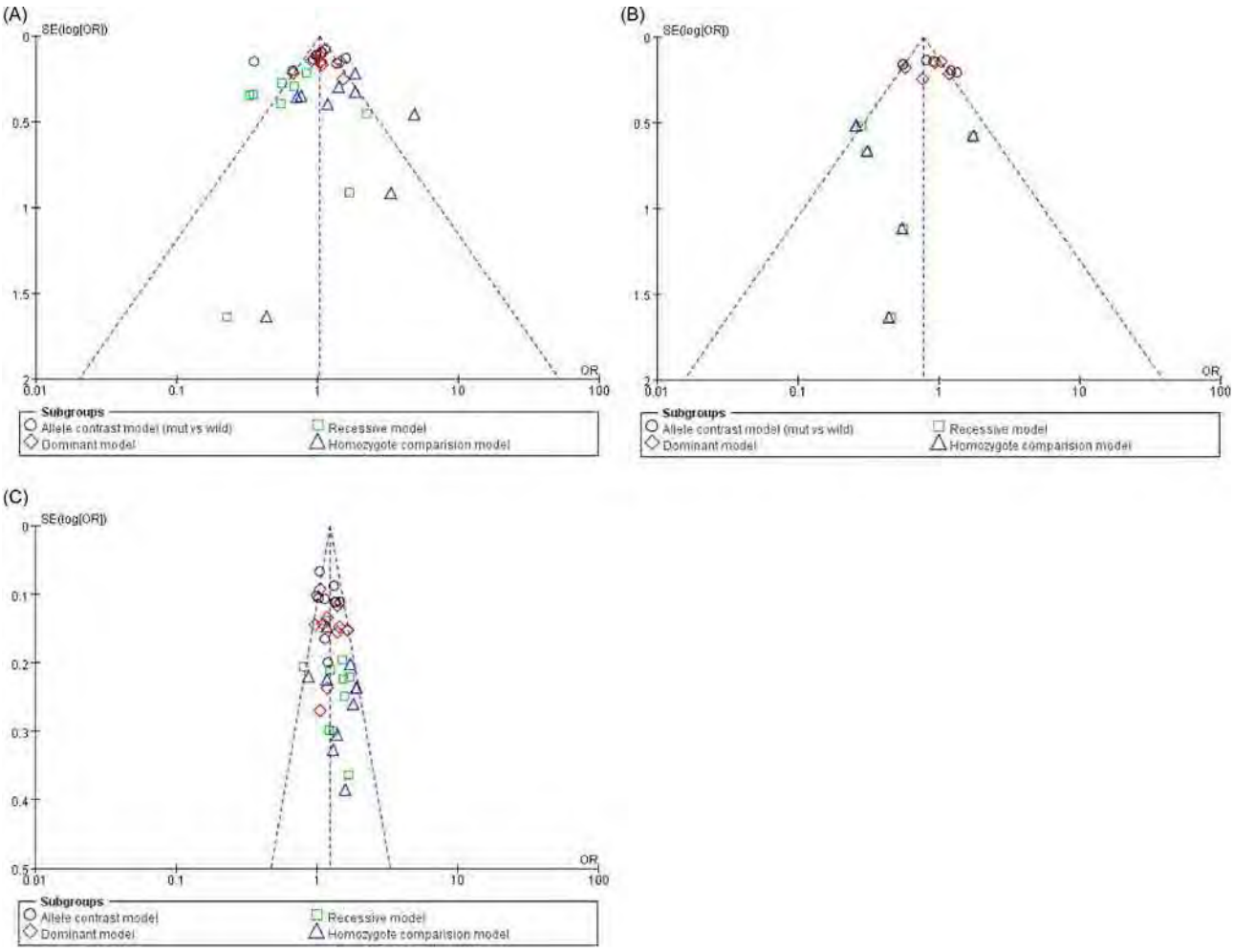

Figure.3 Funnel plot for the assessment of publication bias. (A) *XRCC1* Arg194Trp polymorphism; (B) *XRCC1* Arg280His polymorphism; (C) *XRCC1* Arg399Gln polymorphism.

a synthetic disease and caused by multiple factors, including genetic and environmental factors. Over the course of a lifetime, the body suffers damage from external environments, environments that could lead to the occurrence of a tumor *via* affect the stability of genome DNA. At present, the studies of a tumor's genetic sensitivity mainly focus on chromosome stability, DNA repair, structural changes of oncogene/tumor suppressor gene, genetic polymorphisms, *etc*.[30]. The risk of gene mutation would increase if the ability of DNA to repair was reduced or damaged. The ability of DNA to repair is the body's most important defensive barrier and a critical function, as it can repair damage caused by internal and external environments. Studies suggested that the polymorphisms of the DNA repair gene may be associated with the sensitivity of tumorigenesis and development[31]. Gliomas, which belong to a high degree of malignant tumors and are characterized by rapid growth, strong infiltration, short disease course, and high mortality, are considered a multifactorial disease. Nowadays, the causes of gliomas are still not fully understood, but one of the generally accepted causes is exposure to ionizing radiation. Hence, it is essential to understand the pathogenesis and make a risk evaluation on a genetic basis.

XRCC1 (X-ray repair cross complementing group 1) was first cloned from the gene library of a Chinese hamster ovary EM9 cell line, and was the main participant in repairing the damage induced by ionizing radiation and chemical mutagen through base-excision repair (BER) and single-stranded break repair. Single nucleotide polymorphisms (SNP), where the distribution of different genotypes in a population are caused by the insertion, deletion, or substitution of a nucleotide, were usually used to compare the nucleotide differences between patients and health populations, so as to find the corresponding genetic site and provide the preliminary research basis for gene targeted therapy[32,33]. At present, the genetic polymorphisms of *XRCC1*, which would reduce the repairing ability of the *XRCC1* gene, mainly contains Arg194Trp polymorphisms, Arg280His polymorphisms, and Arg399Gln polymorphisms. The association between genetic polymorphisms and tumors has been studied for several decades, and most results showed that the nucleotide polymorphisms were closely associated with cancer sensitivity, such as lung cancer, esophageal cancer, colon cancer, and colorectal cancer[34]. There are also numerous studies from all over the world that have reported the relationship between *XRCC1* polymorphisms and glioma risk[11-29]. For the relationship between *XRCC1* genetic polymorphisms and glioma risk, many researchers had analyzed via evidenced-medical analysis. For example, in Lu's study[35], their meta-analysis results showed that the Arg194Trp polymorphisms of XRCC1 could noticeably increase glioma risk in Asians.

In this meta-analysis, in order to make a fairer, more comprehensive and accurate evaluation of the association between *XRCC1* polymorphisms and glioma sensitivity, we made a meta-analysis that included three polymorphism sites (Arg194Trp polymorphism, Arg280His polymorphism and Arg399Gln polymorphism). The cumulative meta-analysis showed there was no obvious association between *XRCC1* Arg194Trp polymorphism or *XRCC1* Arg280His polymorphism and a high risk of gliomas (**Table.3** and **Table.4**), but the *XRCC1* Arg399Gln polymorphism could noticeably increase the risk of gliomas (**Table.5** and **Figure.2**). Moreover, epidemiological studies have shown that the incidence rates of gliomas are different among different races. Pinarbasi, *et al*[36] reported that the incidence rate of brain tumors in Caucasians was obviously higher than that in Asians and Africans; among those, the glioma incidence rate in Caucasians increased twofold compared with Africans, while no difference existed in the incidence rate of a meningioma, pituitary tumors, lymphomas and other brain tumors between white and black people[37]. A Japanese study indicated that the incidence rate in Japanese populations is 50% less than that of the incidence rate in American populations[38]. According to the investigation on the epidemiology of immigrants, the incidence rate of white people settled in Africa was also higher than that of African black people[39]. An incomplete set of statistical results taken in Shanghai from 1980 to 2006 showed the inci-

dence rate of malignant tumors, most notably how the occurrence of gliomas increased year by year[40]. Hence, we further made a subgroup analysis according the ethnic differences, and the results showed that, in Asians and Caucasians, there was no association between the *XRCC1* Arg194Trp polymorphism, *XRCC1* Arg280His polymorphism, and the incidence risk of gliomas, but the subgroup analysis of the *XRCC1* Arg399Gln polymorphism showed this polymorphism could noticeably increase the incidence risk of gliomas in Asians, but not necessarily in Caucasians. In this study, the result of the *XRCC1* Arg194Trp polymorphism was not consistent with the results in Lu's study, in which the author suggested that the Arg194Trp polymorphism of *XRCC1* was associated with increased risk for gliomas, especially in Asians. Finally, to ensure the robustness of this meta-analysis, we further performed a sensitivity analysis, and the results indicated that the outcome of this meta-analysis was stable and reliable.

Overall, the results of this accumulated meta-analysis suggested that there is no association between the *XRCC1* Arg194Trp or Arg280His polymorphisms and the high risk of gliomas, but the *XRCC1* Arg399Gln polymorphisms may be an important factor in the development of gliomas, especially in Asians. However, an evidence-based medical analysis needs continuous development. In this study, we made a systematic assessment based on existing data resources. In the future, along with the progress of medical research and a gradual increase of studies related to *XRCC1* polymorphisms and gliomas, this meta-analysis will be further refined.

Conclusion

There is no association between the *XRCC1* Arg194Trp or Arg280His polymorphisms and the high risk of gliomas, but the *XRCC1* Arg399Gln polymorphisms may be an important factor in the development of gliomas, especially in Asians.

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To Evaluate the Association between Gln223Arg, Lys-109Arg Polymorphisms in Leptin Receptor Gene and Essential Hypertension: an Update Meta-analysis

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Abstract

Studies have shown that the single nucleotide polymorphisms of the leptin receptor gene may increase the risk of essential hypertension. However, there still no consistent conclusion has been drawn. In this study, the meta-analysis was used to summarized the association in *LEPR* Gln223Arg, Lys109Arg polymorphisms and the risk of essential hypertension based on 16 case–control studies retrieved from Pubmed, Web of Science, CNKI, VIP, CBM. Statistical analyses were carried out with the Stata12.0 and Revman5.3 software. In the 13 articles that covered the Gln223Arg polymorphism, a significant association was found between Gln223Arg gene polymorphism and essential hypertension in allelic model (OR=1.36; 95% CI:1.10-1.69), dominant genetic model (OR=1.48; 95% CI:1.14-1.93) and recessive genetic model (OR=1.27; 95%CI: 1.04-1.55). In the 10 articles which were related to Lys109Arg polymorphism, no significant association was found between the Lys109Arg polymorphism and essential hypertension risk under allelic model (OR= 1.02; 95% CI: 0.86-1.21), dominant genetic model (OR=1.00; 95% CI:0.81-1.23) and recessive genetic model (OR=1.11; 95% CI:0.86-1.44). In summary, the variation of Gln223Arg locus can increase the risk of essential hypertension significantly, but there is no evidence shows association between Lys109Arg polymorphism and essential hypertension.

Keywords: Hypertension; Leptin receptor; Gene polymorphisms; Risk; Meta-analysis

Introduction

Essential hypertension is a worldwide health problem. It is not only a risk factor for stroke, myocardial infarction, and kidney disease, but also often comes with other metabolic diseases like high cholesterol, obesity, insulin resistance and so on. Hypertension is currently considered a disease caused by varies factors, including the environment and lifestyle, such as diet, exercise, smoking and drinking, as well as the impact of polygenes.

In recent years, more and more people believe that hypertension is associated with leptin and its receptor. Previous studies have shown that leptin and leptin receptor can regulate blood pressure and adipose tissue metabolism directly, or indirectly lead to obesity and hypertension[(1)]. In addition, Leptin can increase the sympathetic nerve activity through the leptin receptor and further affect the blood pressure. The human leptin receptor gene (*LEPR*) is localized at 1P31, which consists of 20 exons and 19 introns. Studies have shown that mutation of *LEPR* gene may have a direct impact on the biological function of leptin[(2)]. As for Gln223Arg (rs1137101) and Lys109Arg (rs1137100), which are located on the 6th and 4th exons of leptin receptor gene, many studies were done on the relationship between hypertension and these two loci. However, no conclusion has been drawn. To further clarify this relationship, we performed a meta-analysis on the published studies based on the principles of evidence-based medicine and explored whether the mutation of Gln223Arg and Lys109Arg

locus can affect the incidence risk of essential hypertension preliminarily.

Materials and Methods

Search Strategy

Computer-based retrieval along with manual retrieval were done, and all the published articles related to the association between essential hypertension and leptin receptor gene were collected from databases including: CNKI, VIP, CBM, Pubmed, Web of Science. Using keywords: “polymorphism”, “variant”, “variation”, “mutation", "SNP”, “hypertension”, “hypertensive”, “blood pressure”, “BP”, “Leptin Receptor”, “LEPR Protein”, “CD295 Antigens”, “LEPR”. The searching date is updated to August 1st, 2017.

Data Extraction and Quality

The inclusion criteria were as follows:(1) Articles included case-control studies which reported the relationship between the polymorphism of leptin receptor gene and hypertension; (2) Articles that covered *LEPR* Gln223Arg (rs1137101) polymorphism or Lys109Arg (rs1137100) polymorphism; (3) All the cases have been included were essential hypertension (systolic blood pressure ≥ 140mm Hg or diastolic blood pressure ≥ 90mm Hg; (4) The frequency distribution of genotypes in both case group and control group were provided either directly or indirectly; (5) For studies with repetitive data, include the one which provided the largest amount of information.

The exclusion criteria were as follows: (1) Studies were experimental research or review; (2) Studies were designed as cohort studies or cross-sectional studies; (3) Subjects in the studies were secondary hypertension patients; (4) Studies did not report the comparison of hypertension and non-hypertension; (5) There was a defect in the study design, or the data provided in the results were incomplete or incorrect, or an inappropriate statistical method was used.

Inclusion and Exclusion Criteria

Based on the pre-design data extraction table, one of the two researchers responsible for extracting and entering data, the other make sure everything is on the right track. In case of disagreement, it can be discussed by the two researchers or turn to the third party.

Data that were extracted from each study included: name of the first author, year of publication, race of the subjects, age, gender, blood pressure, the matching variable, research method, source of the case group and the control group, as well as the frequency distribution of each genotype and loci in both case group and control group. In the meantime, Chi-square was used to test whether the frequency of genotype distribution in case group and control group were corresponding to the Hardy-Weinberg Equilibrium. The quality of the included case-control studies was evaluated using the Newcastle-Ottawa Scale (NOS) document.

Statistical Analysis

Stata12.0 and Review Manager5.3 were used to analyze the included data which were input and analyzed independently by two reviewers. In this study, continuous variables, such as age, were expressed in terms of “mean ± standard deviation’. The incidence risk of hypertension in different genotype populations was expressed as Odds Ratio (OR) and its 95% Confidence Interval (CI). P-value which was less than 0.05 indicated statistical significance.

I^2 and Q tests were used to perform heterogeneity testing of the included literature. If I^2<50% and P>0.10, then it was considered as heterogeneity and fixed effect model (the Mantele–Haenszel method) should be used for the pooled OR. Otherwise, a random-effects model would be applied (the DerSimonian and Laird method). Moreover, the source of heterogeneity should be found. If there was no way to find out the source of heterogeneity, then no meta-analysis should be performed but using description analysis instead. The funnel plot was used to assess the publication bias.

At the same time, the Hardy-Weinberg Equilibrium was tested for the control group using

Chi-square test., if $P>0.05$, then it indicated the chosen population in this study is consistent with the genetic balance status. According to the matching condition of the case and control groups or to the HWE of the control group, a sub-group analysis was performed to find out the source of heterogeneity. By sequentially omitting one article at a time, the influence of a single article on pooled effect estimation was evaluated in the sensitivity analysis. The publication bias was estimated by the funnel plot, in which the funnel plot asymmetry was assessed by the method of Egger's linear regression test, a linear regression approach for measuring funnel plot asymmetry on the natural logarithm scale of OR.

Results

Study Identification and Selection

According to the retrieval strategy, 614 articles were included initially after retrieving from online database and endnote auto duplicate checking, as well as manual duplicate checking. Next, applied the exclusion and inclusion criteria mentioned in 2.2 on the titles and abstract, 490 articles were excluded, including reviews, animal experimental studies, clinical randomized controlled trials, conference reports, letters and so on. Finally, read through the full text carefully, excluding the studies with repetitive data, not case-control or with subjects that are not essential hypertension patients. Eventually, 16 studies were included into this meta-analysis[3-18]. A flow diagram is provided in **Figure 1** for more details of the articles screening process.

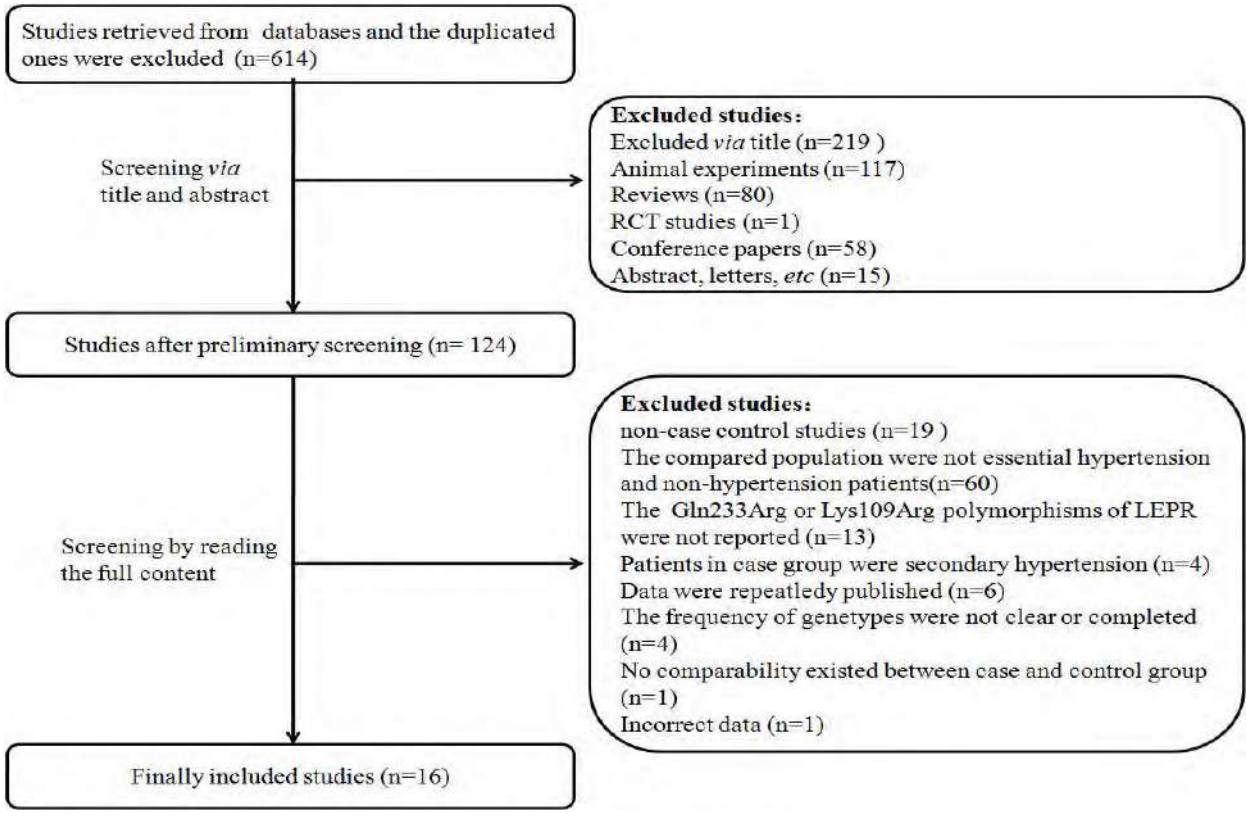


Figure.1 A flow diagram of the study selection process.

Study Characteristics

All included articles were published between 2007 and 2017, have covered 5037 cases in the case group and 2987 in the control group. Among these articles, 13 of them were related to LEPR Gln223Arg polymorphism[4-7,10-14]. Within these 13 articles, the frequency distribution of the control group in 3 of the articles is not consistent with the HWE[8,14,15]; There are 11 articles talked about leptin receptor Lys109Arg locus polymorphism[3,7-12,14,15,17,18]. Within these 11 articles, the frequency distribution of the control group in 3 of the articles is not consistent with the HWE[3,7,15]. Refer to **Table.1** for more basic characteristics of the included articles.

Table.1 Characters of included studies.

Study	Case			Control			Matching	LEPR polymorphism	HWE
	No.	Male %	Mean age	No.	Male %	Mean age			
Zhao,2007	392	56.4	51±5	**252**	51.2	53±5	age, sex	Gln223Arg	No
Wu, 2017	267	52.8	70.45±7.13	**255**	59.6	68.09±6.48	age, sex	Gln223Arg, Lys109Arg	Yes
Wang, 2010	283	46.6	65.94±7.49	153	45.1	66.43±9.20	age, sex, blood lipid	Gln223Arg, Lys109Arg	Yes
Wang, 2007	90	56.7	52.0±10.5	**52**	55.8	53.0±9.5	sex	Gln223Arg	Yes
Wang, 2010	90	56.7	52.0±10.5	**52**	55.8	53.0±9.5	age, sex	Lys109Arg	Yes
Shi, 2007	90	54.4	64.8±11.6	**53**	66	63.8±11.2	sex	Lys109Arg	No
Pan, 2008	210	54.8	62±5.3	**111**	47.7	56.4 ±7.2	age, sex	Gln223Arg	Yes
Liu, 2017	753	63.8	51.09±9.40	**489**	59.3	50.5±7.92	age, sex	Gln223Arg	Yes
Li, 2012	283	46.6	55.94±7.49	**153**	45.1	48.43±9.20	age, sex	Gln223Arg, Lys109Arg	Yes
Gu, 2009	239	NA	NA	141	NA	NA	Age, sex, TC, FBG	Gln223Arg, Lys109Arg	No
Cai, 2011	170	50.6	61.3±8.6	77	59.7	60.4±8.8	age, sex, TC, TG, HDL-C, LDL-C, BG	Gln223Arg, Lys109Arg	Yes
Bao, 2010	270	52.6	59.0 ±5.3	**111**	47.7	56.4 ±7.2	age, sex	Lys109Arg	Yes
Zheng, 2013	190	54.7	56.1	**88**	52.3	52.2±9.6	age	Gln223Arg	Yes
Liu, 2014	808	64.5	51.40±9.38	490	59.7	50.47±7.91	age, sex	Gln223Arg, Lys109Arg	Yes
Jiang, 2014	358	31.3	68.5	153	42.6	67.1 ± 7.1	No	Gln223Arg, Lys109Arg	Yes
Gu, 2012	544	55	56.95±8.93	357	60.2	57.02±11.47	age, sex	Gln223Arg, Lys109Arg	No

Note: Abbreviating Words: NA, not available; FBG, fasting blood-glucose; BG, blood-glucose; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Quality Assessment

Beside the samples in study by Wu, JH2017[14] were from Chinese residents nutrition and health status survey, the cases in the rest of the studies were collected continuously and all from the hospitals. The control groups were chosen from the same hospitals with non-essential hypertension. The diagnose criteria was the same and the process and outcome were in fine detail. (Blood pressure was measured after a 10 min rest at a sitting position. Hypertension was defined at a mean systolic blood pressure ≥140 mmHg and/or a mean diastolic blood pressure ≥90 mmHg. Patients taking any antihypertensive medication were defined as hypertensive. [17]).In addition, there is no explanation on the matching scale of the case group and the control group in the study by Shi, YW2007. However, based on the gender and age distribution, Chi-square test showed there was no statistical difference between the case group and the control group (P>0.05).

Quantitative Synthesis

By calculating the pooled OR and its 95% CI of the allele genetic model (A allele *vs* G allele), recessive genetic model (AA *vs* AG+GG) and dominant genetic model (AA+AG *vs* GG), a meta-analysis on the *LEPR* Gln223Arg and Lys109Arg polymorphisms was performed[19].

Table. 2 The distribution of genotypes and alleles of LEPR Gln223Arg in the case and control group, and the results of meta-analysis for essential hypertension risk under the three genetic models.

Study	Case AA/AG/GG	Control AA/AG/GG	Case A/G	Control A/G	A *vs* G	(AA+AG) *vs* GG	AA *vs* (AG+GG)
Zhao LS, 2007	156/152/84	72/62/118	464/320	192/312			
Wu JH, 2017	7/51/209	2/54/199	65/469	58/452			
Wang XM, 2010	4/150/129	2/47/104	158/408	51/255			
Wang N, 2007	21/45/24	11/26/15	87/93	48/56			
Pan RW, 2008	3/69/138	1/21/89	75/345	23/199			
Liu Y, 2017	9/210/534	14/116/359	228/1278	144/834	OR=1.36 95% CI: [1.10, 1.69] P=0.004 I^2=81% P_{het}<0.00001 Model: R	OR=1.48 95% CI: [1.14, 1.93] P=0.003 I^2=82% P_{het}<0.00001 Model: R	OR=1.27 95% CI: [1.04, 1.55] P=0.02 I^2=43% P_{het}=0.05 Model: F
Li CC, 2012	4/150/129	2/47/104	158/408	51/255			
Gu P, 2009	35/21/183	17/20/104	91/387	54/227			
Cai ZY, 2011	2/56/112	0/15/62	60/280	15/139			
Zheng H, 2013	10/57/123	2/23/63	77/303	27/149			
Liu Y, 2014	8/192/608	14/116/360	208/1408	144/836			
Jiang B, 2014	6/73/279	3/33/117	85/631	39/267			
Gu P, 2012	86/98/360	42/49/266	270/818	133/581			

Note: OR: odds ratio; 95% CI: 95% confidence interval; *P: P* values of test for pooled OR; I^2, inconsistency index; Phet: *P* values of heterogeneity test; R: Random model; F: Fixed model.

Association between LEPR Gln223Arg Polymorphism and the Incidence Risk of Hypertension

As shown in **Table 2**, for the site of *LEPR* Gln223Arg, heterogeneity test showed that: heterogeneity existed in the allele genetic model and the dominant genetic model, but not in the recessive genetic model. Therefore, the random effects model should be applied to the first two models and for the recessive genetic model, fixed effects model should be used (P<0.00001, I^2=81%; P<0.00001, I^2=82%; P=0.05, I^2=43%). In the meantime, the allele genetic pattern showed that the risk of developing essential hypertension in the population carrying A allele (mutant gene) was 1.36 times higher than those carrying the G gene (wild-type gene) (OR = 1.36; 95% CI: 1.10-1.69); The results of the dominant model showed that the risk of developing essential hypertension in population with (AA+AG) genotype was 1.48 times higher than those with GG genotype (OR=1.48; 95% CI: 1.14, 1.93); In the recessive model, the risk of developing essential hypertension in the AA genotype was 1.27 times higher than that in the (AG+GG) genotype (OR = 1.27; 95% CI:1.04, 1.55).

Association between LEPR Lys109Arg Polymorphism and the Incidence Risk of Hypertension

As shown in **Table 3**, for the leptin receptor Lys109Arg locus, heterogeneity test showed that there was a moderate heterogeneity both in the allele genetic model and dominant genetic model (P=0.006, I^2=59%; P=0.004, I^2=61%), and there was no heterogeneity in the recessive genetic model (P=0.55, I^2=0%). Therefore, random effects modal was applied to the first two genetic model and fixed effects model was applied to the recessive genetic model. After performing the accumulated analysis on the OR value of included studies, results showed that the effect of *LEPR* Lys109Arg polymorphism under the 3 genetic models were all not statistical significant.

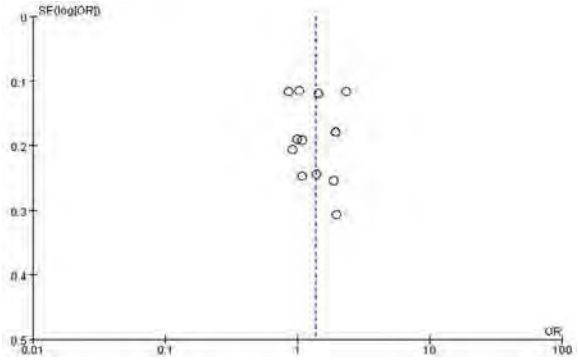
Table.3 The distribution of genotypes and alleles of LEPR Lys109Arg in the case and control group, and results of meta-analysis for essential hypertension risk under the three genetic models.

Study	Case	Control	Case	Control	A *vs* G	(AA+AG) *vs* GG	AA *vs* (AG+GG)
	AA/AG/GG	AA/AG/GG	A/G	A/G			
Wu JH, 2017	12/67/188	4/73/178	91/443	81/429			
Wang XM, 2010	5/115/163	3/52/98	125/441	58/248			
Wang N, 2010	6/36/48	4/21/27	48/132	29/75			
Shi YW, 2007	18/15/57	4/5/44	51/129	13/93			
Li CC, 2012	5/115/163	3/52/98	125/441	58/248	OR=1.02 95% CI: [0.86, 1.21] P=0.83 I^2=59% P_{het}=0.006 Model: R	OR=1.00 95%CI: [0.81, 1.23] P=0.99 I^2=61% P_{het}=0.004 Model: R	OR=1.11 95%CI: [0.86, 1.44] P=0.42 I^2=0% P_{het}=0.55 Model: F
Gu P, 2009	34/38/167	19/25/97	106/372	64/219			
Cai ZY, 2011	1/39/130	0/29/48	41/299	29/125			
Bao BK, 2010	9/50/211	3/35/73	68/472	41/181			
Liu Y, 2014	22/246/547	18/149/323	290/1340	185/798			
Jiang B, 2014	4/76/186	3/35/72	84/448	41/179			
Gu P, 2012	62/119/363	38/59/260	243/845	135/579			

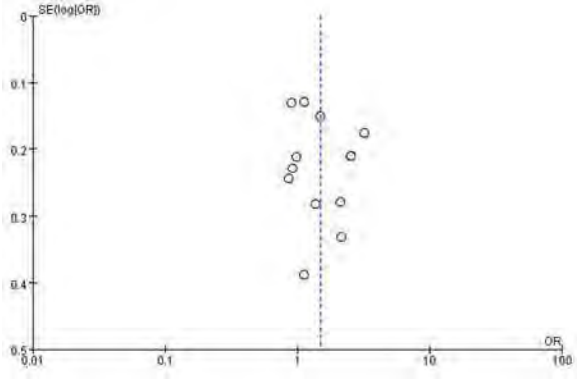
Publication Bias of the Literature

A bias analysis on the results computed from the 3 genetic models of GLn223Arg locus and Lys109Arg locus were performed respectively, where the outcomes were shown in **Figure 2** and **Figure 3**. The funnel plot of each of the genetic models all showed symmetry, means no obvious bias.

(A)



(B)



(C)

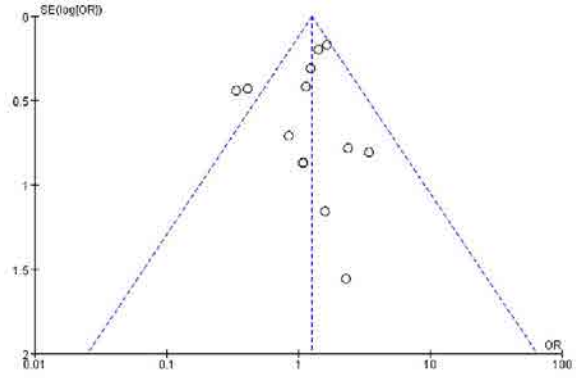
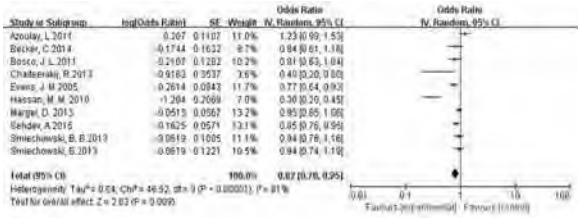
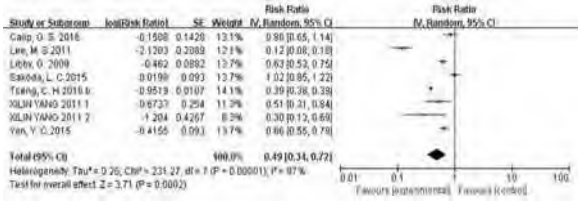


Figure. 2 Funnel plot of allele genetic model (A), dominant genetic model (B) and reccesive genetic model (C) for LEPR Gln223Arg polymorphism.

(A)



(B)



(C)

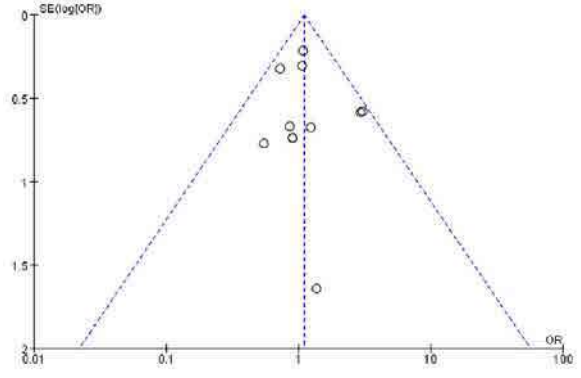


Figure.3 Funnel plot of allele genetic model (A), dominant genetic model (B) and reccesive genetic model (C) for LEPR Lys109Arg polymorphim.

Discussion

There were 16 references included in this meta-analysis, 13 of which refer to *LEPR* Gln223Arg polymorphism, including 4,587 patients with essential hypertension and 2,771 patients with non-essential hypertension, all the population were Chinese. Among those, the results of 10 references showed that the incidence of essential hypertension was closely associated with the mutation of *LEPR* Gln223Arg polymorphism[4-6,10-13,15,16,18], while other 3 references reported no relationship existed, which had no statistical significance. Meanwhile, 11 references refer to the *LEPR* Lys109Arg polymorphism, including 3,317 patients with essential hypertension and 1,952 patients with non-essential hypertension. During the process of literature screening, the disease type of all the patients were secondary hypertension in the researches of JIANG LY2006[20], Gao L2009[21], Wiedemann, A2010[22], Tennekoon, KH2012[23], therefore, these references were not included in this paper.

For the *LEPR* Gln223Arg polymorphism, the meta-analysis results of these 13 references showed that there was no heterogeneity in recessive genetic model, while a large heterogeneity in allelic genetic model and dominant genetic model were found. However, all these 3 genetic models had statistical significance, and had a small *P* value (allelic genetic model: P=0.004; dominant genetic model: P=0.003; recessive genetic model: P=0.02), so we supposed that the mutation of *LEPR* Gln223Arg would increase the risk of essential hypertension. Besides, HWE was assessed only in controls, since cases may not be in HWE if there was indeed an association between genotype and disease outcome. So the references which are not satisfy with HWE were excluded, and the residual references were analyzed for the sources of heterogeneity, and the result of which showed as below: there were statistical significance in the meta-analysis results of allelic genetic model and dominant genetic model, which indicate that the SNPs associate with the increasing risk of hypertension. But there still were some heterogeneity among these researches, which implied the heterogeneity was not from these 3 references[5,7,15]. To specified the sources of heterogeneity, we used the method of sensitivity analysis via excluding the references which from the 13 references one by one, and compared the pooled odds ratio (OR) before excluded and after, and finally found the heterogeneity came from Liu, Y2014[18] and Liu, Y2017[13]. In these two researches, Liu, Y2014 and Liu, Y2017 used TaqMan genotyping technique while others used polymerase chain reaction-restricted fragment length polymorphisms PCR-RLFP), which might be the source of heterogeneity.

For the *LEPR* Lys109Arg polymorphism, the meta-analysis results of these 11 references showed that there was no heterogeneity in recessive genetic model (P=0.55, I^2=0%), while there was a medium heterogeneity both in allele genetic model and dominant genetic model (P=0.006, I^2=59%; P=0.004, I^2=61%). But in the meta-analysis results of all these 3 genetic models, the difference of pooled OR between two groups had no statistical significance. For allelic genetic model, OR=1.02, P=0.83; for dominant genetic model, OR=1.00, P=0.99; for recessive genetic model, OR=1.11, P=0.42. Therefore, meta-analysis results indicated there was no association between the mutation of *LEPR* Lys109Arg and the incidence risk of essential hypertension.

However, in this meta-analysis, the subjects in the control group were the healthy people in a same period in the hospitals, rather than in communities, which might be lack of representation; in addition, for the lack of the severity state of illness, there might be an influence on the results of research. And our analyzing only selected two SNPs of *LERP* for the incidence of essential hypertension which was influenced by multi-genes. But in our study, we strictly restrained the patients as essential hypertension, excluded the sources of heterogeneity by sensitivity analysis, and got a stable result, which objectively and fairly reflected the relationship between the SNPs of *LEPR* Gln223Arg/Lys109Arg and the incidence risk of essential hypertension.

Finally, evidence-based medical analysis is a

kind of research that needs to develop continuously. Even though there are deficiency, this study has systematically evaluated this issue based on the existing data resources. Considered the included references are retrospective case-control research with various potentially mixed factors, the result of this study also requires a large sample to deal with, and also need the study with more representative population sample to verify.

Conclusion

In this study, the result of the meta-analysis showed that no significant association between the *LEPR* Lys109Arg polymorphism and essential hypertension is observed. However, *LEPR* Gln223Arg polymorphism is highly related to the risk of developing essential hypertension. The risk of essential hypertension increases significantly in the population with A allele gene (mutant gene).

Acknowledgement

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To Explore the Changes of Gene Expression Profiles in Metastatic Uveal Melanoma

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Abstract

Uveal melanoma (UVM) is the most common malignant ocular tumor among adults, with the characteristics of strong infiltration, prone to distant metastasis, and poor prognosis after metastasis. Up until now, many studies suggested the metastasis of uveal melanoma is associated with multiple factors, such as the abnormalities of chromosomes, gene expressions and signaling pathways. In this study, to further understand the underlying mechanism and molecular basis of the UVM metastasis, we selected the datasets of GSE27831 from GEO database, and made a comparative analysis between the 11 cases of UVM metastasis samples and 17 cases of non-metastasis samples using the bioinformatics methods. According to the results of differentially expressed gene screening: compared with the non-metastasis samples, which were considered as control group, 46 up-regulated genes and 323 down-regulated gene existed in the UVM metastasis group. The GO results showed system development (GO:0048731, 1.05E-10), cell periphery (GO:0071944, 9.26E-08), brain-derived neurotrophic factor binding (GO:0048403, 1.12E-09), *etc*. were significantly enriched, and the hierarchical tree of GO category suggested motor activity (GO: 0003774, 9.14e-4), calmodulin binding (GO: 0005516, 2.070e-4), and cis-trans isomerase activity (GO: 0016859, 8.921e-5), *etc*., may associated with the UVM metastasis process. Meanwhile, the Kegg analysis showed that 369 DEGs were involved in 19 signaling pathways, including protein digestion and absorption, focal adhesion, ECM-receptor interaction, PI3K-Akt signaling pathway, p53 signaling pathway, *etc*. In addition, the network of protein- protein interaction indicated 129 nodes existed and multiple hub proteins were identified, including *POMC, HIST2H2BE, CABP7, CACNA1A, NGFR, etc*. Throughout the study, we were able to preliminarily explore the changes of gene expression profile of metastatic UVM, and hopefully we can generate more accurate UVM transcriptome profiles based on large sample size, and investigates some potential biomarkers and therapy targets for metastatic UVM in the future.

Keywords: Uveal melanoma; GEO database; DEGs; Go analysis; KEGG analysis

Introduction

Uveal melanoma (UVM) is the most common primary malignant tumor in adult eyes, predominantly found in Caucasians[1]. The UVM morbidity is the second most prevalent ocular malignant tumor in China. UVM mainly originates from the pigment cells and nevi cells of uveal tissues and is associated with a high incidence of local infiltration and distant metastasis, key characteristics of this disease[2]. Among that, local infiltration can occur outside the eye, and the distant transfer usually occurs in liver, lungs, kidneys, brain tissue, etc. Statistical data indicates that in clinical, the majority of the UVM patients are at risk of distant metastasis, especially in the common site of liver[3]. In addition, for the UVM patients, the prognosis is very poor once metastasis is diagnosed, and patients would die within several months (median survival time varies from <4-12.5 months), and despite the improvements in diagnosis and treatments of the primary tumor, no effective treatment for metastatic UVM has been found[4,5].

Different from the skin melanoma, due to the lack of lymphatic vessels in eyes, the metastasis of UVM is mainly through bloodstream,

which results in the patients not able to spot the early metastasis in time, and among some of the patients, the UVM metastasis had already happened before the diagnose[6]. However, the invasion and metastasis of tumor tissue is a multi-cascade reaction process, including the abnormal tumor cells proliferation, morphological changes, invasion of the basement membrane, transferred to the blood vessels, the blood transmission, and the formation of metastases in the target sites[7]. Recently, more and more studies show the process of UVM metastasis is closely associated with a variety of factors such as chromosomal abnormalities, gene mutations, cytokines, miRNAs and signal transduction pathway abnormalities, *etc*.[8]. Among these factors, studies of UVM cytogenetics indicate that the abnormalities mainly occur in Chromosome 1, 3, 6, 8, 11, 13[9-12]. The loss of chromosome 3 is the most important risk factor for UVM metastasis, and the theory is widely accepted. Meanwhile, the hypothesis, which states that the loss of chromosome 3 increases the incidence of UVM metastasis and mortality rate, has been proven in several studies[13,14]. Moreover, metastasis related genes, such as *Bcl-2*, *MDM-2*, *p53*, *GNAQ*, *GNA11*, *BRAF*, *BAP1*, *c-Kit*, *c-Met*, *PTP4A3*, *etc*., is also an important study focus of UVM[15,16]. For example, in Pópuloh 's study, the vivo experiments of mouse showed *GNAQ* mutation could promote the metastasis of UVM[17]; Laurent's study found *PTP4A3* overexpression in uveal melanoma cell lines significantly increased cell migration and invasiveness in vivo, suggesting a direct role for this protein in metastasis[18]. Hence, to further evaluate the roles of metastasis related genes is important to explore the underlying mechanism in UVM.

Microarray, which is a highly efficient technology and large-scale access to biological information, can detect and analyze the differentially expressed genes between normal and tumor tissues. In this study, to reveal the mechanism of pathogenesis and metastasis from the genome level and provide new targets and marker for UVM treatment, we performed the data excavation of UVM metastasis related genes and make a bioinformatics analysis using the Gene Expression Omnibus (GEO) database.

Materials and Methods

Data Source

In this study, we selected the GSE27831 datasets from NCBI GEO database and screened the differentially expressed genes (DEGs), which are related with the metastasis of UVM. In the GSE27831 datasets, researchers collected 29 UVM samples, and conducted the miroarray experiments by using the Affymetrix Human Genome U133 Plus 2.0 Array.

Evaluation of Data Quality

In the miroarray experiments, not all of the experiments would be successful, and many factors can cause failure. Among the multiple influential factors, the main causes are possibly idue to technology, such as the quality of chipself, experiments design, or the samples itsself existed degradation, so the quality evaluation is an important operation before the followed up array analysis.

To ensure the reliability of the array data analyzed in the study, we used R software and Bioconductor to process, analyze, annotate, and visualize the CEL raw file data provided in the GEO datasets. For the quality evaluation of Arrays, we used the methods of average and advanced data fit, and combined with affyPLM, simpleaffy R packages to conduct; meanwhile, affy, CLL R packages and AffyRNAdeg function were used to analyzed the sample quality, and the results were visualized *via* RNA degradation curves.

Data Normalization

According to the quality evaluation of the chip data, the unqualified samples were excluded. Then the sample data included in the analysis usually need to subject three steps including background correction, standardization and aggregation to obtain the gene expression matrix for the next differential gene expression analysis. In this study, we used the RMA integration algorithm to preprocess the chip data and obtain a gene expression matrix.

DEGs Screening

Samples of UVM non-metastasis group and metastasis group were considered as control and experiment group respectively. Using t test, R software and limma package were applied to calculate differently expressed probe sets between control and experiment group. During the screening process, the genes with $P<0.05$ and $|\log2(\text{Fold change})|>1$ were selected as the significantly differentially expressed genes (DEGs). A heat map analysis was conducted using the "pheatmap" function of R/ Bioconductor package "ggplot"[19].

GO and KEGG Analysis

The Gene ontology (GO) was analyzed via the online Gene Ontology Enrichment Analysis Software Toolkit (GOEAST) (http://omicslab.genetics.ac.cn/GOEAST/) to facilitate the interpretation of biological roles of DEGs [20], and the GO functions were performed according to three categories including biological process, molecular function, and cellular components. In addition, the pathway enrichment of DEGs was analyzed using the online tool of KOBAS (http://kobas.cbi.pku.edu.cn/annotate.php).

Protein Protein Interaction (PPI) Network Construction

In order to find candidate genes involved in the process of UVM metastasis, PPI network of DEGs were constructed according to the data from STRING database (https://string-db.org/). Furtherly, the PPI network of DEGs were visualized via Cytoscape[21].

Results

Quality Evaluation

In this study, before the DEGs screening, to ensure the reliability of the array data, we divided the 29 samples of the GSE27831 datasets into metastasis group and non-metastasis group based on the clinical information of Rosaria's study, and made a quality evaluation of each chip experiment. Among that, there were 11 samples of metastasis and 18 samples of non-metastasis. As shown in **Figure.1**, the normalized unscaled standard errors (NUSE) box showed the NUSE values of each sample in metastasis group distributed around 1, which indicated the quality of microarrays were good (**Figure.1A**); however, in the non-metastasis group, the NUSE value of MU16.CEL sample appeared an offset, which exceeded 1 and suggested a problem may exist in this microarray experiments (**Figure.1C**). Meanwhile, we also made a fit for the data of microarray CEL files, and we found that there was a serious RNA degradation in the sample of MU16.CEL, while the RNA quality of all other samples were well **(Figure1B, 1D**). Based the analysis above, we believed a serious problem of RNA quality exists in the MU16.CEL sample, and this sample should be excluded in the subsequent analysis. And the clinical data of the 28 included samples are shown in details in **Table.1**.

DEGs Screening and Cluster Analysis

Finally, 11 datasets of UVM metastasis samples including GSM685472_MU9, GSM685473_MU10, GSM685523_MU15, GSM685601_MU_3, GSM685602_MU8, GSM685603_MU4, GSM685652_MU_7, GSM686985_MU_31, GSM686988_MU_34, GSM686989_MU_36, GSM687003_MU21 and 17 datasets of UVM non-metastasis samples including GSM685471_MU1, GSM685474_MU11, GSM685475_MU12, GSM685522_MU13, GSM685650_MU5, GSM685651_MU6, GSM686961_MU_17, GSM686962_MU_22, GSM686963_MU_25, GSM686984_MU_30, GSM686986_MU_33, GSM686986_MU_33, GSM686990_MU_40, GSM686991_MU18, GSM687001_MU_2, GSM687002_MU20, GSM687004_MU23 were included in this study. The results of the screening DEGs analysis showed that comparing with the non-metastasis UVM samples, our control group, 369 genes expressions had significant changes in UVM metastasis group, including 46 up-reg-

ulated genes and 323 down-regulated genes. Meanwhile, the DEGs was analyzed by the two-way cluster, as shown in **Figure.2**, there was a difference in the expression of the same gene in cancerous tissues of different samples. The significantly top 10 down-regulated and up-regulated genes are listed in **Table.2**.

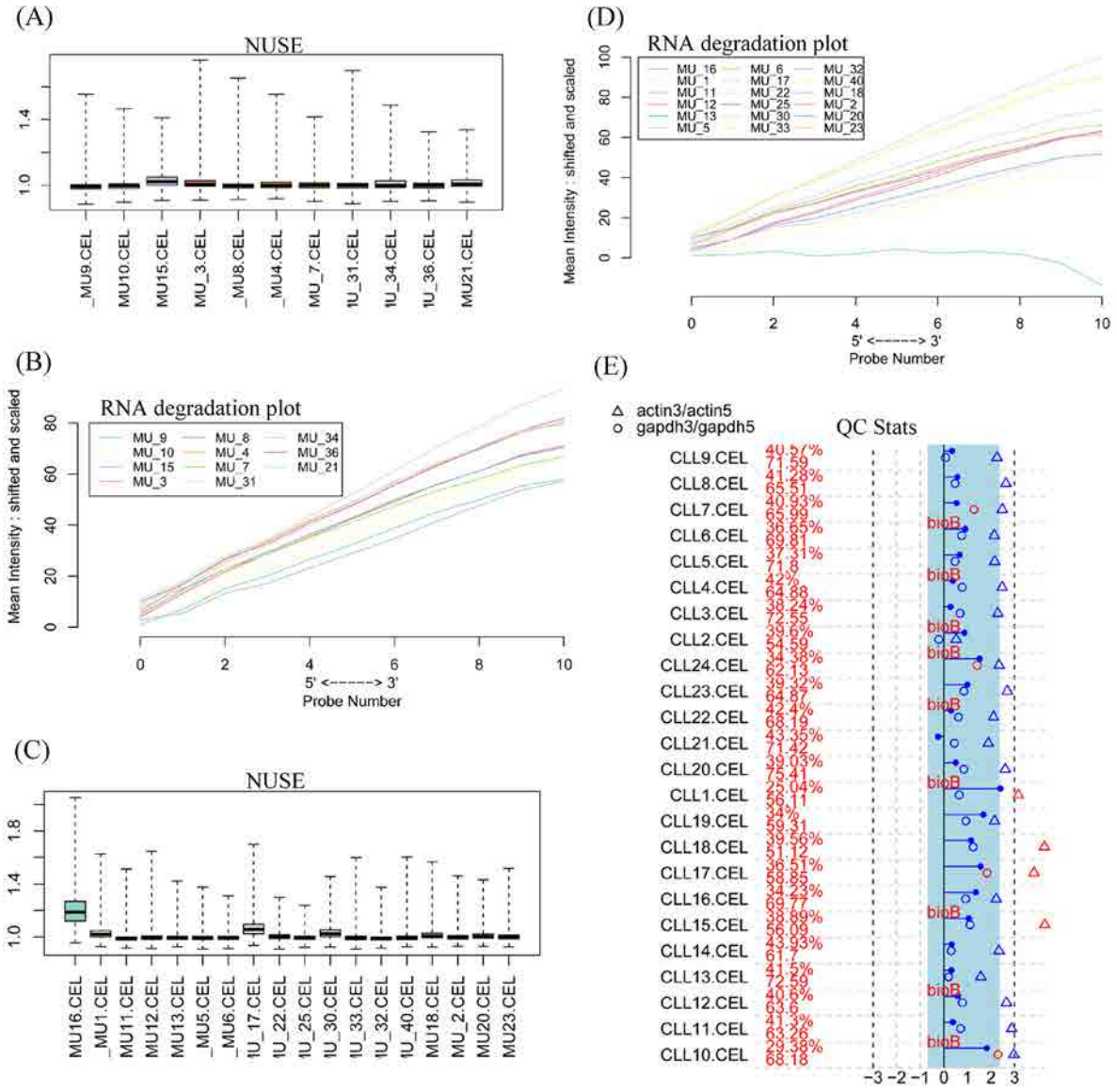

Figure.1 The quality evaluation of GSE27831 datasets (including the metastasis and non-metastasis UVM samples), which selected from GEO database. (A, C) the affyPLM R package was used to make a fitting and regression for the Chip raw data, and the results were visualized through normalized unscaled standard errors (NUSE) box to evaluate the quality of microarray itself; (B, D) the RNA degradation plots of the samples in metastasis group and non-metastasis group; (E) overview plot of microarrays quality control.

Table.1 Clinicopathologic characteristics of 28 uveal melanoma patients included in this study.

Characteristic	Non-metastatic patients (n=17)	Metastatic patients (n=11)
Mean age (years)	64.94 ± 3.264	66.73 ± 3.681$^{(n.s)}$
Mean primary tumor thickness (mm)	7.861 ± 0.9407	9.855 ± 1.240$^{(n.s)}$
Mean tumor largest diameter (mm)	13.29 ± 1.053	14.73 ± 1.502$^{(n.s)}$
Gender, No. of patients (Male/Female)	10:7	7:4$^{(n.s)}$
Disease-free survival (months)	43.76 ± 3.074	25.64 ± 3.123***
Location of tumor		
anterior	2	2
posterior	3	4
middle	10	5
NP	2	0
Histopathologic cell type		
epithelioid	3	3
spindle	8	1
mixed	4	7
NP	2	0

Notes: compared with non-metastasis patients, ***P<0.001, and "*n.s*" represents P>0.05.

Table.2 Most obviously dysregulated genes sorted by P value in metastatic uveal melanoma compare to non-metastatic tumors.

Genesymbol	Log_2(Fold Change)	*P*.Value	adj.*P*.Val
Down-regulated			
ANKRD65	-1.66756↓	5.66E-19	1.16E-14
COX6A2	-2.68557↓	2.10E-15	1.43E-11
TAF1L	-1.23861↓	5.76E-15	2.84E-11
LOC100652824	-1.15841↓	6.35E-14	1.44E-10
LUZP4	-1.26167↓	5.64E-14	1.44E-10
KCNK12	-1.03134↓	9.34E-14	1.91E-10
GIPR	-1.08615↓	2.18E-13	3.43E-10
HHIPL2	-1.07116↓	8.79E-13	8.28E-10
GHRL	-1.01515↓	1.68E-12	1.27E-09
ERVH48-1	-1.02828↓	2.38E-12	1.62E-09
Up-regrulated			
OLFML1	1.088928↑	5.52E-08	1.02E-06
LOC102724689	1.404867↑	1.14E-07	1.75E-06

NT5DC3	1.292795↑	1.81E-07	2.47E-06
LOC100130429	1.067554↑	2.57E-07	3.22E-06
ASAP1-IT2	1.884606↑	5.54E-07	5.93E-06
NEURL1B	1.097672↑	1.91E-06	1.58E-05
FAM64A	1.05693↑	1.90E-06	1.58E-05
DDX43	1.617977↑	5.05E-06	3.43E-05
KIF20A	1.334202↑	6.86E-06	4.41E-05
CALHM2	1.167053↑	1.15E-05	6.72E-05

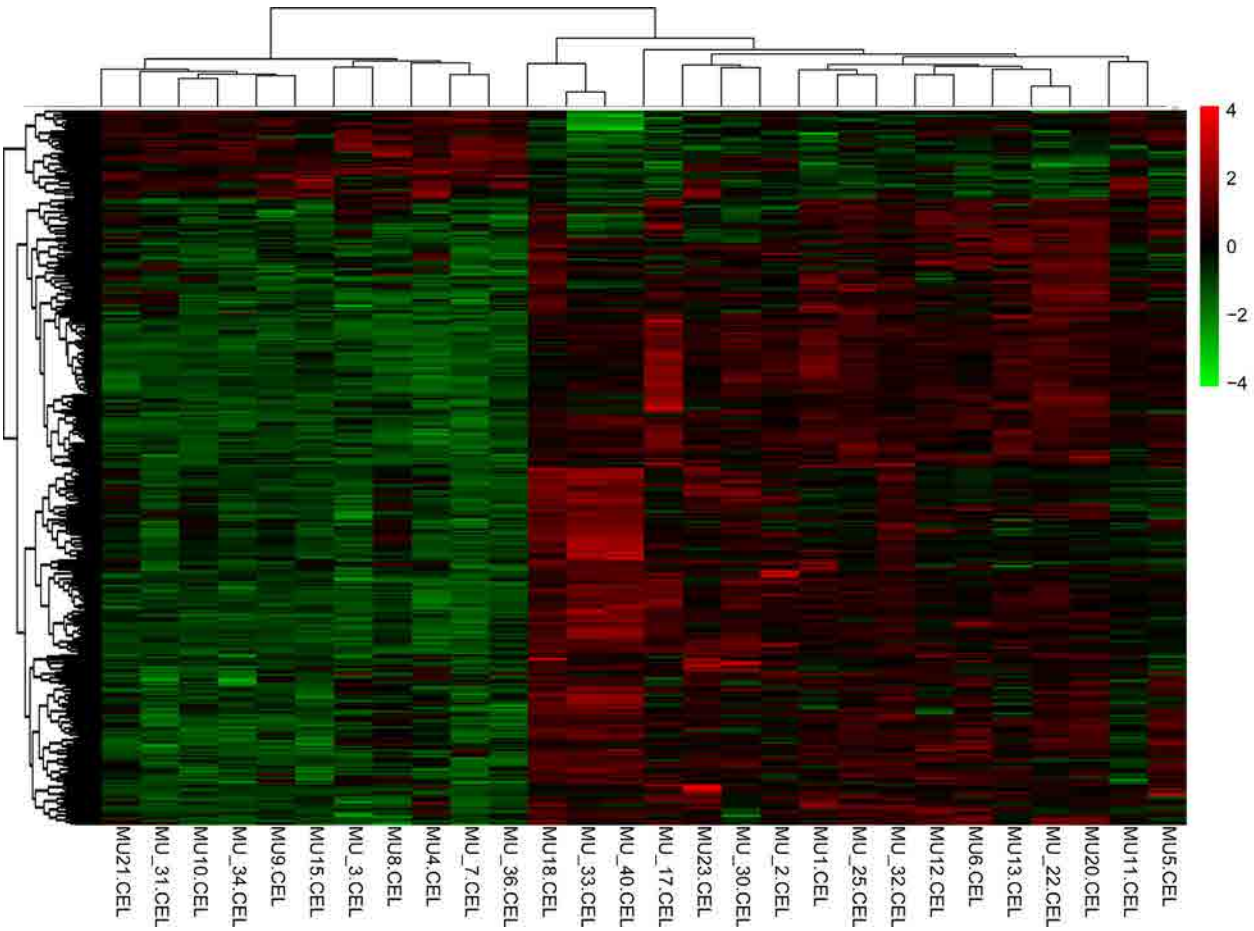

Figure.2 Heat-map image of the significantly DEGs in UVM samples of metastasis and non-metastasis group. Expression data are described as a data matrix in which each row represents a gene and each column represents a sample. Expression level are described according to the color scale shown at the topright. Red and green indicate high and low expression levels, respectively.

GO enrichment and KEGG enrichment analysis

System development (GO:0048731, 1.05E-10), single-organism developmental process (GO:0044767, 2.05E-10), anatomical structure development (GO:0048856, 2.05E-10) and

developmental process (GO:0032502, 4.73E-10) were significantly enriched upon the category of GO biological process; cell periphery (GO:0071944, 9.26E-08), extracellular region (GO:0005576, 9.49E-08), presynaptic active zone (GO:0048786, 1.65E-07) and plasma membrane (GO:0005886, 2.08E-07) were significantly enriched upon the category of GO cellular component; while for the category of GO molecular function, brain-derived neurotrophic factor binding (GO:0048403, 1.12E-09), protein tyrosine kinase activator activity (GO:0030296, 6.10E-08), neurotrophin receptor activity (GO:0005030, 7.41E-07) and neurotrophin binding (GO:0043121, 5.88E-06) were notably enriched. Meanwhile, the hierarchical tree of each GO category (biological process, molecular function and cellular component) shows motor activity (GO: 0003774, 9.14e-4), calmodulin binding (GO: 0005516, 2.070e-4), peptidyl-prolyl cis-trans isomerase activity (GO: 0003755, 8.921e-5) and cis-trans isomerase activity (GO: 0016859, 8.921e-5) may related with the metastasis of UVM (**Figure.3**).

In addition, the results of KEGG analysis showed: the 369 DEGs were involved in 19

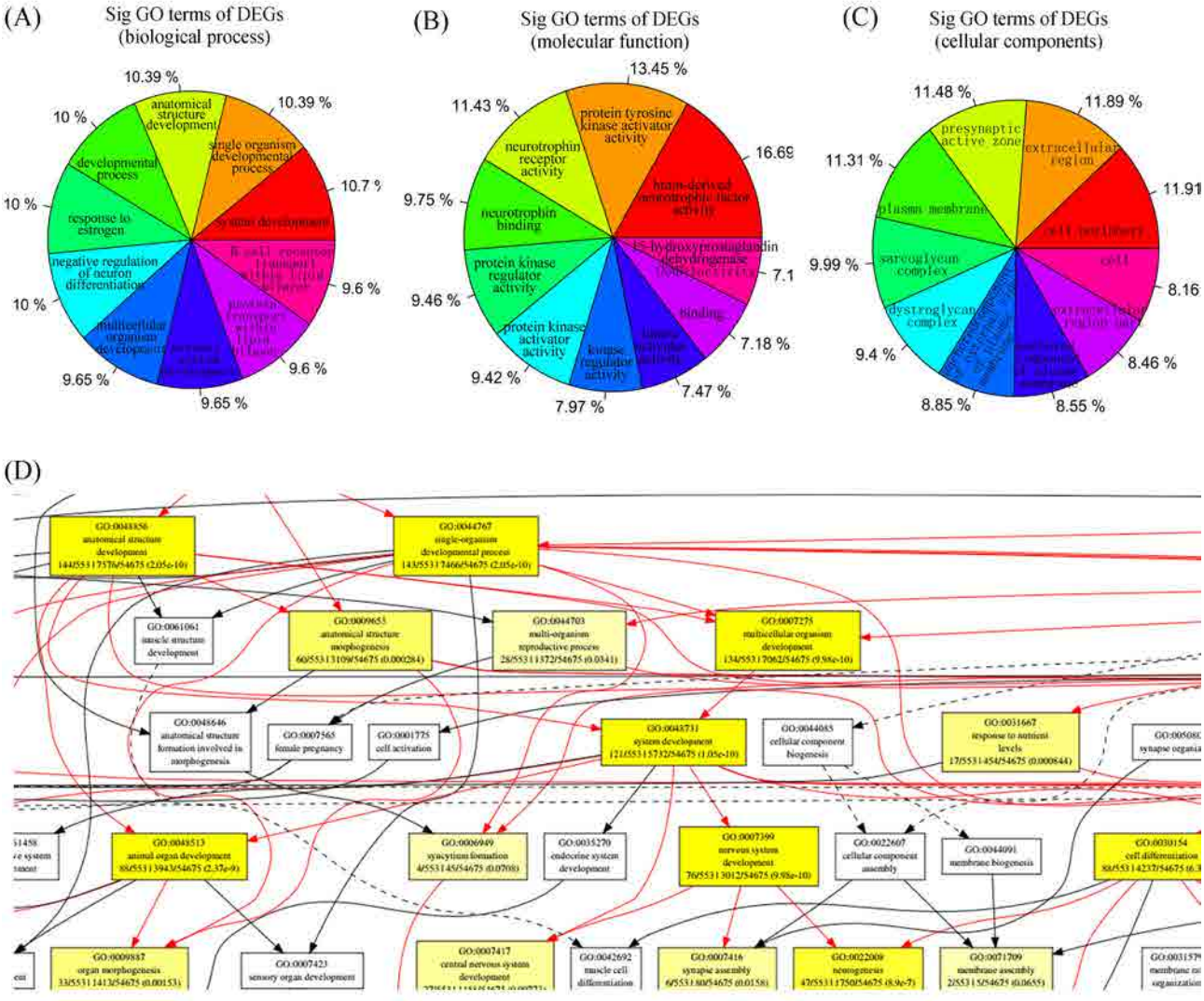

Figure.3 GO enrichments. The enrichment was conduct based on the P value of each category, and the enrichment score equates -log10(*P*-value). Top 10 significantly enriched categories of GO biological process (A), GO cellular component (B), and GO molecular function (C); (D) the hierarchical tree of each GO category.

signal pathways, including protein digestion and absorption, focal adhesion, ECM-receptor interaction, Axon guidance, Axon guidance, Cysteine and methionine metabolism, cAMP signaling pathway, PI3K-Akt signaling pathway, p53 signaling pathway, *etc*. (**Table.3**)

PPI Network of DEGs

The PPI network of significantly DEGs consisted 129 nodes. And multiple hub proteins were identified in this network, including *POMC*, *HIST2H2BE*, *ACTC1*, *KIT*, *ADCY8*, *CABP7*, *CACNA1A*, *NGFR*, *etc*. (**Figure.4**).

Table.3 Kegg enrichment of DEGs: top 15 terms with high enrichment scores.

Kegg ID	Term	*P*-Value	Input (Entrez Gene ID)
hsa04974	Protein digestion and absorption	2.42E-04	6519|1302|1282|255631|1297|9056
hsa04510	Focal adhesion	7.10E-04	7058|857|5063|1282|3915|1297|399694|6696
hsa04512	ECM-receptor interaction	1.17E-03	3915|1297|7058|6696|1282
hsa04360	Axon guidance	6.39E-03	5063|10154|22854|10512|6091|3983
hsa00270	Cysteine and methionine metabolism	9.33E-03	1036|23382|64902
hsa04024	cAMP signaling pathway	1.11E-02	51738|6662|114|5139|2696|84152
hsa00120	Primary bile acid biosynthesis	1.25E-02	8309|10858
hsa00480	Glutathione metabolism	1.35E-02	6241|119391|2940
hsa04916	Melanogenesis	1.48E-02	5443|114|8323|3815
hsa04151	PI3K-Akt signaling pathway	1.48E-02	7058|4804|1282|3815|3915|1297|2256|6696
hsa05034	Alcoholism	2.62E-02	55506|84152|8349|4915|399694
hsa05202	Transcriptional misregulation in cancer	2.67E-02	4211|7704|3248|4804|5087
hsa04115	p53 signaling pathway	2.75E-02	6241|1647|575
hsa03320	PPAR signaling pathway	3.05E-02	335|28965|8309
hsa01100	Metabolic pathways	3.88E-02	55753|349565|1339|8395|337876|50617|5446|1373|23382|64902|7941|64900|197258|3294|6241|150763|1036|8309

Discussion

For the uveal melanoma, once it is spread to distant organs, the disease will be largely resistant to currently available therapies[22]. Nowadays, It is generally accepted that the altered gene expression pattern of a cancer tissue should be associated with the initiation and progress of malignant phenotype. In our study, we selected the GSE27831 datasets from NCBI GEO database for analysis, attempting to reveal which genes expression significantly changed in the process of UVM metastasis from the genome level, and which biological processes and signal pathways these dysregulated genes participated, in order to provided a reliable theoretical basis for the further study of mechanism in UVM metastasis. The present study followed microarray-based 28 uveal melanoma patients, who are not significantly different in ages,

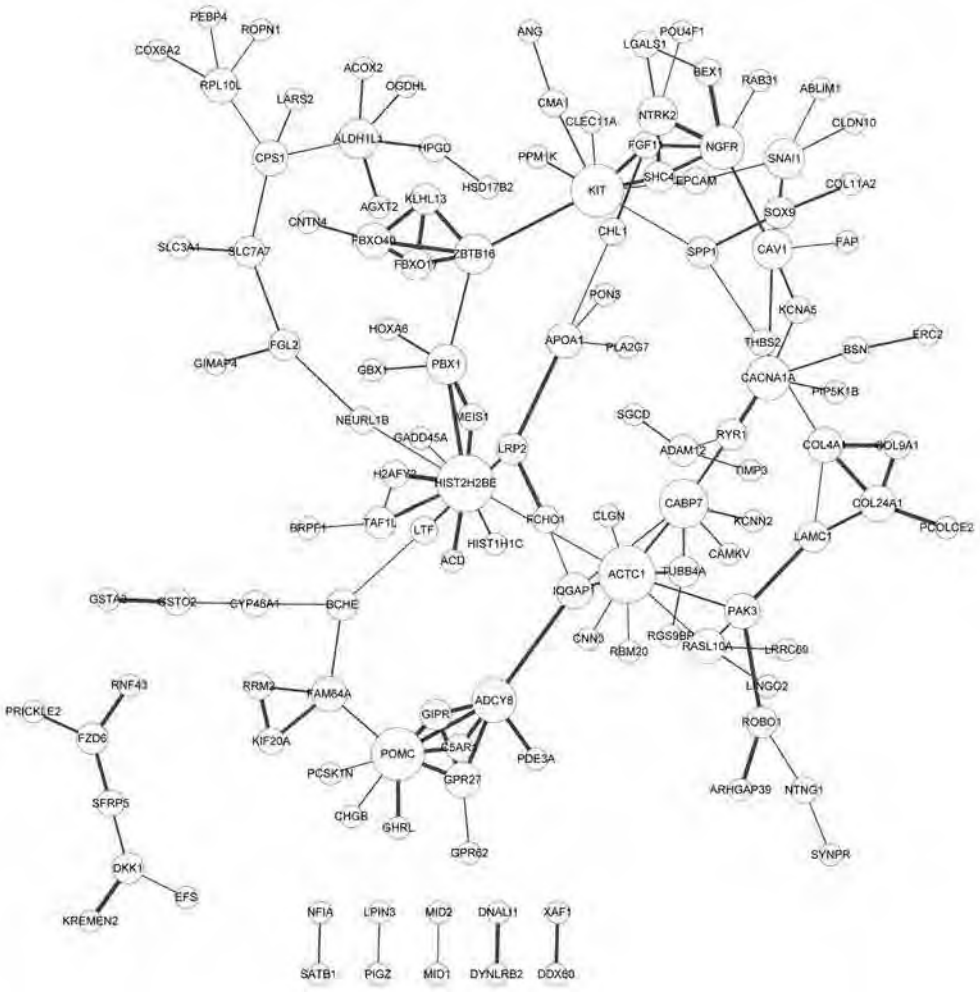

Figure.4 PPI network of DEGs. The size of the nodes and the width of the edges show their significance (larger indicates lower *P* value).

gender, primary tumor thickness, tumor largest diameter, but are significantly different in the disease-free survival (DFS). The screening results of differentially expressed genes (DEGs) showed that 369 DEGs exists in the metastatic UVM comparing with non-metastatic UVM, which is involved in 278 categories of GO biological process, 47 categories of GO cellular component, 27 categories of GO molecular functions, 19 signal pathways. At the same time, the analysis of PPI network indicates the existence of 129 nodes and multiple hub proteins, including *HIST2H2BE, KIT, NGFR, etc*.

In bioinformatics, the gene ontology is widely considered as the primary tool for organization and functional annotation of the molecular aspects of cellular systems[23]. In this study, after DEGs screening, we conducted a GO analysis and the hierarchical tree of each GO category (biological process, molecular function and cellular component) showed the motor activity (GO: 0003774, 9.14e-4), calmodulin binding (GO: 0005516, 2.070e-4), peptidyl-prolyl cis-trans isomerase activity (GO: 0003755, 8.921e-5) and cis-trans isomerase activity (GO: 0016859, 8.921e-5) may be related with the metastasis of UVM. Among those, for the GO category of calmodulin binding (GO: 0005516, 2.070e-4), Van *et al*[24] used Chromatographic procedures and amino acid sequence analysis to identify the series of calcium-binding proteins d in both primary tumors and cell lines of uveal melanoma and found calcium-binding proteins may endow tumor cells with properties related to their malignancy and metastatic phenotype; and Wagner *et al*[25] suggests both normal and

neoplastic uveal melanocytes require an intracellular signal or signals which involves calcium and calmodulin in the few minutes following cell binding to ECM proteins in order for successful cell attachment to occur. For the peptidyl-prolyl cis-trans isomerase activity, studies showed multiple proteins which have peptidyl-prolyl cis-trans isomerase activity play an important role in protein folding, transportation, signal transduction, inflammation, immune regulation, apoptosis and other biological processes. As is well known, the protein phosphorylation at Ser/Thr-Pro site is an important step of intracellular signal transduction. Recent studies found the protein phosphorylation at Ser/Thr-Pro site is just the first step for the changing of protein structure, many proteins must be getting the second change in structure, after which the corresponding protein could exert its function normally, and this structurual change is carried out under the regulation of peptidyl-prolyl cis-trans isomerase (PPIase) [26,27]. For example, the protein of Pin, which contains two functional structures including phosphorylation region and peptidyl-prolyl cis-trans isomerase activity region, participates the regulation of multiple signal pathways and could catalyze many protein like p53, β-catenin, cyclinD1, *etc*., which are closely associated with the occurrence and progression of tumor[28,29].

Kegg pathway can find the significant signaling pathways that DEGs participate in, and provide a comprehensive understanding about interactions of genes and relations between up and down stream. In this study, the kegg analysis showed protein digestion and absorption, focal adhesion, ECM-receptor interaction, PI3K-Akt signaling pathway, p53 signaling pathway, *etc*., were enriched. In previous studies, numerous studies have proven PI3K-Akt signaling pathway participated in several cancers' metastasis[30-32]; meanwhile, it was reported that the pathways of focal adhesion, ECM-receptor interaction were also associated with the invasion of cancer cells. In addition, in the PPI network of DEGs, compared with the non-metastatic UVM samples, the expression of hub protein *KIT* up-regulated 2.82-fold, and the expression of hub proteins *NGFR*, *HIST2H2BE* down-regulated 2.05-fold and 2.11-fold, respectively. Among these proteins, in 2004, All-Ericsson *et al*[16] reported that c-kit was vastly expressed in uveal melanoma, and the c-kit molecular pathway may be important in uveal melanoma growth, and pointed to its use as a target for therapy with *STI571*; in Calipel's study(33), in UVM patients, Ninety-five percent of liver metastases expressed *KIT* at the protein level, which suggested *KIT* may play an important role in the metastasis process; however, in Lüke's study[34], it was proved that c-Kit expression was not found to be associated with metastasis formation. Hence, it is necessary to further study if the hub protein *KIT* is really associated with the metastasis of UVM.

All above results suggest that there are differences in gene expression between the metastatic and non-metastatic uveal melanoma. The proteins encoded by these genes involved in multiple GO categories and signal pathways, the dysregulation of which may contribute to the UVM metastasis. Despite a similar study conducted by Zhang *et al*[35], a quality evaluation was not performed and all samples of GSE27831 datasets were included in their study, which may cause an error. In this study, based on the accurate quality evaluation and analysis, the identified DEGs, the related GO terms and signal pathways that DEGs enriched here provide an important theoretical basis for clinical investigation, meanwhile, all these results also need to be further studied and confirmed in a larger sample size containing more patients by other clinic-related studies.

Conclusion

Bioinformatics can excavate and analyze large amounts of data in microarrays by the methods of rigorous experimental planning, scientific statistical analysis and collection of completed data. In our study, we evaluated the quality of GSE27831 datasets, analyzed the DEGs screened from non-metastatic and metastatic UVM samples using bioinformatics, and preliminary provided partial new targets for di-

agnosis and theoretical basis for studying the mechanism of uveal melanoma metastasis.

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Metformin Reduces the Incidence of Cancer in Type 2 Diabetes Patients: an Update Meta Analysis

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Abstract

To evaluate the association between metformin treatment and the incidence risk of cancer in diabetes patients. Firstly, literatures related to metformin treatment and type 2 diabetes were searched in CBM, CNKI, VIP, wanfang, Cochrane Library, PubMed and Web of science databases *via* computer-based retrieval and manual retrieval, the searching date was updated to April 2017. Then according to the eligibility criteria to screen literatures, and twenty-five studies were included in this study. Finally, the related data were extracted and analyzed using Revman 5.3 software. The meta results of fifteen cohort studies showed: the incidence of cancer in type 2 diabetes patients who were treated with metformin was obviously lower than that in type 2 diabetes patients without metformin treatment, and RR=0.49, 95% CI [0.34, 0.72]. The meta results of ten case-control studies showed the reduction of cancer incidence in type 2 diabetes patients is associated with the use of metformin, and OR=0.82, 95% CI [0.7, 0.95]. In conclusion, metformin treatment can effectively decrease the incidence of cancer in type 2 diabetes patients.

Keywords: Metformin; type 2 diabetes; Cancer incidence risk; Meta analysis

Introduction

Diabetes is one type of the most common chronic diseases. As the diseases progress, lesion of the large vessel and the micro vessel could be induced by long-term hyperglycemia, which would eventually lead to a variety of complications, seriously affecting the patients on their daily life. Moreover, people living with diabetes are at a higher risk of getting various diseases and tend to have higher molarity than the general population. Over the past few decades, the incidence of diabetes is gradually increasing year by year as quality of people's life improves. According to International Diabetes Federation (IDF), as of 2015, the number of people with diabetes has exceeded 450 million in the world. This figure is expected to continue growing for the following decades and would eventually reach about 642 million in 2040[(1)]. Type 2 diabetes mellitus (T2DM), also known as adult-onset diabetes, mostly onset around age 35 to 40, which make up more than 90% of diabetic patients. In recent years, the incidence of type 2 diabetes (T2DM) kept rising and showed young trend. It not only endangers the health of patients severely but also puts the patients and their family, or even the whole society, under huge economic burden[(2)]. Epidemiological studies have shown that T2MD is mainly caused by the factors of high-fat diet, lack of exercise and obesity, *etc*., and the islet cell dysfunction, decreased insulin secretion and insulin resistance are the main symptoms of most patients. A large number of studies have shown that the risk of cancer in patients with diabetes was significantly higher than the general population, in fact, it is far above the expectation, especially for T2MD patients. The incidence liver cancer, pancreatic cancer and endometrial tumor of them are 2 times higher, as for the risk of renal cancer, bladder cancer, and breast cancer are 1.2-1.5 times higher[(3-5)].

Metformin, a biguanide drug, which is a preferred early treatment for T2MD due to its effectiveness, safety, and affordable price. It is used since it is successfully developed from the 50s of 20th century. According the 2017 edition of the American "Diabetes Medical Diagnostic Criteria", released by American Diabetes Asso-

ciation (ADA): If no contraindications and it is tolerable, metformin should be the first choice of early treatment for patients with type 2 diabetes Metformin can treat T2MD through improving the utilization of glucose, inhibit carbohydrate absorption and gluconeogenesis, which play a similar role as insulin[6]. In 2005, a retrospective study has found that T2MD patients who received metformin as treatment were found under significantly lower risk of cancer, comparing to patients who were on other medication[7]. Since then, using metformin as an anti-cancer drug had become a research hotspot. Over the years, some studies have shown that with the use of metformin can reduce the risk of getting various cancers including pancreatic cancer, rectal cancer, ovarian cancer, prostate cancer, lung cancer, thyroid tumors, laryngeal cancer etc. There are also studies the risk of cancer is not related to the use of metformin. However, long-term use of metformin can increase the survival of T2MD patients with breast cancer[8]. Never the less, in the study of Kowall et al[9] shows there isn't any significant protective effect on colorectal cancer, lung cancer, breast cancer and prostate cancer. In the meantime, using metformin can neither increase the survival rate of patients with pancreatic cancer nor improve the prognosis[10].

Therefore, to better assess whether metformin has a protective effect on T2MD patients, to prevent them from tumorigenesis, we are going to use the systematic evaluation method in this study to give an objective and impartial evaluation.

Materials and Methods

Inclusion Criteria

(1) Subjects are type 2 diabetes patients; (2) Comparison in the risk of cancer between patients receive metformin and control is performed; (3) OR value/HR value is reported in the result.

Exclusion Criteria

(1) Animal research or cytology experimental study; (2) Article type is review; (3) Cross-sectional study; (4) The results of the study did not give the adjusted value of disease risk, meanwhile, it cannot be calculated from known data; (5) Design flaws, incomplete data and incorrect statistical method exist in study.

Search Strategy

Searched in CNKI, CBM, VIP, and Wanfang using “metformin”, “diabetes”, “cancer” as keywords; Using keywords: “Metformin”, “Diabetes Mellitus”, “Neoplasms” to search in PubMed, Cochrane, Web of science. The searching date is updated to April 2017.

Data Extraction

Following the pre-design data extraction table, two researchers, one was responsible for entering the information extraction and the other make sure everything was on the right track. In case of disagreement, it could be discussed within the two researchers or judged by the third party. Lack of information should be made up by contacting with the author by phone or email. Information that were extracted including: (1) General information: title, name of author(s), date published, and resource of articles; (2) Research method; (3) The total number of patients with diabetes and the number of people with cancer within them, which region they are from, what is the diagnostic criteria, and also their sex, age, nationality, is there any interference is done etc.; (4) Outcome and time; (5) Outcome and effect indicators.

Statistical Analysis

Revman 5.3 software was used in this meta-analysis. In case-control and cohort studies, the incidence rate is represented as odds ratio (OR) and relative risk (RR), respectively. If the value of incidence rate is less than one (OR<1 or RR<1), and 95% CI does not overlap with the value of one, it suggests that metformin is a protective factor for T2MD patients. That is, taking metformin can reduce the risk of tumorigenesis; The heterogeneity was detected by I^2 and Q test. If $I^2 < 50\%$ and $P > 0.10$, then there is no heterogeneity, which means the fixed effect model should be used, otherwise, choose random effect model. If there is significant heterogeneity

and the source of it cannot be determined, then no meta-analysis should be performed but using descriptive analysis instead.

Results

Characters of Included Studies

In this meta-analysis, total 708 related literatures were retrieved. Twenty-five of them were included in the analysis by carefully reading the title, abstract and full text, excluding those cross-sectional studies, repeated publications, animal or cytology experiments[7,11-34], and research not based on T2MD patients, *etc*. The literature screening process is shown in **Figure 1**. Subjects of all 25 studies are T2MD patients along with exact OR of the incidence rate of cancer. Within these studies, 15 of them are cohort study[14,17,18,20,24-34], and 10 of them are case-control study[7,11-13,15,16,19,21-23]; Notice that Tseng, C. H.[24-32] in 2014 to 2016, has published 9 retrospective cohort study articles, respectively, on the risk of T2MD patients in colon cancer, prostate cancer, thyroid cancer, bladder cancer, breast cancer, ovarian cancer, endometrial cancer, oral cancer, renal cell carcinoma. Only one of them is listed in **Figure 1**. See more details of the articles included in the study in **Table 1**.

In addition, in the data extraction process of this meta-analysis, if both the pre-adjusted and post-adjusted OR are provided in the articles, the post-adjusted value is adopted due to the fact that post-adjusted value has taken the confounding factors into consideration, which makes it more precise than the pre-adjusted value. When the incidence of cancer in T2MD patients is low in case-control studies (such as studies of Azoulay, L.[11], Smiechowski, B. B.[23] and Smiechowski, B. [22]), since the effect of OR is close to RR, RR value can be used as OR value for analysis.

Meta analysis

The result of this accumulated meta-analysis which included 10 case-control studies showed that there was a significant heterogeneity (I^2=81%, P<0.00001), therefore, the random effect model was adopted here. The result of the forest plot showed that: The incidence of cancer is lower in the T2MD patients who were treated with metformin, with OR=0.82, 95% CI [0.7, 0.95] (**Figure 2**).

Similarly, the results of the accumulated meta-analysis which included 15 cohort study showed that there is a significant heterogeneity between each study (I^2=97%, P<0.00001), and random effect model was used. According to the forest plot: administration of metformin can reduce the incidence of cancer in T2MD patients, with RR=0.49, 95% CI [0.34, 0.72] (Figure 3).

Subgroup Analysis

Since the degree of heterogeneity in the accumulated meta-analysis was high, we performed a subgroup analysis based on the type of cancer furtherly. However, the type of cancer in the 10 case-control studies was all different from each other, therefore, only the 15 cohort studies are accounted for this subgroup analysis.

Within the 15 cohort studies, 3 of them have analyzed the incidence of colorectal cancer in T2MD patients and 3 have analyzed the incidence of breast cancer.

The results of the subgroup analysis for the 3 studies on colorectal cancer showed that there was no heterogeneity between the studies (I^2=7%, P=0.34), and fixed effect model was used. Meanwhile, the result of forest plot suggested that taking treatment of metformin can reduce the incidence risk of colorectal cancer in T2MD patients, with RR=0.68, 95% CI [0.56, 0.84] (**Figure 4A**).

The results of the subgroup analysis for the 3 studies on breast cancer showed that the heterogeneity between the 3 studies was I^2=57% (P=0.10), so random effect model was adopted. And the forest plot indicated that the reduction of the incidence risk of breast cancer in T2MD patients is related to metformin administration, with RR=0.69, 95% CI [0.55, 0.87] (**Figure 4B**).

Sensitivity Analysis

In addition, in order to assess the stability of the meta-analysis, a sensitivity analysis using a single variable sensitivity analysis was used to test: whether the study had a significant effect

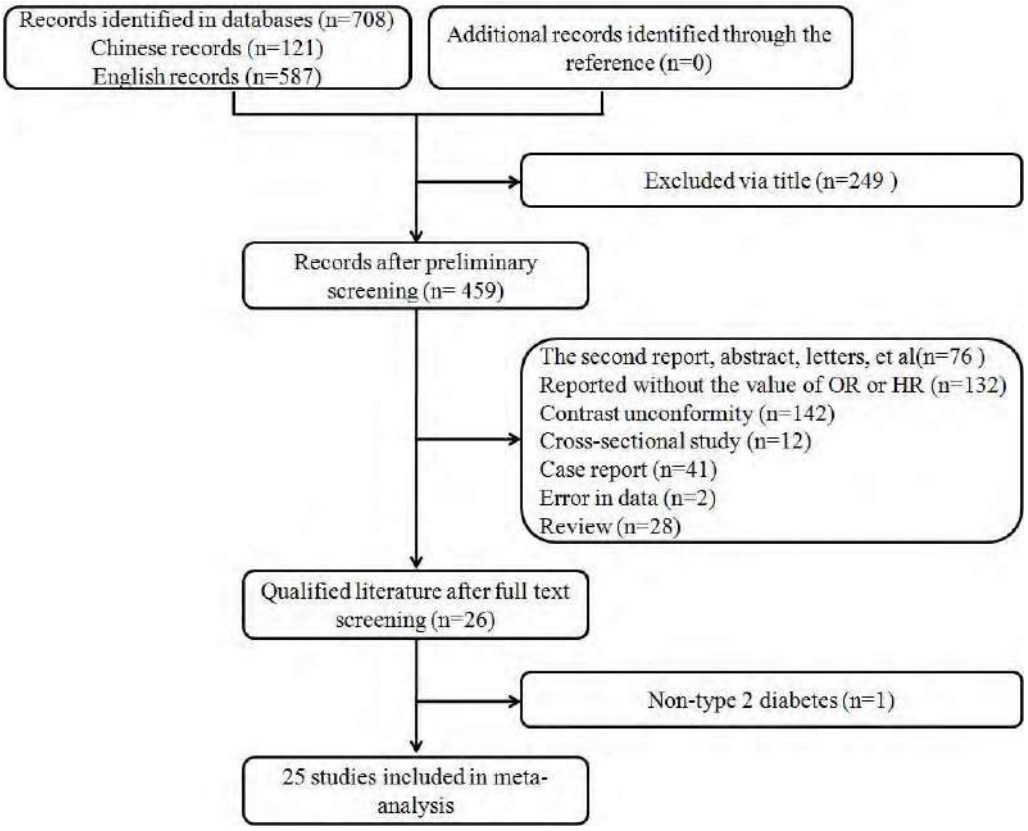

Figure. 1 A flow diagram of the study selection process.

Table.1 Characters of Included Studies.

Studies	Study type	Cancer type	OR	RR	95% CI
Azoulay, L.2011[11]	Nested case-control	Prostate Cancer	-	1.23	0.99-1.52
Becker, C.2014[12]	Case-control	Head-neck carcinoma	0.84	-	0.61-1.15
Bosco, J. L.2011[13]	Nested case-control	Breast cancer	0.81	-	0.63-0.96
Calip, G. S. 2016[14]	Cohort study	Invasive breast cancer	-	0.86	0.65-1.12
Chaiteerakij, R.2013[15]	Case-control	Intrahepatic cholangiocarcinoma	0.4	-	0.2-0.9
Evans, J. M.2005[7]	Case-control	Mixed	0.77	-	0.64-0.92
Hassan, M. M. 2010[16]	Case-control	Liver cancer	0.3	-	0.2-0.6
Lee,M. S.2011[17]	Cohort study	Mixed	-	0.12	0.08-0.19
Libby,G. 2009[18]	Cohort study	Mixed	-	0.63	0.53-0.75
Margel,D.2013[19]	Nested case-control	Prostate Cancer	0.95	-	0.85-1.07
Sakoda, L. C.2015[20]	Cohort study	Lung cancer	-	1.02	0.85-1.22
Sehdev, A.2015[21]	Case-control	Colorectal cancer	0.85	-	0.76-0.95
Smiechowski, B. B.2013[23]	Nested case-control	Lung cancer	-	0.94	0.76-1.17
Smiechowski, B.2013[22]	Nested case-control	Colorectal cancer	-	0.94	0.74-1.19
Tseng, C. H.2016[24-32] *	Cohort study	Mixed	-	0.386	0.378-0.394
Yang, X 2011[33]	Cohort study	Mixed	-	0.51	0.31-0.82
Yen, Y. C.2015[34]	Cohort study	Head-neck carcinoma	-	0.66	0.55-0.79

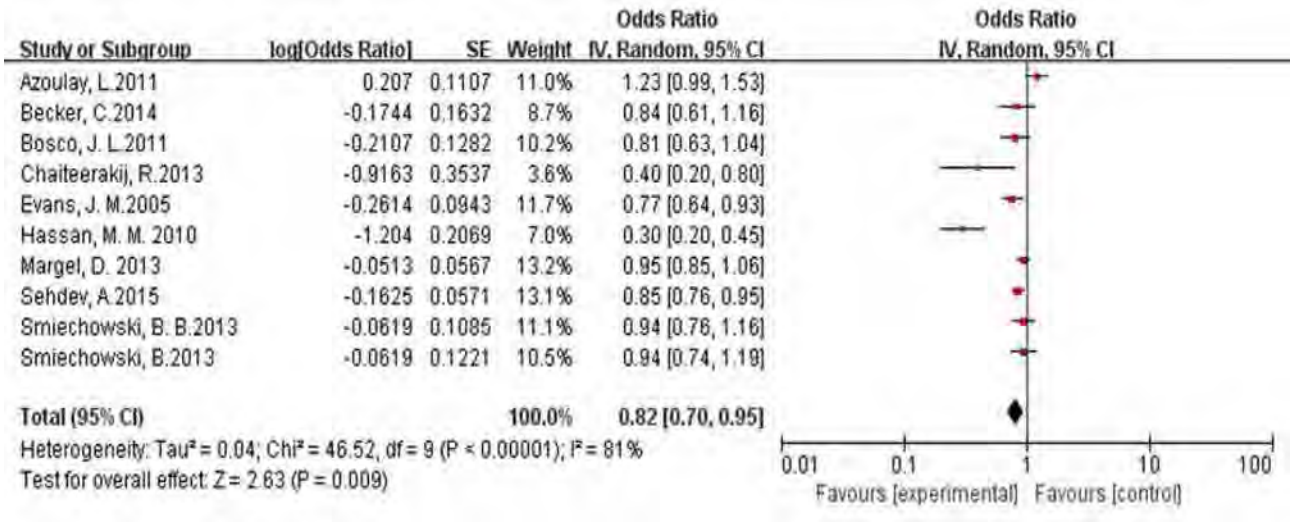

Figure 2 In the 10 case-control studies, the incidence rate of cancer in T2MD patients treated with or without metformin were compared.

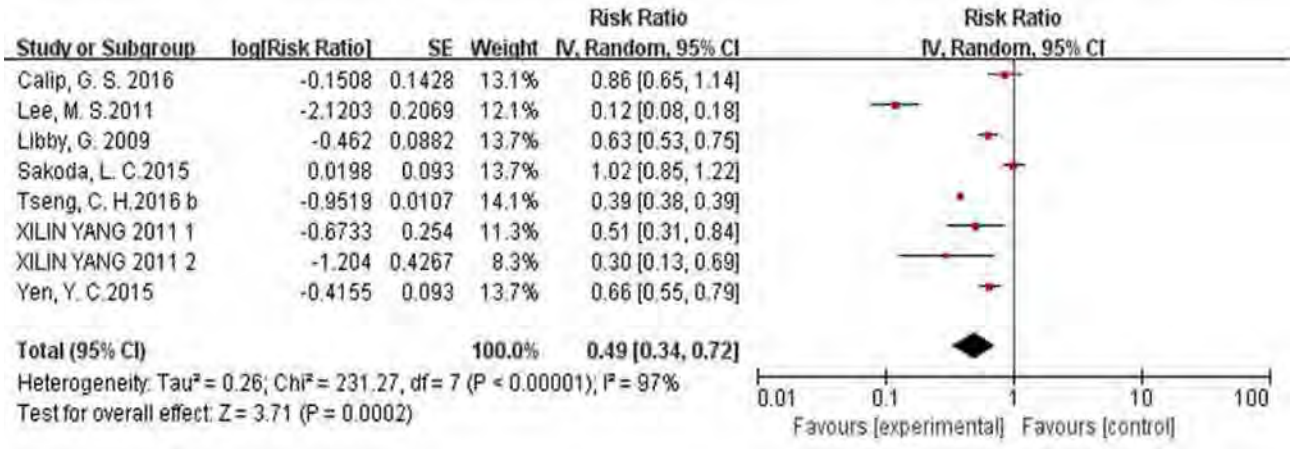

Figure 3 Comparison of cancer incidence in metformin treatment group and non-metformin treatment group of T2MD patients, in the 15 cohort studies. Among that, "Tseng, C. H 2016 b" represented the total RR value which accumulated by the RR value of 9 studies published by Tseng, *et al* from 2014 to 2016. "XILIN YANG 2011 1" and"XILIN YANG 2011 2" represented the RR value of effects when treated with two different concentrations of metformin in YANG's study, respectively.

on the overall outcome. The results suggested the pooled ORs were statistically robust and reliable.

Discussion

Diabetes is a common chronic metabolic disorder characterized by hyperglycemia and could be induced by varies of causes, often leading to three major metabolic disorders in patients, which eventually give rise to dysfunction of multiple organs[35]. Never the less, high blood sugar, insulin resistance and the other diseases that induced by metabolic disorders can easily cause the risk of a variety of cancer to increase[36,37]. In recent years, as the incidence of diabetes keeps increasing and continuous research shows that type 2 diabetes is closely related to the risk of cancer. Both clinical and basic research on the relationship between diabetes medication and the incidence of cancer has become a research focus in a wide range.

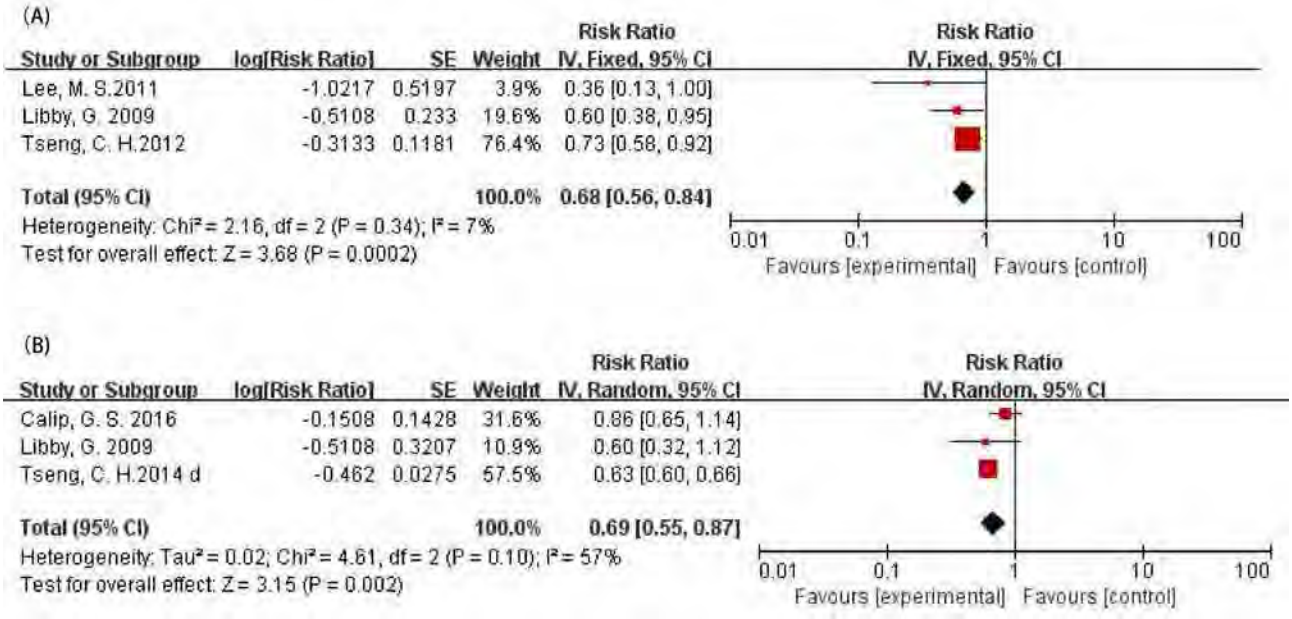

Figure 4 Subgroup analysis: (A) Comparison of colorectal cancer incidence in metformin treatment group and non-metformin treatment group of T2MD patients; (B) Comparison of breast cancer incidence in metformin treatment group and non-metformin treatment group of T2MD patients.

Metformin as the preferred medication for treating T2MD, it can reduce the blood sugar in patients by reducing the body's resistance to insulin, which enhances the efficiency of using insulin for the body. With the widespread use of metformin in treating T2MD, some recent epidemiological studies found that metformin not only have extraordinary hypoglycemic effects but also has some significant effect on treating a variety of cancers and could be a potential anticancer drug[38]. A large number of experimental studies have shown that the cell proliferation of esophageal cancer[39,40], gastric cancer[41,42], liver cancer[43,44], pancreatic cancer[45], breast lobular carcinoma[46] and another tumor cells can be inhibited in T2MD patients after metformin administration, which helps reduce the risk of incidence of cancer. In 2016, Amin *et al*. have analyzed 1916 diabetic patients with pancreatic cancer (PDAC) and found that the risk of death is reduced by 12% in patients who are on metformin treatment[47]. In addition, in vitro cell experiments have also confirmed that metformin can activate AMPK and inhibits P13K/AKT/mTOR signaling pathways which help regulate cell cycle[48], or inhibits cancer cells from metastasis by regulating EMT expression through inhibiting the COX/PGE2/STAT3 signal pathways[49], which can also reach the goal of reducing the incidence and development of tumors [50].

Although the prevention of metformin on malignant is highly supported by many epidemiological investigations and clinical research, there still not yet has a conclusion about if metformin can really reduce the incidence rate of cancer in T2MD patients. In order to clarify this relationship, this study has quantitatively synthesized the published result of the incidence of cancer in T2MD patients and analyzed them with meta-analysis, based on the principle of evidence-based medicine, try to reduce the impact of offset on the outcome and improve the accuracy and reliability. In this study, 25related studies with high quality were included in the meta-analysis, after retrieval and screening based on the established literature exclusion and inclusion criteria. Within these studies, 15 of them are cohort study, and 10 of them are case-control study. The result of the accumulated meta-analysis of the case-control studies showed that: compared with the T2MD patients treated without metformin, the incidence of cancer is lower in the T2MD patients who treated

with metformin, with OR=0.82, 95% CI=[0.7, 0.95] (I^2=81%, P<0.00001). Since the result of this meta had a high heterogeneity, we checked for the heterogeneity by excluding one study each time and found that Hassan, M. M.'s study in 2010 [16] was the main source of heterogeneity. When this study was excluded, OR=0.89, 95% CI [0.80, 0.99], (I^2=58%, P=0.01). The result of cohort studies of meta-analysis showed: total RR=0.49, 95% CI [0.34, 0.72] (I^2=81%, P<0.00001), the heterogeneity was high and the heterogeneity had no significant change while using the exclusion method; therefore, subgroup analysis was applied, and the result showed that: metformin can effectively reduce the risk of colorectal cancer and breast cancer in T2MD patients. To ensure the robustness of the meta-analysis, we took a step further and presented the sensitivity analysis, which showed that the results of this study were relatively stable and reliable.

In summary, this study has preliminarily confirmed that metformin treatment can effectively reduce the risk of cancer in T2MD patients. However, due to the fact that most of the study on using metformin as hypoglycemic treatment are observational studies which are case-control study or cohort study. There is a certain degree of limitation in the verification, and the reliability of the results is relatively low. But notice the fact that evidence-based medicine analysis is still developing and despite the above flaws, this study has conducted a systematic evaluation to this problem, based on existing data resources. The number and the quality of the articles that were taken into account are relatively large and high, as well ascomprehensive. The conclusion can still be a guide for preventingand treating cancer in type 2 diabetes patients.

Conclusion

This study has done a meta-analysis on the data which is extracted from the selected articles and the results show that: Metformin treatment can reduce the incidence rate of cancer in type 2 diabetes patients.

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Research Progress of Nanotechnology Treating for Brain Glioma

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Abstract

With its highly proliferative, infiltrative, and invasive properties, glioma is the most common malignant primary brain tumor, and 57% of them are glioblastoma multiforme, with a median survival time of only 18 months. However, facing the great challenges from blood-brain barrier and blood-brain tumor barrier, the drug treating for glioma remain non-targeted with low efficiency, which urgently need a new drug. Due to its plastic, low-toxic, targeted nature, nanotechnology brings hope to glioma treatment. In this review, we will discuss the potential possibility of nanoparticles in the treatment of gliomas based on targeted drug delivery system.

Keywords: Nanotechnology; Brain glioma; Treatment

Introduction

The incidence of malignant primary brain tumor is 5.03-8.84 per 100,000. Glioblastoma accounts for 8% of all intracranial tumors. Glioblastoma multiforme (GBM) is the most common, aggressive, and malignant form of astrocytomas, which originate in the brain. The 5-year relative survival rate for patients with GBM is lower than 5%[1], and the standard clinical treatment of GBM consists of maximal surgical resection, chemotherapy, and radiotherapy. However, the resistance of GBM towards the chemotherapy and radiotherapy leads to aggressive tumor relapse as well as poor convalescence, and the median survival of GBM patient is only 18 month[2,3].

Currently, the most critical challenge of treating for GBM is the poor drug penetration over the body barrier, including blood-brain barrier (BBB) and blood-brain tumor barrier (BBTB). The BBB isolates the central nerve system and blood-borne substances through the enzymatic barrier, cell-cell tight junction, immunological barrier, and diffusion barrier, while the BBB allows the liposoluble or small hydrophilic to pass through[4]. The BBTB, on the other hand, is the barrier between brain tumor and blood-borne substance, the permeability processes the apparent heterogeneity. Usually, due to the high metabolic demand of tumor cells, the tumor vasculature is highly enriched and contributes to the high permeability of the barrier, which is in favor of the drug aggregation. However, although the core portion of GBM has the abundant blood vessels supply as well as high BBTB permeability, the BBTB surrounded the tumor edge has similar low permeability as the complete BBB. Therefore, the concentration of drug penetrated into the brain tumor is still low[5,6].

Recently, the application of nanotechnology has drawn extensive attention from researchers in the medical science. The nanomaterial is readily degradable in *vivo*, which can be used as a delivery system of a lot of medicine. With the application of nanotechnology as drug delivery system, we can improve the drug administration: make the drugs more peculiar to the targets, decrease the side effects, better monitor the concentration, and easier design the drug construct[7]. As we all know, the major potential mechanism of nanotechnology in the drug delivery system is enhancing the permeability and retention effect (EPR)[8]. Therefore, during the process of the nanoparticle drug cross the BBB, on one hand, the nanoparticle reagents indirectly increase the drug concentration in the cerebrospinal fluid by enhancing the BBB

permeability; On the other hand, the nanoparticles can cross the BBB through active and passive transportation, and the receptor-mediated nanoparticle endocytosis is the most accepted pathway[9]. The nanocarrier consists of polymer nanoparticles, nanoscale liposomes, and carbon nanotubes, *etc*[10]. Although there are several nanoparticle drugs are currently approved in the market for cancer treatment, the study of the application of nanotechnology in GBM is lagging behind.

In the studies of nanoparticle and GBM, the nanoscale liposome is the relatively mature carrier than the others. Some drugs have been already approved for phase I and phase II clinical trial such as polyethylene glycol liposomal adriamycin DepoCyt®, liposomal daunoXome ®, and other types of vectors are in preclinical trial[11,12]. In this paper, we will review the nanotechnology in the treatment of GBM, imaging and diagnosis field based on the characteristics of the properties of nanoparticles and their function in the targeted drug delivery system.

Application of Organic Nanoparticles in the Treatment of GBM

Organic nanoparticles, according to the composition and structural trait can be categorized to high molecular polymer nanoparticles, biomaterial nanoparticles, micellar nanoparticles, dendrimer, and *etc*.[10].

High Molecular Polymer Nanoparticles

Micellar Nanoparticles

Micellar nanoparticles are composed of an amphiphilic macromolecule polymer. Amphiphilic molecules can spontaneously assemble and form the hydrophobic core to encapsulate the hydrophobic particles. According to the animal study, based on ERP effect, the convection-enhanced delivery of paclitaxel can extend the survival period of rat glioma model by using the polyether Nano microsphere as carriers[13]. Interestingly, polyether nanoparticles themselves have the ability to prevent the drug from clearance out[14]. The in *vitro* study of human glioblastoma cells indicates that the combination of the acid-labile groups and the nanomicrosphere polymers has the advantage of rapidly reaching and maintaining the effective plasma concentration[15]. After binding to the acid-labile groups, the nanomicrosphere polymers can be not only structurally stable in the physiological environment of pH=7.4 but also be able to load the doxorubicin; and then release the doxorubicin under the acidic conditions (e.g. Lysosomes). What's more, the release is biphasic, the nanomicrosphere polymer release the doxorubicin at a high rate during the first phase (pH>5); while during the second phase (pH<5), the release is slow but stable. However, for the in vitro study, the physiological environment of the pH is relatively consistent. While in the actual human body, the pH of the tumor microenvironment is usually lower than the normal tissue (pH=6~7). The glioblastoma tumor tissues are highly heterogeneous, the elevated metabolism of rapidly growing tumor results in the release of various acidic metabolites, which lower the pH of the tumor microenvironment, contributing to the complexity of microenvironmental pH[16]. Taken together, the pH-dependent sustained release system needs further studies. In addition, an Star-branched amphiphilic copolymers can transport chemotherapeutic drugs as well as genes, which offers better treatment than the microsphere with single agents. This combination therapy broadens the horizon of nanotechnology in terms of cancer treatment[17].

Due to the EPR effect, the nano-agents have a higher concentration in the tumor tissue compared to normal tissue, called passive targeting[16]. However, elevated levels of drug concentration in the tumor site are not equivalent to the elevated level of drug internalization, whereas the latter has a greater biological significance[18]. Based on this, we can bind specific ligands to the surface receptor on the nano-agents to achieve the active targeting. Currently, the ligands of active targeting are generally those overexpressed in the tumor cells such as folic acid, transferrin, EGFR, glycoprotein receptor, *etc*., or tumor endothelial cells such

as *VEGF*, avb3 integrin, *VCAM-1*, *MMP*[16]. Thus, in the course of the treatment of the tumor, in order to enhance the target specificity, on the one hand, we can co-target two ligands of tumor cells and tumor endothelial cells; on the other hand, we can target the common ligand of tumor cell and endothelial cell[19,20]. For example, the cRGD peptide is a class of short peptides containing the Arg-Gly-Asp sequence that can be used as a recognition site for certain integrins and their ligands to mediate the cell interaction. Thus, its application in targeted therapy has become a new research focus. The cRGD peptide targeting drug loading primarily based on the precursor microsphere which consists of amphoteric macromolecule polymer that contains abundant surface functional groups binds to the sulfhydryl group of cRGD. In addition, some studies used nuclear magnetic resonance (NMR) to analyze the effect of the microsphere with drugs and ligands loaded, based on EPR theory, and compared the drug concentration inside U87MG cells before and after the drug treatment. They found the drug concentration inside the cells was higher when drug and ligand were both loaded. At the same time, as to the efficacy, Miura *et al.*[21] found that the cRGD peptides could use endocytosis to cross the BBTB by observing the tumor growth on the glioma BALB/c nude mice model, therefore, increase the target binding efficiency and drug concentration, and provide the extraordinary anti-tumor effect. Above is the general idea of studying the application of nanocarrier in terms of target therapy on the tumor. Conventional models and techniques are shown in **Table 1**. (See supplemental data)

However, in the nano-agent experiment of tumor drugs, although the EPR effect has been tested in the animal model and achieved expected results, most of the clinical trials are not successful. This is due to the huge difference between the mouse tumor model and human tumors. The mouse tumor progresses faster than the human tumor, and mouse tumor and blood vessel are less heterogeneous, the individual difference cause to the inconsistency of mouse and human experiment results[22]. Nevertheless, nanotechnology has a great potential for tumor treatment. On the one hand, some applications, for example, low toxicity (assuming that the drug has low toxicity, can sustain low concentration yet effective without the EPR effect.), local medication and diagnosis do not rely on the EPR effect. On the other hand, the concept of personalized therapy is still lagging behind, and the application of Nano-agent will be the solution to such problems[8].

Dendrimer

Nano dendrimers, as the name suggests, consists of dendrimer polymers, highly branched, radially, and have a large number of surface groups. Due to its particular dendritic structure, this particle has a high drug load and versatility, and the transport efficiency of the drug is related to the size and configuration of the particles[18]. The most studied nano-dendrimers are polyamide amines (PAMAM) particles[19,20,23], which are ideal for cationic multimers and transport vectors[24]. The versatility is mainly due to its ability to combine a variety of functional materials to achieve targeted transport protein and BBB permeability changes. For example, in addition to binding to specific ligands such as Angiopep-2[20], transferrin[19], *etc*. to achieve "active targeting"; the combination with magnetic fluorescent materials can also achieve in vivo positioning, in Agrawal *et al.*[23], the use of MRI targeting targeted transporter siRNA could inhibit the expression of tumor cell epidermal growth factor to treat glioblastoma. In addition, studies[19] show that nano dendrimers can also achieve biphasic binding, that is, internal binding of specific molecules such as tamoxifen, externally binding specific ligands, thereby reducing cell efflux to drugs and increasing drug intake of the cell.

In addition to nanospheres and nano dendrimers, other polymeric nanoparticles also include PLGA particles, which can also bind to specific ligands such as nucleic acid[25], transferrin[26], and even magnetic Silica[26] to achieve targeted drug delivery to glioma.

However, despite the fact that specific ligands increase the efficacy of drug administration, but the “off-target” problem is still unavoidable. In *vitro* study[27] showed that af-

ter the drug entering the body, the transferrin group on the surface of the nanoparticles would interact with the proteins in the medium and form a protein "crown," thereby preventing the aggregation of the drugs in the non-target site. Although the study only reported the transferrin, it is reasonable to suspect that other ligands will also present this kind of issue.

Nano-biomedical Material

Biomaterial nanoparticles are made up of natural polymers such as polysaccharides and proteins. In essence, they are macromolecule polymer. Biomaterial nanoparticles have multiple advantages including low cell toxicity, abundant surface functional groups, high drug affinity, and excellent target cells absorption[28]. Animal natural proteins include albumin, gelatin, elastin, polysaccharides such as chitosan can be used as ligands or as nanoparticle skeletons[28]. Natural proteins, compared to the artificial proteins, will attain better bioactivity. When the natural proteins bind to the receptors on the cell surface, they can activate endocytosis while the artificial proteins' abilities to activate endocytosis are limited. Therefore, even to the same receptor, the natural protein and artificial protein will trigger different signaling pathways[29].

Nano-liposomes

The liposome is a synthetic spherical structure with the lipid bilayer, also composed of amphiphilic molecule. Unlike the monolayer nanomicrosphere that forms a hydrophobic core, the bilayer nanomicrosphere can form a hydrophilic core in the liquid phase which is in favor of the transport of small hydrophilic molecules. Indeed, the nano-liposomes provide the potential solution to the rapid metabolism, poor absorption, and high toxicity of conventional liposome application[30].

Compared with other nano-agent, liposomes have the disadvantages such as low clearance rate, and low transportation rate. Therefore, the liposomes usually bind with polyethylene glycol (PEG), APMP (acting on GLUT-1, which favors the cell uptake of nanoparticles)[31], RGD peptides[32], transferrin[33], and other specific ligands. Among those ligands, PEG can escape the reticuloendothelial system, reduce the phagocytosis of macrophages[34], reduce the antigenicity of liposomes in order decrease the clearance of immune system[35], prolong the circulatory time in the body[36], and increase the drug concentration on the tumor site. Thus, PEG conjugated liposomes are called "stealth liposomes"[37].

As TPGS (an amphoteric copolymer material) is increasingly used in liposome and microsphere cell skeletal synthesis[37,38], studies have found that TPGS (PEGylated Vitamin E) Nano-agents have a greater toxicity to tumor cells while showing a higher packaging efficiency to drugs, cell uptake rate is also higher[39]. TPG is likely to overcome the multidrug resistance of tumor cells as a P-glycoprotein inhibitor[39]. Due to the TPGS is more conducive to be uptaken by cell and the TPGS does not bind ligands causes poor targeting, the side effects will be more severe than other liposomes reagents.

In recent years there comes out a kind of theranostic liposomes, integrated with the imaging and treatment as a whole, not the only save the time of patients, but also reduce the toxicity of contrast agent, thereby is safer than traditional imaging technology. For example, the quantum dots (QD) contain heavy metals, which are toxic, but it degrades less after encapsulating into the liposomes, so that make sure the harmful substances in the body can be maintained within the human safety range[37,38].

Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) take advantage of nanoscale liposomes and polymeric macromolecules. The major differences between SLNs and nanoscale liposomes are (1) solid structure, a solid lipid core matrix, can dissolve lipophilic substances; (2) composed of solid natural or synthetic lipid, so the biocompatibility is high, and the cell Toxicity is minimal. Studies have shown that SLNs with ligand folate and APMP loaded can enhance the transport efficiency of etoposide in vitro to achieve targeted delivery[40]. Also, somatostatin recep-

tors, particularly type 2, are overexpressed in neovascular endothelial cells and glioma cells in gliomas. Therefore, by constructing Tyr containing paclitaxel (PTX) -3-octreotide-modified SLNs can act on both cells simultaneously to achieve "double-targeted" therapy[41].

Inorganic Nanoparticles in the Treatment of Brain Gliomas

Inorganic nanoparticles include magnetic nanoparticles, carbon nanotubes, *etc*. Compared to organic nanoparticles, inorganic nanoparticles have better physical properties and play a unique role in thermotherapy.

Magnetically-responsive Nanoparticles (MNP)

MNP consists of magnetic cores (composed of iron, manganese, cobalt, nickel and their respective oxides) and a biocompatible polymer shell, which is noninvasive and can be administered orally[42]. On the one hand, it can use its magnetic field to manipulate an external magnetic field such as MRI to accurately reach the organs with the tumors, reduce the side effects of the drug concentration in the non-lesion site while maintaining the external magnetic field to allow the residence time of the nanomaterials in the tumor site to be prolonged sufficient time to release the drug. The animal study found that the turnover time of MNP in the tumor site can be up to 150 min, and it took 60 min to release all the drugs[43]. On the other hand, studies have shown that thermotherapy as adjuvant therapy can improve the survival rate of patients with recurrent pleomorphic glioblastoma[44]. MNP magnetic field energy can be converted into heat; alternating magnetic field of local heat can be applied to the treatment of thermotherapy to achieve the purpose of treating tumor[45]. At the same time, external thermal effects can also improve tissue permeability and increase the plasma drug concentration. MNP can also be combined with heat-sensitive materials to "guard" the release of drugs[46].

Currently, MNP has been shown to be non-toxic and well-tolerated in preclinical and clinical studies of solid tumors[47,48]. However, due to MNP requires high magnetic field control, has low biocompatibility, and limited targeting ability, intracarotid drug administration will cause vessels clotting and therefore leads to the risk[49]. Mohammed *et al.*[50] found that the polymer coating could prevent particle aggregation and improve biocompatibility to some extent. Therefore, the magnetic field controlled MMPs still needs to be further improved. The limited targeting is that the magnetic field is localized to local organ levels with limited fineness and can further bind to specific ligands to increase the targeting of magnetic nanoparticles at the cellular level[51].

Carbon Nanotubes (CNTs)

CNTs are a kind of nano-material with cylindrical structure, which can be separated into single-walled nanotubes (SWNTs) and Multi-walled nanotubes (MWNTs), which is a good gene therapy drug carrier. In the targeted drug delivery studies, CNTs can bind with specific ligands such as angiopep-2 to achieve target therapy[52], and can also bind other ligands and fluorescent markers to achieve a simultaneous diagnosis and treatment[53]. In addition, studies have found that with the addition of the glioma inhibitor, CNT can significantly enhance the ability of inflammatory cells (such as macrophages) to uptake CpG oligodeoxynucleotide and activate the intracellular receptor TLR9, and then cause a series of immune responses such as increased NK cells, more effectively inhibit tumor growth[54]. Ouyang *et al.*[55] also found that CNTs combined with TMZ chemotherapy had better anti-tumor effects.

In addition, CNT-Mediated Thermal Therapy (CNMTT) is also a research hotspot in recent years. The principle is that carbon nanotubes can convert electromagnetic radiation (such as internal infrared rays) into heat and cause apoptosis. SWNTs and MWNTs are both used in thermotherapy of the tumor, and MWNs is more commonly used[56]. However, the applica-

tion of CNMTT not only need to fully consider the precise mode of administration, CNT structural characteristics, and physical/chemical properties; but also to evaluate its cytotoxic effect on tumor cells and cytogenetic and acquired heat-resistant treatment[57,58].

At present, the difficulty of CNMTT application is mainly on how to accurately administer the drug and overcome the tumor cell self-protection mechanism - heat shock reaction (HSR). To achieve local administration without damage to other intracranial tissue, the intracranial catheter can be used for External Collection Devices (ECD), but because the ECD method is still in the study, there is not enough data to prove its feasibility in CNMTT while the evaluation of HSR is mainly based on the expression of heat shock protein. Eldridge *et al.*[57] used the transient heating method to induce stress in the cells to simulate acquired heat resistance and found that PEG phospholipids encapsulated MWNTs did not induce cell production of HSR. TMZ chemotherapy is commonly used in patients with glioma, and whether TMZ will affect the acquired heat resistance of tumor cells needs further analysis[57].

In addition, gold nanoparticles can also be used for thermotherapy; the basic principle is similar to carbon nanotubes. In comparison, carbon nanotubes have the wider absorption spectrum, higher energy efficiency[59], and less temperature distortion[60], and the potential for thermotherapy is much greater than that of gold nanoparticles.

Application of Nanotechnology in the Diagnosis and Imaging of Brain Glioma

Brain glioma, especially GBM, shows strong aggressiveness, rapid development, which makes the early clinical diagnosis and treatment very important. MRI is a fundamental and sensitive method to diagnose a brain tumor, but its sensitivity highly relies on the quality of contrast agents[61]. Conventional contrast agent has several problems such as low contrast and high toxicity, while the imaging technology is closely related to diagnostic accuracy.

In MRI imaging, the common nanomaterial is a nano contrast agent and common nanocarrier[61]. The nano contrast agent is a superparamagnetic iron oxide (SPIO), including ferumoxides (Feridex in the USA, Enodorem in Europe) with a particle size of 120 to 180 nm, and ferucarbotran (Resovist) with a particle size of about 60nm, which are clinically approved and on the clinical trial[62]. In addition, other than SPIO, carbon nanotubes as nano contrast agent still under clinical trial[63]. For carbon nanotubes, as conventional nanocarrier of contrast agent, it contains toxic substance gadolinium. Therefore, the PS80 nanoparticle[64], PAMAM dendrimers molecule[61] can be used as a carrier to lower the toxicity. There are studies have shown that human glioblastoma will continue to shed some specific cells into the blood of microbubbles, so antibody can be used to label cell microbubbles and mark the magnetic nano-cell microbubbles. Micronuclear magnetic resonance (μNMR) detection can also mark cell signal strength and sensitively reflect the blood cell microbubble level, which provides a promising glioma prospective to glioma diagnosis and treatment[65].

Conclusion and Prospect

Due to the high aggressiveness of glioma to BBB and BBTB, the progression of glioma is complicated, which brings the great challenge to the treatment. Compare to conventional drugs, the advantages of nano-agent are highly specific to the targets, and low toxicity. On the one hand, the microenvironment of glioma is very complex, it can interact with the nano-agent to form a protein crown, resulting in the off-target of the drug. What's more, glioma is highly heterogeneous, and cannot be simulated by the in-*vitro* study. On the other hand, glioma cells express diverse cell surface protein, so it is important to select appropriate ligands. At the same time, the application of nano technology in the diagnosis and treatment of glioma began relatively late. Although the animal study

of EPR effect broadens the horizon of diagnosis and treatment. However, with the failure of more and more nano-agent on the clinical trial, we found EPR effect is highly heterogeneous in human. Hopefully, in the future, there will be better clinical glioma model to boost the development of nanotechnology in glioma treatment.

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