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Original Article

Effects of gene polymorphisms in the endoplasmic reticulum stress pathway on clinical outcomes of chemoradiotherapy in Chinese patients with nasopharyngeal carcinoma

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Abstract

There is considerable inter-individual variability in chemoradiotherapy responses in nasopharyngeal carcinoma (NPC) patients receiving the same or similar treatment protocols. In this study we evaluated the association between the gene polymorphisms in endoplasmic reticulum (ER) stress pathway and chemoradiation responses in Chinese NPC patients. A total of 150 patients with histopathologically conformed NPC and treated with concurrent chemoradiotherapy were enrolled. Genotypes in ER stress pathway genes, including VCP (valosin-containing protein) rs2074549, HSP90B1 rs17034943, CANX (calnexin) rs7566, HSPA5 [heat shock protein family A (Hsp70) member 5] rs430397, CALCR (calcitonin receptor) rs2528521, and XBP1 (X-box binding protein 1) rs2269577 were analyzed by Sequenom MassARRAY system. The short-term effects of primary tumor and lymph node after radiotherapy were assessed based on the Response Evaluation Criteria in Solid Tumors (RECIST) of WHO. And acute radiation-induced toxic reactions were evaluated according to the Radiation Therapy Oncology Group or European Organization for Research and Treatment of Cancer (RTOG/EORTC). The effects of gene polymorphisms on clinical outcomes of chemoradiotherapy were assessed by chi-square test, univariate and multivariate logistic regression analyses. We found that CT and CT+CC genotypes of CANX rs7566 was significantly correlated with primary tumor treatment efficacy at 3 months after chemoradiotherapy and with occurrence of radiation-induced myelosuppression in Chinese NPC patients. CT and CT+CC genotypes of CALCR rs2528521 were significantly correlated with cervical lymph node efficacy at 3 months after chemoradiotherapy. And CC and CT+CC genotypes of VCP rs2074549 were significantly associated with occurrence of myelosuppression. In conclusion, SNPs of VCP rs2074549, CANX rs7566 and CALCR rs2528521 in ER stress pathway genes may serve as predictors for clinical outcomes of chemoradiotherapy in Chinese NPC patients.

Keywords: nasopharyngeal carcinoma; chemoradiotherapy; endoplasmic reticulum stress pathway; calnexin; calcitonin receptor; SNPs; valosin-containing protein; short-term effect; toxicity reaction

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Introduction

Nasopharyngeal carcinoma (NPC) is a head and neck epithelial malignant tumor that is located on the surface of the nasopharynx and has distinct ethnic and regional characteristics. The incidence of NPC is much greater in southeast Asia and southern China than in the United States[1]. In high-prevalence areas, such as southern China, 90%–95% of NPC cases are classified as nonkeratinized undifferentiated carcinoma[2]. Owing to its pathological features and the radiosensitivity of NPC,

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radiotherapy has been a major modality in the treatment of NPC[3]. Concurrent chemoradiotherapy (CCRT) either with or without induction (neoadjuvant) chemotherapy is more beneficial than radiotherapy alone for the treatment of advanced NPC patients[4–6].

There is little doubt that chemoradiotherapy improves treatment outcomes and reduces recurrence and death rates. However, there is also considerable inter-individual variability in chemoradiotherapy reactions in tumor patients receiving the same or similar treatment protocols[7, 8]. Because TNM stage is the main criterion for predicting prognosis in NPC patients, patients with the same TNM stage still exhibit variable efficacy and toxic effects, owing to heterogeneity among the tumors and the hosts[9]. Xerostomia, myelosuppression, dermatitis and oral mucositis are common side effects induced by chemoradiotherapy. Myelosuppression occurs with leucopenia, neutropenia, anemia and thrombocytopenia, and sometimes is even life threatening[10]. In addition to the clinical and demographic factors, genetic background plays an important role in inter-individual variations in response to chemoradiotherapy[11–13].

Protein synthesis and folding, intracellular calcium levels, and lipid and sterol synthesis are all regulated by the endoplasmic reticulum (ER)[14]. ER stress may be activated by perturbation of the tumor microenvironment by factors such as hypoxia, pH changes, and oxidative stress induced by both chemotherapy and radiotherapy[15, 16]. ER stress is also involved in cancer cell proliferation, cytoprotective autophagy, apoptosis, angiogenesis, and the inflammatory response mediated by the unfolded protein response (UPR), thereby resulting in cancer development and metastases[17]. Functional polymorphisms in ER stress pathway related genes, especially single nucleotide polymorphisms (SNPs) in the coding regions, may affect the expression or activity of proteins. The expression level of glucose-regulated protein of molecular mass 78 (GRP78, a major ER chaperone) has prognostic value in neoadjuvant chemoradiotherapy and laparoscopic surgery for locally advanced rectal cancer[16]. Peng *et al* have found that the G/G genotype at rs2269577, compared with the homozygous C/C genotype, is significantly associated with severe gastrointestinal toxicity in advanced non-small cell lung cancer (NSCLC) patients undergoing platinum-based chemotherapy[18]. The SNP rs2074549 in the valosin-containing protein (VCP) gene is significantly associated with severe neutropenia in Chinese advanced NSCLC patients on platinum-based regimens[19]. As a molecular folding chaperone responding to ER stress, heat shock protein 90 beta family member 1 (HSP90B1) has been implicated in chemoresistance and radioresistance and often is an anticancer therapeutic target[20, 21]. These findings suggest that the ER stress pathway may play an important role in cancer development and metastases. However, there is a lack of direct clinical evidence of the relationship between ER stress gene SNPs and chemoradiotherapy efficacy or toxic reactions, thus leading to difficulties in determining which genes are risk factors.

In this study, we speculated that SNPs in ER stress pathway genes might be predictive factors for chemoradiotherapy efficacy and toxic reactions in NPC patients and may account for the inter-patient heterogeneity in clinical efficacy and toxic reactions. Thus, we explored the association between SNPs in ER stress response-related genes, including VCP (valosin-containing protein) rs2074549, HSP90B1 rs17034943, CANX (calnexin) rs7566, HSPA5 (heat shock protein family A (Hsp70) member 5) rs430397, CALCR (calcitonin receptor) rs2528521, and XBP1 (X-box binding protein 1) rs2269577, and clinical efficacy and toxic reactions.

Materials and methods

Study population

This study was approved by the Ethics Committee of Jiangxi Cancer Hospital (China) and was registered on the official website of the Chinese Clinical Trial Registry (www.chictr.org, registration number: ChiCTR-OPC-14005257). Between April 2014 and September 2015, a total of 150 patients with histopathologically confirmed NPC were enrolled at the Radiology Department of Jiangxi Cancer Hospital, China. Only patients whose performance status (PS) scores were ≤1.0 without severe heart, lung, liver or kidney dysfunction and whose lesions were measurable according to the WTO evaluation criteria, were eligible for this study. The other inclusion criteria included no anti-tumor treatment prior to chemoradiotherapy and the completion of radical treatment. The exclusion criteria included pregnancy or lactation, active infection before treatment, history of cancer, or complication with other tumors. All patients provided signed informed consent. Basal demographic and clinical information was collected.

Treatment regimen

All patients received intensity-modulated radiotherapy (IMRT) with 6-MV photons at a dose of 2 Gy/d, 5 times weekly. The doses were prescribed on the basis of the gross tumor volume (GTV), clinical target volume (CTV), and planning target volume (PTV). The accumulated doses for nasopharyngeal primary lesions and positive cervical lymph nodes were 66–77 Gy and 54–60 Gy, respectively, and were applied to the cervical prevention area in 30–33 fractions.

All patients received concurrent chemoradiotherapy (CRT). Platinum-based chemoradiotherapy was used as induction chemotherapy and adjuvant chemotherapy.

Evaluation of treatment efficacy and toxic reactions

On the basis of the Response Evaluation Criteria In Solid Tumors (RECIST) of the World Health Organization (WHO), the short-term effects on the primary tumor and lymph node after RT were evaluated with computed tomography (CT) and magnetic resonance imaging (MRI) immediately and 3 months after RT. The RECIST definition of efficacy included complete remission (CR), partial remission (PR), stable disease (SD) and progression disease (PD). In this study, for specific comparison with the CR group, the PR, SD and PD groups were consolidated into a single non-CR group.

Acute radiation-induced toxic reactions were evaluated

according to the guidelines of the Radiation Therapy Oncology Group or European Organization for Research and Treatment of Cancer (RTOG/EORTC). In this study, acute toxic reactions of xerostomia, myelosuppression, dermatitis and oral mucositis were evaluated. On the basis of the RTOG/EORTC scores of the toxic reactions, the patients were further divided into subgroups. Patients with xerostomia of grade 2 or higher (grades 2–3) were defined as the chemoradiation sensitive subgroup, whereas those with mild toxicity (grade 1) were classified as the chemoradiation insensitive subgroup. For myelosuppression, patients were subdivided into the grade 0–2 (grades 0, 1, and 2) group and the grade 3+ (grades 3 and 4) group. For dermatitis, patients were subdivided into a grade 1–2 group and a grade 3+ (grades 3 and 4) group. For oral mucositis, patients were subdivided into a grade 0–2 (grades 0, 1, and 2) group and a grade 3+ (grades 3 and 4) group.

Genotyping assay

Peripheral blood samples (3 mL) were collected for genotyping before radiotherapy and were stored at -20 °C in EDTA anticoagulant tubes. DNA was extracted using a Wizard Genomic DNA Purification Kit (Promega, Madison, USA), per the instructions. Polymorphisms in 6 genes were analyzed with a Sequenom MassARRAY system (BioMiao Biological, Beijing, China).

Statistical analysis

All data were analyzed with SPSS 19.0 software. All statistical tests were two-sided. *P* values <0.05 were considered to be statistically significant. Descriptive statistics were used to report the demographics and treatment characteristics. Chi-square tests were performed to evaluate the effect of clinical factors on the short-term treatment efficacy (including primary tumor efficacy and cervical lymph node efficacy) and the risk of occurrence of xerostomia, myelosuppression, dermatitis and oral mucositis. The association between polymorphisms in ER stress pathway genes and short-term efficacy as well as the risk of acute toxic reactions was evaluated by estimating the odds ratios (ORs) and 95% confidence intervals (CIs) through univariate and multivariate logistic regression analyses. Multivariate analyses were adjusted for age, sex, drinking, smoking, family history, body mass index (BMI), TNM stage and clinical stage.

Results

Population characteristics and clinical outcome

A total of 150 NPC patients with a defined pathological diagnosis of non-keratinized nasopharyngeal carcinoma (NPC) were recruited. There were 100 males and 50 females with a mean age of 49.56 years (ranging from 14–71 years). On the basis of the clinical criteria of the 7th Union for International Cancer Control (UICC) staging standard, there were no NPC patients at stage I, 12 patients at stage II, 67 patients at stage III, and 71 patients at stage IV. Their demographics and treatment characteristics are summarized in Table 1. Epstein-Barr virus (pEBV) was detectable in the plasma of 109 patients (72.7%; pEBV-positive population). However, the pEBV-positive rate after CRT decreased to 2.2%.

Table 1. Baseline demographics and clinical characteristics.

Characteristics	Number of patients (%) (*n*=150)
Age at diagnosis (mean±SD)	49.56±11.00
Gender	
Male	100 (66.7)
Female	50 (33.3)
Drinking	
Yes	38 (25.3)
No	112 (74.7)
Smoking	
Yes	67 (44.7)
No	83 (55.3)
Family history of tumor	
Yes	19 (12.7)
No	131 (87.3)
BMI	
≤24.0	98 (65.3)
>24.0	52 (34.7)
Tumor size	
TI–T2	36 (24.0)
T3–T4	114 (76.0)
Lymph node metastasis	
N0–N1	68 (45.3)
N2–N3	82 (54.7)
Distant metastasis	
M0	145 (96.7)
M1	5 (3.3)
Clinical stage	
I–II	12 (8.0)
III–IV	138 (92.0)
EBV-DNA	
Positive	109 (72.7)
Negative	41 (27.3)

SD, standard deviation; BMI, body mass index; EBV-DNA, plasma Epstein-Barr virus (EBV) DNA. TNM staging is based on Clinical stage 7th AJCC/UICC staging system.

The associations between population characteristics and short-term effects in NPC patients immediately after and 3 months after CRT are listed separately in Table S1 (supplementary materials) and Table 2. We primarily evaluated primary tumor efficacy and cervical lymph node efficacy in NPC

Table 2. Association of clinical factors and the short term treatment effect 3 months after CRT.

Clinical characteristics	Primary tumor efficacy (*n*=110)			Cervical lymph node efficacy (*n*=99)		
	CR	Non-CR	*P*	CR	Non-CR	*P*
Age (year)			0.786			0.828
≤60	87	9		78	9	
>60	13	1		11	1	
Gender			0.799			0.691
Male	66	7		59	6	
Female	34	3		30	4	
Drinking			0.777			0.075
Yes	24	2		22	0	
No	76	8		67	10	
Smoking			0.626			0.978
Yes	42	5		36	4	
No	58	5		53	6	
Family history			0.171			0.176
Yes	16	0		14	0	
No	84	10		75	10	
BMI			0.751			0.746
≤24.0	65	7		58	6	
>24.0	35	3		31	4	
Tumor size			0.314			0.593
T1–T2	24	1		20	3	
T3–T4	76	9		69	7	
Lymph node metastasis			0.176			0.814
N0–N1	42	2		30	3	
N2–N3	58	8		59	7	
Distant metastasis			0.260			0.494
M0	97	9		85	10	
M1	3	1		4	0	
Clinical stage			0.826			0.703
I–II	8	1		6	1	
III–IV	92	9		83	9	

CRT, chemoradiotherapy; CR, complete remission; Non-CR, not complete remission; BMI, body mass index.

patients. For short-term effects immediately after CRT, the distribution of primary tumor efficacy was significantly different in the two tumor-size groups (T1–T2 group versus the T3–T4 group; *P*=0.036) in that the T3–T4 group showed poorer efficacy than the T1–T2 group (OR: 3.581; 95% CI: 1.019–12.582; *P*=0.047). The association between cervical lymph node efficacy and stages of lymph node metastasis was nearly significant (OR: 3.358; 95% CI: 0.919–12.272; *P*=0.067; N2–N3 *vs* N0–N1). There was no correlation between the short-term effect 3 months after CRT and clinical factors.

When assessing the associations between clinical factors and the risk of occurrence of toxic reactions such as xerostomia, myelosuppression, dermatitis and oral mucositis, we found that a family history of NPC was the most relevant risk factor for xerostomia (*P*=0.008), and patients who had a family history of NPC were at lower risk of developing grade 2+ xerostomia (OR: 0.239; 95% CI: 0.078–0.735; *P*=0.013). The results are shown in Table 3. Additionally, compared with patients with a body mass index (BMI)≤24.0, patients with a BMI>24.0 had a lower risk of developing grade 3+ oral mucositis (OR: 0.431; 95% CI: 0.215–0.865; *P*=0.018), as shown in Table S2.

Relationship between gene polymorphisms in the ER stress response pathway and short-term effects in NPC patients

As shown in Tables S3 and S4, there were no statistically significant associations between SNPs and primary tumor efficacy or cervical lymph node efficacy immediately after chemoradiotherapy in either the univariate analysis or the multivariate analysis.

The CT and CT+TT genotypes of rs7566 in the CANX gene were significantly associated with better primary tumor efficacy 3 months after CRT, as compared with the CC genotype (CT OR: 0.133; 95% CI: 0.020–0.886; *P*=0.037 and CT+TT OR: 0.122; 95% CI: 0.018–0.815; *P*=0.030). The CT and CT+CC genotypes of rs2528521 in the CALCR gene were significant factors predicting cervical lymph node efficacy 3 months after CRT in NPC patients (CT OR: 15.294; 95% CI: 1.625–143.976; *P*=0.017 and CT+CC OR: 13.478; 95% CI: 1.470–123.575; *P*=0.021). The results are listed in Tables 4 and 5.

Relationship between gene polymorphisms in the ER stress response pathway and the risk of toxicity

The associations between genotypes of ER stress response pathway genes and xerostomia and myelosuppression are shown in Tables 6 and 7. The results showed that the CC and CT+CC genotypes of VCP rs2074549 were associated with a significantly increased risk of developing grade 3+ myelosuppression, by using the TT genotype as a reference (CC OR: 3.040; 95% CI: 1.336–6.914; *P*=0.008 and CT+CC OR: 2.768; 95% CI: 1.243–6.162; *P*=0.013). A significant association was detected between the CT and CT+TT genotypes of CANX rs7566, compared with the CC genotype, and there was an increased risk of occurrence of grade 3+ myelosuppression (CT OR: 2.459; 95% CI: 1.096–5.518; *P*=0.029 and CT+TT OR: 2.382; 95% CI: 1.112–5.104; *P*=0.026). There was no association between the selected SNPs and the risk of occurrence of xerostomia. As shown in Table S5, no relationship was found between SNPs in ER stress response pathway genes and the risk of dermatitis. We identified only a borderline significant association between the CT genotype of rs2074549 in the VCP gene and the risk of developing grade 3+ oral mucositis (OR: 3.939; 95% CI: 0.976–15.902; *P*=0.054), as shown in Table S6.

Table 3. Association of clinical factors and the risk of xerostomia and myelosuppression after CRT.

Clinical characteristics	Xerostomia (*n*=150) Grade 1	Grade 2+	*P*	Myelosuppression (*n*=150) Grade 0–2	Grade 3+	*P*
Age (year)			0.583			0.936
≤60	110	15		89	36	
>60	21	4		18	7	
Gender			0.728			0.523
Male	88	12		73	27	
Female	43	7		34	16	
Drinking			0.646			0.711
Yes	34	4		28	10	
No	97	15		79	33	
Smoking			0.810			0.244
Yes	59	8		51	16	
No	72	11		56	27	
Family history			**0.008***			0.764
Yes	13	6		13	6	
No	118	13		94	37	
BMI			0.831			0.270
≤24.0	86	12		67	31	
>24.0	45	7		40	12	
Tumor size			0.748			0.160
T1–T2	32	4		29	7	
T3–T4	99	15		78	36	
Lymph node metastasis			0.239			0.585
N0–N1	57	11		47	21	
N2–N3	74	8		60	22	
Distant metastasis			0.386			0.663
M0	126	19		103	42	
M1	5	0		4	1	
Clinical stage			0.664			0.709
I–II	10	2		8	4	
III–IV	121	17		99	39	

CRT, chemoradiotherapy; CR, complete remission; Non-CR, not complete remission; BMI, body mass index. *P<0.05 was shown in bold.

Stratification analyses of patients at tumor stages T3 and T4

To evaluate the effects of SNPs in ER stress pathway genes on clinical efficacy and toxic reactions of patients at only tumor stages T3 and T4, we performed additional stratification analyses. As shown in Table 8, the CT and CT+TT genotypes of rs7566 in the CANX gene were significantly associated with better primary tumor efficacy 3 months after CRT in NPC patients at stages T3 and T4 only (CT OR: 0.112; 95% CI: 0.013–0.941; P=0.044 and CT+TT OR: 0.101; 95% CI: 0.012–0.849; P=0.035). Regarding the myelosuppression in patients at only the T3 and T4 stages, we obtained similar results: the CC and CT+CC genotypes of VCP rs2074549 and the CT and CT+TT genotypes of CANX rs7566 were significantly associated with increased risk of grade 3+ myelosuppression (Table 9). In addition, the association was more significant for the entire patient population.

Discussion

In this study, the effects of 6 SNPs in ER stress pathway genes on the chemoradiotherapy responses in NPC patients were evaluated. Our data demonstrated that the T allele of CANX rs7566 was a favorable factor related to primary tumor treatment efficacy 3 months after CRT, but it was also significantly associated with the development of grade 3+ myelosuppression in Chinese NPC patients. The C allele of CALCR rs2528521 was significantly correlated with cervical lymph node efficacy 3 months after CRT. The C allele of VCP rs2074549 was significantly associated with the occurrence of 3+ myelosuppression. To our knowledge, this is the first study to investigate the effects of polymorphisms in ER stress pathway genes on chemoradiotherapy reactions in NPC patients.

Through correlation analysis of population characteristics and clinical outcomes, NPCs classified as T3 and T4 showed poorer primary tumor efficacy after chemoradiotherapy than those classified as T1 and T2. TNM stage is a major prognostic predictor in NPC patients, and patients classified as T3 and T4 often have a poor prognosis[22]. Additionally, patients with a family history of NPC tended to have a lower risk of developing grade 2+ xerostomia as well as an improved survival rate[23]. Thus, it was surmised that patients with a family history of xerostomia might be more tolerant than those without this family history. Our results revealed that patients with a BMI >24.0 were at lower risk of developing grade 3+ oral mucositis. Pretreatment BMI was a significant independent prognostic factor for patients with NPC. Compared with their low-BMI counterparts, high-BMI patients have better 5-year failure-free survival rates (FFS)[24]. High-BMI patients may be more tolerant of oral mucositis than low-BMI patients. However, the underlying mechanism remains unknown, and further studies are wanted.

Valosin-containing protein (VCP) is a conserved structural protein in eukaryotes that belongs to the ATPase family. The expression of VCP has been found to be related to the pathological state and clinical outcome of human tumors such as childhood acute lymphoblastic leukemia[25]. The results from our study indicated that the C allele of VCP rs2074549 was significantly associated with the risk of occurrence of grade 3+ myelosuppression directly after CRT in NPC patients (P=0.008), particularly in the subgroup of patients at stage T3 and T4 (P=0.003). Rs2074549 is located in the intronic region of the VCP gene, and mutations at this SNP may influence the expression of VCP by affecting mRNA splicing, localization and stability[26]. Another conceivable mechanism is that the real functional variant mediating this process is not SNP

Table 4. Association of the selected SNPs with primary tumor efficacy 3 months after CRT.

		CR		Non-CR		Crude	Adjusted[a]
	Genotypes	*n*	f (%)	*n*	f (%)	OR, 95% CI, *P*	OR, 95% CI, *P*
VCP (rs2074549)	TT	48	48.0	4	40.0	1	1
	CT	8	8.0	1	10.0	1.500, 0.148–15.197, 0.731	2.165, 0.146–32.152, 0.575
	CC	44	44.0	5	50.0	1.364, 0.344–5.404, 0.659	1.499, 0.280–8.024, 0.636
	CT+CC	52	52.0	6	60.0	1.385, 0.368–5.207, 0.630	1.603, 0.322–7.976, 0.565
HSP90B1 (rs17034943)	GG	25	25.0	3	30.0	1	1
	AG	45	45.0	3	30.0	0.556, 0.104–2.961, 0.491	0.411, 0.060–2.817, 0.365
	AA	30	30.0	4	40.0	1.111, 0.227–5.439, 0.897	0.806, 0.128–5.074, 0.818
	AG+GG	75	75.0	7	70.0	0.778, 0.187–3.238, 0.730	0.575, 0.110–3.017, 0.513
CANX (rs7566)	CC	48	48.0	8	80.0	1	1
	CT	48	48.0	2	20.0	0.250, 0.050–1.239, 0.090	0.133, 0.020–0.886, **0.037***
	TT	4	4.0	0	0.0	NC	NC
	CT+TT	52	52.0	2	20.0	0.231, 0.047–1.141, 0.072	0.122, 0.018–0.815, **0.030***
HSPA5 (rs430397)	CC	72	72.0	10	100.0	1	1
	CT	27	27.0	0	0.0	NC	NC
	TT	1	1.0	0	0.0	NC	NC
	CT+TT	28	28.0	0	0.0	NC	NC
CALCR (rs2528521)	TT	45	45.0	5	50.0	1	1
	CT	39	39.0	5	50.0	1.154, 0.311–4.283, 0.831	1.328, 0.311–5.671, 0.702
	CC	16	16.0	0	0.0	NC	NC
	CT+CC	55	55.0	5	50.0	0.818, 0.223–3.004, 0.762	0.860, 0.207–3.568, 0.835
XBP1 (rs2269577)	CC	50	50.0	2	20.0	1	1
	GC	40	40.0	7	70.0	4.375, 0.861–22.230, 0.075	4.425, 0.692–28.288, 0.116
	GG	10	10.0	1	10.0	2.500, 0.206–30.293, 0.472	2.051, 0.119–35.279, 0.621
	GC+GG	50	50.0	8	80.0	4.000, 0.809–19.779, 0.089	3.830, 0.635–23.086, 0.143

CR, complete remission; Non-CR, not complete remission. *$P<0.05$ was shown in bold.

rs2074549 but other polymorphisms in linkage disequilibrium (LD) with this SNP[27]. This SNP is significantly associated with severe neutropenia[19]. VCP is supposedly involved in the dissociation and proteasomal degradation of I-κB, an inhibitor of nuclear factor-kappaB (NF-κB)[28]. Interestingly, the NF-κB pathway is related to neutrophil apoptosis[29, 30], but its detailed mechanism requires further study. Variation in this SNP is probably related to the regulation of neutrophil apoptosis, thus leading to myelosuppression. However, there was no association between rs2074549 and treatment efficacy in NPC patients.

Calcitonin receptor (CALCR), one of seven transmembrane-spanning G protein–coupled receptors, is involved in the maintenance of calcium homeostasis and the regulation of bone resorption. The C1377T polymorphism in the CALCR gene has been found to be associated with bone mineral density (BMID) in the lumbar spine in a postmenopausal Han Chinese population[31]. Furthermore, the CT and CT+CC genotypes at rs2528521 were significantly correlated with cervical lymph node efficacy 3 months after CRT both in the whole population and in patients at stages T3 and T4. Rs2528521 is a C/T single-nucleotide variation located in the promoter region of the CALCR gene. SNPs located within promoters might influence overall protein activity or gene expression[32]. Few studies have examined the association between CALCR gene variations and disease, and the relationship between CALCR gene variations and toxic reaction in response to CRT has not been reported. An elevated intracellular calcium level may induce cell apoptosis[33, 34]. We speculate that functional mutation of this SNP may decrease the intracellular concentration of Ca^{2+}, thereby leading to cervical lymph node cell survival.

We also analyzed the relationship between CANX (calnexin) gene polymorphisms and clinical outcomes of chemoradiation in NPC patients. Our data indicated that the C/T single-nucleotide variation in CANX rs7566 is a favorable prognostic factor 3 months after CRT, and it was significantly associated with the development of chemoradiotherapy-induced myelosuppression in Chinese NPC patients. Stratification analyses of NPC patients at stages T3 and T4 yielded similar results regarding the risk of myelosuppression. As a calcium-binding molecular chaperone of ER, CANX interacts with newly synthesized N-linked glycoproteins in a timely manner and thus plays an important role in protein synthesis and folding[35]. Accumulating evidence indicates that CANX is involved in apoptosis induced by ER stress[36, 37]. CANX silencing in murine neonatal cardiomyocytes induces Ca^{2+} cycling defects, ER stress, and apoptosis[38]. The over-expression of CANX provokes apoptosis[39, 40]. In addition, CANX rs7566 is located in the 3′-terminal untranslated region (3′-UTR), which can be fully or partly bound by microRNAs (miRNAs), thereby trig-

Table 5. Association of the selected SNPs with the cervical lymph node efficacy 3 months after CRT.

		CR		Non-CR		Crude	Adjusted[a]
	Genotypes	*n*	f (%)	*n*	f (%)	OR, 95% CI, *P*	OR, 95% CI, *P*
VCP (rs2074549)	TT	42	47.2	5	50.0	1	1
	CT	6	6.7	2	20.0	2.800, 0.440–17.799, 0.275	1.915, 0.259–14.158, 0.524
	CC	41	46.1	3	30.0	0.615, 0.138–2.740, 0.523	0.512, 0.098–2.660, 0.426
	CT+CC	47	52.8	5	50.0	0.894, 0.242–3.304, 0.866	0.761, 0.179–3.236, 0.711
VHSP90B1 (rs17034943)	GG	25	28.1	2	20.0	1	1
	AG	39	43.8	2	20.0	0.641, 0.085–4.848, 0.667	0.836, 0.091–7.694, 0.874
	AA	25	28.1	6	60.0	3.000, 0.552–16.317, 0.204	5.190, 0.664–40.586, 0.117
	AG+GG	64	71.9	8	80.0	1.562, 0.310–7.872, 0.589	2.131, 0.322–14.089, 0.432
CANX (rs7566)	CC	44	49.4	5	50.0	1	1
	CT	41	46.1	5	50.0	1.073, 0.289–3.980, 0.916	0.906, 0.207–3.960, 0.895
	TT	4	4.5	0	0.0	NC	NC
	CT+TT	45	50.6	5	50.0	0.978, 0.265–3.614, 0.973	0.841, 0.192–3.688, 0.818
HSPA5 (rs430397)	CC	68	76.4	9	90.0	1	1
	CT	20	22.5	1	10.0	0.378, 0.045–3.164, 0.369	0.476, 0.051–4.415, 0.514
	TT	1	1.1	0	0.0	NC	NC
	CT+TT	21	23.6	1	10.0	0.360, 0.043–3.007, 0.345	0.412, 0.045–3.776, 0.433
CALCR (rs2528521)	TT	43	48.3	1	10.0	1	1
	CT	33	37.1	8	80.0	10.424, 1.242–87.523, **0.031***	15.294, 1.625–143.976, **0.017***
	CC	13	14.6	1	10.0	3.308, 0.193–56.635, 0.409	6.602, 0.293–148.995, 0.235
	CT+CC	46	51.7	9	90.0	8.413, 1.023–69.213, **0.048***	13.478, 1.470–123.575, **0.021***
XBP1 (rs2269577)	CC	41	46.1	6	60.0	1	1
	GC	39	43.8	4	40.0	0.701, 0.184–2.674, 0.603	0.491, 0.101–2.382, 0.378
	GG	9	10.1	0	0.0	NC	NC
	GC+GG	48	53.9	4	40.0	0.569, 0.150–2.157, 0.407	0.409, 0.086–1.937, 0.260

SNPs, single nucleotide polymorphisms; CRT, chemoradiotherapy; CR, complete remission; Non-CR, not complete remission; OR, odds ratio; CI, confidence interval; NC, not calculated. *P<0.05 was shown in bold. [a]Adjusted for age, gender, dringking, smoking, family history, BIM, TNM stage and clinical stage.

gering specific mRNA cleavage or translational repression[41, 42]. Thus, we speculate that rs7566 located at the miRNA-binding sites may influence the binding ability of miRNAs and consequently the expression of target genes, thereby leading to alterations in susceptibility to CRT and increasing CRT efficacy and the risk of development of myelosuppression.

There were several limitations of this study. First, clinical evaluations of acute radiation-induced toxic reactions, were somewhat dependent on the patients' subjective feelings. For example, reporting and tolerance degree of xerostomia may vary greatly among patients. Second, patients with different CRT regimens may have different tumor responses and side effects and thus might have confounded our analyses. Third, the number of enrolled patients in this study was small, thus potentially limiting the statistical power of the correlation analysis. Nevertheless, we will verify our current findings in our upcoming study with a larger sample size.

In conclusion, we identified the VCP rs2074549, CANX rs7566 and CALCR rs2528521 SNPs as novel chemoradiation associated genes that can be regarded as predictors of clinical outcome of CRT in NPC patients. Knowledge of chemoradiation-related genes may aid in identification of targets for innovative therapies and promote individualized CRT. To our knowledge, our study is the first attempt to explore the association between ER stress pathway gene variations and clinical outcomes of chemoradiation. The underlying mechanism by which ER stress pathway genes modulate the response to chemoradiation in NPC patients requires further study.

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Author contribution

Xiao-bin GUO, Wan-le MA, and Cheng-xian GUO were responsible for the research design, data analysis and manuscript composition; Li-juan LIU, Yu-ling HUANG, and Jing WANG performed case collection, data entry and data interpretation; and Shao-jun CHEN, Li-hua HUANG, Hui WANG, and Xiang-dong PENG were responsible for verifying and analyzing the clinical data; Ji-ye YIN, Jin-gao LI, and Guo-ping YANG provided logistic assistance.

Table 6. Association of the selected SNPs with the risk of xerostomia after CRT.

		Grade 1		Grade 2+		Crude	Adjusted[a]
	Genotypes	*n*	f (%)	*n*	f (%)	OR, 95% CI, *P*	OR, 95% CI, *P*
VCP (rs2074549)	TT	42	47.2	5	50.0	1	1
	CT	6	6.7	2	20.0	2.800, 0.440–17.799, 0.275	1.915, 0.259–14.158, 0.524
	CC	41	46.1	3	30.0	0.615, 0.138–2.740, 0.523	0.512, 0.098–2.660, 0.426
	CT+CC	47	52.8	5	50.0	0.894, 0.242–3.304, 0.866	0.761, 0.179–3.236, 0.711
VCP (rs2074549)	TT	61	46.6	6	31.6	1	1
	CT	11	8.4	2	10.5	1.848, 0.330–10.367, 0.485	1.425, 0.219–9.257, 0.711
	CC	59	45.0	11	57.9	1.895, 0.659–5.455, 0.236	1.853, 0.606–5.670, 0.279
	CT+CC	70	53.4	13	68.4	1.888, 0.676–5.270, 0.225	1.777, 0.602–5.246, 0.298
HSP90B1 (rs17034943)	GG	30	22.9	8	42.1	1	1
	AG	59	45.0	8	42.1	0.508, 0.174–1.488, 0.217	0.456, 0.146–1.425, 0.177
	AA	42	32.1	3	15.8	0.268, 0.066–1.094, 0.067	0.293, 0.068–1.269, 0.101
	AG+GG	101	77.1	11	57.9	0.408, 0.151–1.108, 0.079	0.394, 0.137–1.127, 0.082
CANX (rs7566)	CC	63	48.1	12	63.2	1	1
	CT	55	42.0	6	31.6	0.573, 0.202–1.628, 0.296	0.648, 0.214–1.961, 0.442
	TT	13	9.9	1	5.3	0.404, 0.048–3.383, 0.403	0.299, 0.031–2.916, 0.299
	CT+TT	68	51.9	7	36.9	0.540, 0.200–1.459, 0.225	0.560, 0.198–1.582, 0.273
HSPA5 (rs430397)	CC	97	74.0	14	73.7	1	1
	CT	32	24.4	5	26.3	1.083, 0.362–3.241, 0.887	0.923, 0.284–3.005, 0.895
	TT	2	1.5	0	0.0	NC	NC
	CT+TT	34	25.9	5	26.3	1.019, 0.341–3.040, 0.973	0.879, 0.272–2.839, 0.830
CALCR (rs2528521)	TT	58	44.3	8	42.1	1	1
	CT	56	42.7	8	42.1	1.036, 0.364–2.949, 0.948	0.984, 0.329–2.947, 0.977
	CC	17	13.0	3	15.8	1.279, 0.305–5.361, 0.736	1.258, 0.284–5.571, 0.762
	CT+CC	73	55.7	11	57.9	1.092, 0.413–2.893, 0.859	1.050, 0.382–2.886, 0.924
XBP1 (rs2269577)	CC	56	42.7	10	52.6	1	1
	GC	64	48.9	5	26.3	0.438, 0.141–1.357, 0.152	0.453, 0.138–1.485, 0.191
	GG	11	8.4	4	21.1	2.036, 0.540–7.681, 0.294	2.850, 0.660–11.922, 0.162
	GC+GG	75	57.3	9	47.4	0.672, 0.256–1.763, 0.419	0.733, 0.267–2.016, 0.548

SNPs, single nucleotide polymorphisms; CRT, chemoradiotherapy; OR, odds ratio; CI, confidence interval; NC, not calculated. [a]Adjusted for age, gender, dringking, smoking, family history, BIM, TNM stage and clinical stage.

Supplementary information

Supplementary information is available at Acta Pharmacologica Sinica's website.

References

1 Cao SM, Simons MJ, Qian CN. The prevalence and prevention of nasopharyngeal carcinoma in China. Chin J Cancer 2011; 30: 114–9.
2 Kamran SC, Riaz N, Lee N. Nasopharyngeal carcinoma. Surg Oncol Clin North Am 2015; 24: 547–61.
3 Wang W, Feng M, Fan Z, Li J, Lang J. Clinical outcomes and prognostic factors of 695 nasopharyngeal carcinoma patients treated with intensity-modulated radiotherapy. BioMed Res Int 2014; 2014: 814948.
4 Qiu WZ, Huang PY, Shi JL, Xia HQ, Zhao C, Cao KJ. Neoadjuvant chemotherapy plus intensity-modulated radiotherapy versus concurrent chemoradiotherapy plus adjuvant chemotherapy for the treatment of locoregionally advanced nasopharyngeal carcinoma: a retrospective controlled study. Chin J Cancer 2016; 35: 2.
5 Zhang S, Lin S, Hu L. Lobaplatin combined with docetaxel neoadjuvant chemotherapy followed by concurrent lobaplatin with intensity-modulated radiotherapy increases the survival of patients with high-risk lymph node positive nasopharyngeal carcinoma. J BUON 2016; 21: 161–7.
6 Airoldi M, Gabriele P, Gabriele AM, Garzaro M, Raimondo L, Pedani F, *et al*. Induction chemotherapy with carboplatin and taxol followed by radiotherapy and concurrent weekly carboplatin+taxol in locally advanced nasopharyngeal carcinoma. Cancer Chemother Pharmacol 2011; 67: 1027–34.
7 Liu H, Huang PY, Tang LQ, Chen QY, Zhang Y, Zhang L, *et al*. Functional polymorphisms of matrix metalloproteinase-9 and survival in patients with locoregionally advanced nasopharyngeal carcinoma treated with chemoradiotherapy. Med Oncol 2013; 30: 685.
8 Ozdemir S, Akin M, Coban Y, Yildirim C, Uzel O. Acute toxicity in nasopharyngeal carcinoma patients treated with IMRT/VMAT. Asian Pac J Cancer Prev 2015; 16: 1897–900.
9 Qin L, Wu F, Lu H, Wei B, Li G, Wang R. Tumor volume predicts survival rate of advanced nasopharyngeal carcinoma treated with concurrent chemoradiotherapy. Otolaryngol Head Neck Surg 2016; 155: 598–605.
10 Dechaphunkul T, Pruegsanusak K, Sangthawan D, Sunpaweravong P. Concurrent chemoradiotherapy with carboplatin followed by carboplatin and 5-fluorouracil in locally advanced nasopharyngeal carcinoma. Head Neck Oncol 2011; 3: 30.
11 Wang TL, Song YQ, Ren YW, Zhou BS, Wang HT, Bai L, *et al*. Dual

Table 7. Association of the selected SNPs with the risk of radiation-induced myelosuppression after CRT.

	Genotypes	Grade 0–2 *n*	Grade 0–2 f (%)	Grade 3+ *n*	Grade 3+ f (%)	Crude OR, 95% CI, *P*	Adjusted[a] OR, 95% CI, *P*
VCP (rs2074549)	TT	55	51.4	12	27.9	1	1
	CT	10	9.3	3	7.0	1.375, 0.328–5.765, 0.663	1.540, 0.348–6.812, 0.569
	CC	42	39.3	28	65.1	3.056, 1.392–6.709, **0.005***	3.040, 1.336–6.914, **0.008***
	CT+CC	52	48.6	31	72.1	2.732, 1.269–5.882, **0.010**	2.768, 1.243–6.162, **0.013**
HSP90B1 (rs17034943)	GG	28	26.2	10	23.3	1	1
	AG	53	49.5	14	32.6	0.740, 0.291–1.878, 0.526	0.716, 0.274–1.871, 0.496
	AA	26	24.3	19	44.2	2.046, 0.805–5.204, 0.133	2.072, 0.780–5.502, 0.144
	AG+GG	79	73.8	33	76.8	1.170, 0.511–2.678, 0.711	1.144, 0.486–2.696, 0.758
CANX (rs7566)	CC	60	56.1	15	34.9	1	1
	CT	38	35.5	23	53.5	2.421, 1.124–5.213, **0.024***	2.459, 1.096–5.518, **0.029***
	TT	9	8.4	5	11.6	2.222, 0.649–7.610, 0.204	2.107, 0.581–7.647, 0.257
	CT+TT	47	43.9	28	65.1	2.383, 1.144–4.966, **0.020***	2.382, 1.112–5.104, **0.026***
HSPA5 (rs430397)	CC	82	76.6	29	67.4	1	1
	CT	24	22.4	13	30.2	1.532, 0.690–3.398, 0.294	1.353, 0.583–3.137, 0.481
	TT	1	0.9	1	2.3	2.828, 0.171–46.683, 0.467	5.871, 0.266–129.419, 0.262
	CT+TT	25	23.3	14	32.5	1.583, 0.726–3.452, 0.248	1.463, 0.645–3.319, 0.363
CALCR (rs2528521)	TT	48	44.9	18	41.9	1	1
	CT	45	42.1	19	44.2	1.126, 0.525–2.413, 0.760	1.248, 0.566–2.751, 0.582
	CC	14	13.1	6	14.0	1.143, 0.381–3.430, 0.812	1.188, 0.381–3.702, 0.766
	CT+CC	59	552.	25	58.2	1.130, 0.552–2.311, 0.738	1.233, 0.590–2.578, 0.578
XBP1 (rs2269577)	CC	50	46.7	16	37.2	1	1
	GC	45	42.1	24	55.8	1.667, 0.787–3.528, 0.182	1.588, 0.735–3.433, 0.239
	GG	12	11.2	3	7.0	0.781, 0.196–3.120, 0.727	0.725, 0.174–3.025, 0.659
	GC+GG	57	53.3	27	62.8	1.480, 0.716–3.058, 0.289	1.412, 0.668–2.985, 0.366

SNPs, single nucleotide polymorphisms; CRT, chemoradiotherapy; OR, odds ratio; CI, confidence interval. **P*<0.05 was shown in bold. [a]Adjusted for age, gender, dringking, smoking, family history, BIM, TNM stage and clinical stage.

Table 8. Association between rs7566 and primary tumor efficacy 3 months after CRT for patients at T3 and T4 stage.

Genotype	CR *n*	CR f (%)	Non-CR *n*	Non-CR f (%)	OR	95% CI	*P*
CC	34	44.7	8	88.9			
CT	38	50.0	1	11.1	0.112	0.013–0.941	**0.044***
TT	4	5.3	0	0.0	NC	NC	NC
CT+TT	42	55.3	1	11.1	0.101	0.012–0.849	**0.035***

CRT, chemoradiotherapy; CR, complete remission; Non-CR, not complete remission; OR, odds ratio; CI, confidence interval; NC, not calculated. **P*<0.05 was shown in bold.

Table 9. Association between rs2074549, rs7566 and risk of myelosuppression for patients at T3 and T4 stage after CRT.

	Genotype	CR *n*	CR f (%)	Non-CR *n*	Non-CR f (%)	OR	95% CI	*P*
VCP (rs2074549)	TT	40	51.3	8	22.2			
	CT	6	7.7	2	5.6	1.677	0.284–9.797	0.572
	CC	32	41.0	26	72.2	4.062	1.621–10.181	**0.003***
	CT+CC	38	48.7	28	77.9	3.684	1.494–9.084	**0.005**
CANX (rs7566)	CC	45	57.7	11	30.6			
	CT	25	32.1	20	55.6	3.273	1.353–7.917	**0.009***
	TT	8	10.3	5	13.9	2.557	0.699–9.357	0.156
	CT+TT	33	32.4	25	69.5	3.099	1.339–7.175	**0.008***

CRT, chemoradiotherapy; CR, complete remission; Non-CR, not complete remission; OR, odds ratio; CI, confidence interval. **P*<0.05 was shown in bold.

specificity phosphatase 6 (DUSP6) polymorphism predicts prognosis of inoperable non-small cell lung cancer after chemoradiotherapy. Clin Lab 2016; 62: 301–10.
12 Chen F, He B, Yan L, Qiu Y, Lin L, Cai L. FADS1 rs174549 polymorphism may predict a favorable response to chemoradiotherapy in oral cancer patients. Int J Oral Maxillofac Surg 2017; 75: 214–20.
13 Sebio A, Salazar J, Paez D, Berenguer-Llergo A, Del Rio E, Tobena M, *et al.* EGFR ligands and DNA repair genes: genomic predictors of complete response after capecitabine-based chemoradiotherapy in locally advanced rectal cancer. Pharmacogenomics J 2015; 15: 77–83.
14 Araki K, Nagata K. Protein folding and quality control in the ER. Cold Spring Harb Perspect Biol 2012; 4: a015438.
15 Drake TM, Ritchie JE, Kanthou C, Staves JJ, Narramore R, Wyld L. Targeting the endoplasmic reticulum mediates radiation sensitivity in colorectal cancer. Exp Mol Pathol 2015; 98: 532–9.
16 Lee HY, Jung JH, Cho HM, Kim SH, Lee KM, Kim HJ, *et al.* GRP78 protein expression as prognostic values in neoadjuvant chemoradiotherapy and laparoscopic surgery for locally advanced rectal cancer. Cancer Res Treat 2015; 47: 804–12.
17 Giampietri C, Petrungaro S, Conti S, Facchiano A, Filippini A, Ziparo E. Cancer microenvironment and endoplasmic reticulum stress response. Mediators Inflamm 2015; 2015: 417281.
18 Peng J, Chen YY, Yang LX, Zhao XY, Gao ZQ, Yang J, *et al.* XBP1 promoter polymorphism modulates platinum-based chemotherapy gastrointestinal toxicity for advanced non-small cell lung cancer patients. Lung Cancer 2013; 80: 333–8.
19 Peng J, Yang LX, Zhao XY, Gao ZQ, Yang J, Wu WT, *et al.* VCP gene variation predicts outcome of advanced non-small-cell lung cancer platinum-based chemotherapy. Tumour Biol 2013; 34: 953–61.
20 Chehab M, Caza T, Skotnicki K, Landas S, Bratslavsky G, Mollapour M, *et al.* Targeting Hsp90 in urothelial carcinoma. Oncotarget 2015; 6: 8454–73.
21 Gomez-Casal R, Epperly MW, Wang H, Proia DA, Greenberger JS, Levina V. Radioresistant human lung adenocarcinoma cells that survived multiple fractions of ionizing radiation are sensitive to HSP90 inhibition. Oncotarget 2015; 6: 44306–22.
22 M'Hamdi H, Baizig NM, OE EL, M'Hamdi N, Attia Z, Gritli S, *et al.* Usefulness of IGF-1 serum levels as diagnostic marker of nasopharyngeal carcinoma. Immunobiology 2016; 221: 1304–8.
23 Ouyang PY, Su Z, Mao YP, Liang XX, Liu Q, Xie FY. Prognostic impact of family history in southern Chinese patients with undifferentiated nasopharyngeal carcinoma. Br J Cancer 2013; 109: 788–94.
24 Huang PY, Wang CT, Cao KJ, Guo X, Guo L, Mo HY, *et al.* Pretreatment body mass index as an independent prognostic factor in patients with locoregionally advanced nasopharyngeal carcinoma treated with chemoradiotherapy: findings from a randomised trial. Eur J Cancer 2013; 49: 1923–31.
25 Lauten M, Schrauder A, Kardinal C, Harbott J, Welte K, Schlegelberger B, *et al.* Unsupervised proteome analysis of human leukaemia cells identifies the Valosin-containing protein as a putative marker for glucocorticoid resistance. Leukemia 2006; 20: 820–6.
26 Ambros V, Lee RC, Lavanway A, Williams PT, Jewell D. MicroRNAs and other tiny endogenous RNAs in C. elegans. Curr Biol 2003; 13: 807–18.
27 Peng J, Yang LX, Zhao XY, Gao ZQ, Yang J, Wu WT, *et al.* VCP gene variation predicts outcome of advanced non-small-cell lung cancer platinum-based chemotherapy. Tumour Biol 2013; 34: 953–61.
28 Dai RM, Chen E, Longo DL, Gorbea CM, Li CC. Involvement of valosin-containing protein, an ATPase Co-purified with IkappaBalpha and 26 S proteasome, in ubiquitin-proteasome-mediated degradation of IkappaBalpha. J Biol Chem 1998; 273: 3562–73.
29 Choi M, Rolle S, Wellner M, Cardoso MC, Scheidereit C, Luft FC, *et al.* Inhibition of NF-kappaB by a TAT-NEMO-binding domain peptide accelerates constitutive apoptosis and abrogates LPS-delayed neutrophil apoptosis. Blood 2003; 102: 2259–67.
30 Niwa M, Hara A, Kanamori Y, Hatakeyama D, Saio M, Takami T, *et al.* Nuclear factor-kappaB activates dual inhibition sites in the regulation of tumor necrosis factor-alpha-induced neutrophil apoptosis. Eur J Pharmacol 2000; 407: 211–9.
31 Zhang H, Tao X, Wu J. Association of calcitonin receptor gene polymorphism with bone mineral density in postmenopausal Chinese women: a meta-analysis. Arch Gynecol Obstetrics 2015; 291: 165–72.
32 Mottagui-Tabar S, Faghihi MA, Mizuno Y, Engstrom PG, Lenhard B, Wasserman WW, *et al.* Identification of functional SNPs in the 5-prime flanking sequences of human genes. BMC Genomics 2005; 6: 18.
33 Muhammad SN, Mokhtar NF, Yaacob NS. 15d-PGJ2 induces apoptosis of MCF-7 and MDA-MB-231 cells via increased intracellular calcium and activation of caspases, independent of ERalpha and ERbeta. Asian Pac J Cancer Prev 2016; 17: 3223–8.
34 Yang X, Jiang J, Yang X, Han J, Zheng Q. Licochalcone A induces T24 bladder cancer cell apoptosis by increasing intracellular calcium levels. Mol Med Reports 2016; 14: 911–9.
35 Cameron PH, Chevet E, Pluquet O, Thomas DY, Bergeron JJ. Calnexin phosphorylation attenuates the release of partially misfolded alpha1-antitrypsin to the secretory pathway. J Biol Chem 2009; 284: 34570–9.
36 Guerin R, Beauregard PB, Leroux A, Rokeach LA. Calnexin regulates apoptosis induced by inositol starvation in fission yeast. PLoS One 2009; 4: e6244.
37 Delom F, Fessart D, Chevet E. Regulation of calnexin sub-cellular localization modulates endoplasmic reticulum stress-induced apoptosis in MCF-7 cells. Apoptosis 2007; 12: 293–305.
38 Bousette N, Abbasi C, Chis R, Gramolini AO. Calnexin silencing in mouse neonatal cardiomyocytes induces Ca^{2+} cycling defects, ER stress, and apoptosis. J Cell Physiol 2014; 229: 374–83.
39 Guerin R, Arseneault G, Dumont S, Rokeach LA. Calnexin is involved in apoptosis induced by endoplasmic reticulum stress in the fission yeast. Mol Biol Cell 2008; 19: 4404–20.
40 Mohan C, Lee GM. Calnexin overexpression sensitizes recombinant CHO cells to apoptosis induced by sodium butyrate treatment. Cell Stress Chaperones 2009; 14: 49–60.
41 Gu S, Rong H, Zhang G, Kang L, Yang M, Guan H. Functional SNP in 3'-UTR microRNA-binding site of ZNF350 confers risk for age-related cataract. Hum Mutat 2016; 37: 1223–30.
42 Yang P, Tang R, Zhu J, Zou L, Wu R, Zhou H, *et al.* A functional variant at miR-24 binding site in B7-H2 alters susceptibility to gastric cancer in a Chinese Han population. Mol Immunol 2013; 56: 98–103.