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Polymorphisms involved in the *miR-218-LAMB3* pathway and susceptibility of cervical cancer, a case–control study in Chinese women

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ABSTRACT

Objective. Laminin-5 is required in RAS and NF-kappaB blockade induced tumorigenesis of human squamous cell carcinoma and a marker of invasiveness in cervical lesions. MicroRNA-218 (miR-218) can target laminin-5 β3 (LAMB3), but suppressed by HPV-16 E6 protein. Therefore, we hypothesized that single nucleotide polymorphisms (SNPs) in *pri-miR-218* and *LAMB3* may individually and/or jointly contribute to cervical cancer carcinogenesis.

Methods. We identified one SNP rs11134527 located in *pri-miR-218* sequence and one SNP rs2566 in 3′ UTR of *LAMB3* and genotyped these two SNPs in a case–control study of 703 cervical cancer cases and 713 cancer-free controls in Chinese women.

Results. Logistic regression analyses showed that the *pri-miR-218* rs11134527 variant homozygote GG was associated with a decreased risk of cervical cancer compared with the AA genotype (adjusted OR = 0.72, 95% CI = 0.52–0.99), while the *LAMB3* rs2566 variant CT/TT genotypes were associated with a significantly increased risk of cervical cancer (adjusted OR = 1.57, 95% CI = 1.25–1.96), compared with the wild type CC genotype. A significant dose–response effect was observed between the number of risk alleles, rs11134527A and rs2566 T, and the risk of cervical cancer (*P* for trend = 0.0006).

Conclusion. These findings indicate that *pri-miR-218* rs11134527 and *LAMB3* rs2566 may contribute to cervical cancer carcinogenesis, and further validations in diverse populations and functional characterizations are warranted.

Introduction

Cervical cancer is the second most common cancer among women worldwide, with an estimated 493,000 new cases and 274,000 deaths in the year 2002 [1]. It is well established that Human Papillomavirus (HPV) infection is the primary cause of cervical cancer and is indeed deemed as a necessary cause for the disease [1–3]. High-risk HPV E6 and E7 oncoproteins can inactivate critical tumor suppressors, which makes the virus override cell cycle checkpoints and cause cellular transformation [2,4]. It is well characterized that the HPV oncoprotein E6 can degrade p53 through the ubiquitin pathway, and studies also showed that HPV E6/E7 oncoproteins can interact with certain proteins to modify the development of cervical cancer [2–6].

Except protein coding genes, a recent study showed that the expression of the E6 oncoprotein of the high-risk HPV-16 could reduce microRNA-218 (miR-218) expression [7]. Conversely, RNA interference of E6/E7 oncogenes in an HPV-16-positive cell-line could increase miR-218 expression [7]. MicroRNAs (miRNAs) are small noncoding RNAs that may regulate thousands of mRNA targets by binding to their 3′ untranslated regions (3′-UTR) [8], and these targets could be implicated in the regulation of almost all biological processes [9,10]. Laminin-5 β3 (LAMB3) has been verified as a transcriptional target of miR-218 [7] and the expression of LAMB3 is increased in the presence of the HPV-16 E6 oncoprotein and this effect is mediated through miR-218 [7]. Interestingly, laminin-5 is reported as a marker of invasiveness in cervical lesions [11], and is required for RAS and NF-kappaB blockade induced tumorigenesis of human squamous cell carcinoma [12,13]. A recent study also reported that secreted laminin-5 can be used by HPV virus as a transient receptor to aid the virus in the infection of basal cells that express a6b4-integrin [14]. Thus,

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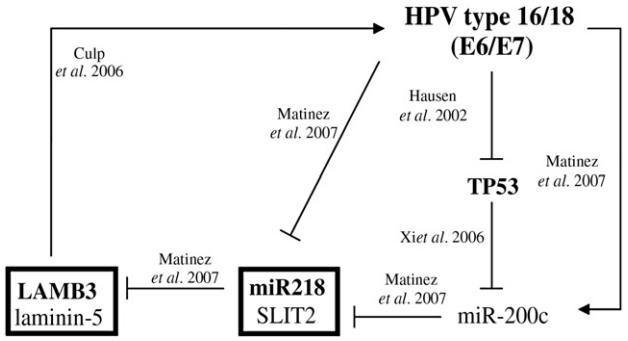

Fig. 1. The HPV-TP53-miRNA-218-LAMB3 loop.

downregulation of miR-218 by E6 and the consequent over expression of LAMB3 may promote viral infection of the surrounding tissue and eventually contribute to cervical carcinogenesis (Fig. 1).

Sequence variations in miRNA genes, including pri-miRNAs (primary miRNAs), pre-miRNAs (precursor miRNAs) and mature miRNAs, could influence the processing and/or target binding of miRNAs [15]. In our previous studies, we found that single nucleotide polymorphisms (SNPs) in pre-miRNAs may contribute to both cancer susceptibility and prognosis [16–18]. In the present study, we hypothesized that polymorphisms involved in the *miR-218-LAMB3* pathway may alter the expression of miRNA-218 or LAMB3 and/or maturation of miRNA-218, and therefore individually and/or jointly contribute to cervical cancer risk. To test the hypothesis, we performed genotyping analysis for 2 SNPs in *pri-miR-218* and *LAMB3* in a case–control study of 703 cervical cancer cases and 713 age frequency-matched cancer-free controls in Chinese women.

Materials and methods

Participants

The study was approved by the Institutional Review Board of Nanjing Medical University and the recruitment of the cases and controls was partly described previously [19]. Briefly, all of the cases and control subjects were unrelated ethnic Han Chinese women. The 703 newly diagnosed, histologically confirmed incident cervical cancer patients were consecutively recruited from the First Affiliated Hospital of Nanjing Medical University and the Nantong Tumor Hospital, Jiangsu, China between March 2006 and Mar 2009. The 713 controls were randomly selected from a pool of more than 30,000 individuals, who participated in a community-based screening program for non-infectious diseases conducted in Jiangsu Province during the same time period as the cases were recruited and had no self-reported cancer history. All of the controls were frequency-matched to the cases on age (±5 years) and residential areas (urban and rural). After informed consent was obtained, each subject was face-to-face interviewed by trained interviewers using a structured questionnaire and a 5-ml venous blood sample was collected.

SNP selection and genotyping

We firstly blasted for common (minor allele frequency, MAF>0.05) SNPs within *pri-miR-218* region and found a SNP rs11134527 with a base change A-to-G 120 bp up from the mature miR-218 sequence. Because LAMB3 is one of the binding targets of miR-218, we selected common SNPs in its 3′UTR and identified a SNP rs2566 with a base change C-to-T.

Both SNPs were genotyped by using PCR-restriction fragment length polymorphism (RFLP) assays. Mismatched T and G were introduced, respectively, into the forward primers of rs11134527 and rs2566, to replace G at −2 bp and T at −5 bp from the polymorphic sites to create *Bcl*I and *Pae*I (New England BioLabs, Beverly, MA) restriction sites. The primers were 5′-GGAGCAGGCCCCCACTGA***T***C-3′ (forward) and 5′-CCCTCCCGTTCTTCTCCCTTCC-3′ (reverse) for rs11134527, and 5′-TCCATCTCCAGGAGACTT***G***CATG-3′ (forward) and 5′-TAACTGTCCCATTGGCTCAGGC-3′ (reverse) for rs2566. The variant allele rs11134527G produces one fragment of 135 bp and the wild type allele rs11134527A generates two fragments of 119 and 16 bp. Similarly, the wild type allele rs2566C produces two fragments of 89 and 23 bp and the variant allele rs2566T results in one fragment of 112 bp.

Quality control for genotyping was described previously [19]. More than 10% of samples were randomly selected for repeating assays, yielding a 100% concordant results. The successful rates of genotyping for rs11134527 and rs2566 were 98.7% and 97.0%, respectively.

Statistical analysis

Differences in the distributions of demographic characteristics, selected variables, and genotypes of rs11134527 and rs2566 between the cases and controls were evaluated using the χ^2 test. We used logistic regression analyses to evaluate the differences in the frequency distributions of genotypes of the two polymorphisms between the cases and controls by computing the crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs). The Hardy–Weinberg equilibrium was tested by a goodness-of-fit χ^2 test to compare the observed genotype frequencies to the expected ones among the control subjects. All the statistical analyses were performed with SAS 9.1.3 software (SAS Institute, Cary, NC).

Results

Selected characteristics of the 703 cervical cancer cases and the 713 cancer-free controls were described in Table 1. As expected, there was similar distribution of age in cases and controls ($P=0.811$). However, compared with the control subjects, the cervical cancer cases had significantly lower age at menarche ($P=0.003$) and at first live birth ($P<0.0001$), had higher proportion of smokers ($P=0.001$), of premenopausal women ($P=0.015$), and of women with higher parity ($P=0.005$) and family history of cancers ($P<0.0001$). Among the 703 cases, 629 (89.5%) were squamous cell carcinoma, 50 (7.1%) adenocarcinoma, 6 (0.9%) adenosquamous carcinoma, and 13 (1.8%) others. Only 5 (0.7%) patients had CIN3, 183 (26.0%) were stage I carcinoma, 370 (52.6%) stage II, 96 (13.7%) stage III, 8 (1.1%) stage IV and 41 (5.8%) unclassified.

The genotype distributions of *pri-miR-218* rs11134527 (A/G) and *LAMB3* rs2566 (C/T) in the cases and controls were described in Table 2. The observed genotype frequencies for these SNPs in the controls were all in Hardy–Weinberg equilibrium ($P=0.568$ for rs11134527 and 0.277 for rs2566). The logistic regression analyses showed that the *pri-miR-218* rs11134527 variant homozygote GG was associated with a significantly decreased risk of cervical cancer (adjusted OR = 0.72, 95% CI = 0.52–0.99), compared with the AA genotype. The *LAMB3* rs2566 variant genotypes were associated with significantly increased risks of cervical cancer, with adjusted OR of 1.57 (95% CI = 1.25–1.99) for CT and 1.54 (95% CI = 1.07–2.23) for TT compared with the common homozygote CC. When we combined the variant genotypes CT/TT, assuming a dominant genetic model, the combined genotypes significantly increased the risk of cervical cancer by 1.57 fold (95% CI = 1.25–1.96). We then performed the combined analyses according to the number of risk alleles for the two SNPs. Cervical cancer risk was significantly increased with the increase of hazardous alleles of rs11134527A and rs2566T in a dose-dependent manner (*P* for trend: 0.0006, Table 2). Compared with the subjects carrying 0 or 1 risk allele, those with 2 to 4 risk alleles had a