Estrogen Receptor Gene Polymorphisms and Lung Adenocarcinoma Risk in Never-Smoking Women

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Introduction: The association between estrogen receptor (ER) gene polymorphism and lung cancer risk is rarely studied. This study aimed to explore the ER gene polymorphisms associated with the lung adenocarcinoma risk in never-smoking women.

Methods: This study evaluated 532 never-smoking female patients with lung adenocarcinoma and 532 healthy controls. The *ESR1* and *ESR2* single nucleotide polymorphism (SNP) data were retrieved from a genome-wide association study. Using a multivariate-adjusted logistic regression assay, the associations of *ESR1* and *ESR2* SNPs with the lung adenocarcinoma risk were estimated. Expression quantitative trait loci analysis was performed to investigate the possible functional roles of ER gene SNPs.

Results: For *ESR1*, seven tagged SNPs were identified. Among them, rs7753153 and rs985192 were associated with lung adenocarcinoma risk (rs7753153: odds ratios [OR], 1.509; 95% confidence intervals [CI], 1.168–1.950; rs985192: OR, 1.309; 95% CI, 1.001–1.712). For *ESR2*, only rs3020450 was associated with lung adenocarcinoma risk (OR, 2.110; 95% CI, 1.007–4.422). Subjects without hormone replacement therapy (HRT) use carrying at-risk genotypes had a significantly higher lung adenocarcinoma risk than subjects with HRT carrying no at-risk genotypes (rs7753153 GG: OR, 2.133; 95% CI, 1.415–3.216; rs985192 AA/AC, OR: 1.752, 95% CI: 1.109–2.768; rs3020450 AG/GG, OR: 7.162, 95% CI: 1.608–31.90). Risk genotypes of rs7753153 ($p = 0.0248$) and rs9479122 ($p = 0.0251$) were associated with decreased *ESR1* expression.

Conclusions: ER gene SNPs are associated with lung adenocarcinoma risk in never-smoking women. The joint effects of ER gene SNPs and HRT use on lung adenocarcinoma risk highlight the importance of the gene–environment interaction in lung carcinogenesis.

Key Words: Estrogen receptor, Polymorphism, Lung cancer, Hormone replacement therapy, Carcinogenesis.

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Lung cancer remains the leading cause of cancer death worldwide.[1] Previous global statistics estimate that one-fourth of lung cancer patients are never smokers, including 53% of women and 15% of men.[2] Another report in Singapore demonstrated that 32.4% of non–small-cell lung cancer patients are never smokers, with 14.6% among men and 72.8% among women.[3] In that study, the major histologic type is adenocarcinoma (69.9%), especially among women (75.0%). In Taiwan, more than 90% of women lung cancer patients were never smokers, with a high percentage of adenocarcinoma (75%).[4] Lung cancer in never smokers is a unique disease entity separate from smoking-related lung cancer. Determining the risk factors associated with lung carcinogenesis in this never-smoking population has become increasingly important.

Several studies support that estrogen could promote lung cancer proliferation, either in vitro or in vivo.[5–7] Estrogens mainly interact with two subtypes of estrogen receptors (ER), ERα[8] and ERβ,[9] to exert molecular action. In breast cancer,

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ERα expression is increased, and ERβ overexpression inhibits its estrodiol-stimulated cancer cell proliferation.[10] Such ERs were found in varying degrees in human non–small-cell lung cancer.[11–15] This indicated that ERα and ERβ may play a role in lung carcinogenesis.

ER gene polymorphisms have been reported to be associated with risks of breast cancer,[16,17] endometrial cancer,[18] and prostate cancer.[19] However, the association between ER gene polymorphism and lung cancer risk is seldom reported. A case–control study in the United States showed no association between *ESR2* haplotypes and lung cancer risk.[20] Another recent study in Taiwan found that ERα gene PvuII and XbaI polymorphisms are associated with non–small-cell lung cancer risk.[21]

To test the hypothesis that there may be an association between ER gene polymorphisms and lung carcinogenesis in never-smoking women, this case–control study was conducted, with *ESR1* and *ESR2* single nucleotide polymorphism (SNP) data retrieved from a genome-wide association study (GWAS) on lung adenocarcinoma in never-smoking woman.[22]

The gene–environment interaction may play a role in lung carcinogenesis. Our previous study demonstrated the impact of the interaction between hormone replacement therapy (HRT) and *epidermal growth factor receptor* (*EGFR*) polymorphism on lung adenocarcinoma risk,[23] based on the crosstalk between EGFR and ER pathways. Because estrogen may interact with ER more directly than with EGFR, we further examined potential joint effects of ER gene SNPs and HRT on the risk of lung adenocarcinoma.

METHODS

Study Population

This case–control study is a part of an ongoing cooperative study in Taiwan, the Genetic Epidemiological Study of Lung Adenocarcinoma (GELAC). From September 2002 to December 2009, patients were recruited from six medical centers. Never-smoking female patients with lung adenocarcinoma confirmed by pathologic or cytologic examination were enrolled. All subjects were Taiwanese and older than 18 years. Lung cancer histology was classified according to the World Health Organization criteria.[24] Patients with a previous history of cancer were excluded.

During the case recruitment period, control subjects were also recruited from the six medical centers. They were cancer-free individuals randomly selected from the health examination clinics of the same hospitals. Controls with a history of cancer were excluded. The control subjects were all never smokers and matched 1:1 to the case subjects based on age and sex. This study is approved by the institutional review board of each hospital.

Genotyping Analysis

The methods of genotyping were described in the previous GWAS.[24] Briefly, genomic DNA was extracted from blood samples, and the Illumina HumanHap610 Quad BeadChip on contract at deCODE Genetics in Iceland was used. A total of 550 cases and 549 controls were genotyped in the GWAS. Quality control metrics were performed to exclude SNPs if (1) there was a minor allele frequency less than 5%; (2) the call rate was less than 90%; and (3) SNPs had a missing rate between 0.02 and 0.1 and nonrandom genotype failure with p less than 0.02, and significant deviation from fitness from the Hardy–Weinberg equilibrium ($p < 0.0001$ in controls). To deal with the population substructure issue so as to avoid spurious association in conducting the GWAS,[22] we applied pairwise population concordance test in PLINK[25] to cluster individuals into homogeneous subsets and to identify outlying individuals (http://pngu.mgh.harvard.edu/purcell/plink/). Based on pairwise population concordance test, we found two clusters; a large cluster consisting of self-reported Han Chinese and a small cluster of 12 people consisting of mainly self-reported aborigines. The 12 subjects in the small cluster were excluded from further analysis. The remaining 532 cases and 532 controls with 457,504 SNPs passed quality control. The *ESR1* and *ESR2* SNPs passing the quality control were retrieved from the GWAS data. To define linkage disequilibrium patterns, SNP genotyping data from the 532 controls were uploaded to HaploView 4.2.[26]

Data Collection

The personal interview was conducted by a trained research nurse. All of the study participants provided written informed consent for blood sample collection and personal interview. Information on age; education level; body mass index (BMI, kg/m^2); active and passive cigarette smoking; cumulative duration of HRT and contraceptive medication; number of pregnancies, deliveries, and miscarriages; menopausal status; history of cooking fume exposure; history of oophorectomy; and family history of malignancies were collected.

The use of HRT was defined as a history of either estrogen replacement therapy or estrogen and progestin combination therapy for a cumulative duration of at least 3 months. Contraceptive use was defined as a history of contraceptive medication for a cumulative duration of at least 3 months. Subjects were defined as "ever cigarette smokers" if she smoked cigarettes regularly for more than 6 months.[27,28] Otherwise, they were defined as never smokers. Passive cigarette smoking was defined as inhalation of other people's cigarette smoke at the workplace or by living with family members who smoked.

The body weight of healthy controls was recorded upon enrollment. For the cases, their body weight was recorded according to the value while in a healthy state, so as to avoid underestimating the BMI because of cancer-related body weight loss. Cooking fume exposure was defined by continuous cooking for no less than 6 months. The sum of cooking fume exposure (cooking time-years) was calculated by multiplying cooking intensity (times per day) by the duration of cooking (years). A family history of breast, ovary, cervix, or endometrial cancer in first-degree female relatives was recorded.

Statistical Analysis

Differences between the case and control groups were analyzed by Pearson's χ^2 test in variables including education; passive smoking status; HRT use; contraceptive medication;