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REVIEW

Genetic Polymorphism, Telomere Biology and Non-Small Lung Cancer Risk

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ABSTRACT

Recent genome-wide association studies (GWAS) have identified a number of chromosomal regions associated with the risk of lung cancer. Of these regions, single-nucleotide polymorphisms (SNPs), especially rs2736100 located in the telomerase reverse transcriptase (*TERT*) gene show unique and significant association with non-small cell lung cancer (NSCLC) in a few subpopulations including women, nonsmokers, East Asians and those with adenocarcinoma. Recent studies have also linked rs2736100 with a longer telomere length and lung cancer risk. In this review, we seek to summarize the relationship between these factors and to further link the underlying telomere biology to lung cancer etiology. We conclude that genetic alleles combined with environmental (e.g., less-smoking) and physiological factors (gender and age) that confer longer telomere length are strong risk factors for NSCLC. This linkage may be particularly relevant in lung adenocarcinoma driven by epidermal growth factor receptor (*EGFR*) mutations, as these mutations have also been strongly linked to female gender, less-smoking history, adenocarcinoma histology and East Asian ethnicity. By establishing this connection, a strong argument is made for further investigating of the involvement of these entities during the tumorigenesis of NSCLC.

KEYWORDS: Telomere; Non-small cell lung cancer; Cancer risk; Polymorphism; *EGFR* mutations

INTRODUCTION

Lung cancer is one of the most common cancer types worldwide in terms of incidence and mortality. Global statistic data show that lung cancer alone accounts for 13% of all newly diagnosed cancers and is responsible for 18% of all cancer-related deaths (Jemal et al., 2011). Behavioral factors such as tobacco smoking and environmental exposure are well-known risk factors of lung cancer (Clapp et al., 2008). Beyond this, genetic factors also play a crucial role in increasing susceptibility to lung cancer (Lichtenstein et al., 2000). However, the etiology of lung cancer based on different histology types is still largely unknown. It is important to find genetic factors which are essential for carcinogenesis.

The most predominant subtype of lung cancer is non-small cell lung cancer (NSCLC), which accounts for over 85% of all lung cancers (Breathnach et al., 2001) and is further classified into three main subtypes: adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma. The major risk factor of lung cancer is tobacco smoking. Exposure to tobacco accounts for 80%−90% of lung cancer cases. 10%−15% of lung cancer cases occur without tobacco smoking (Tsao, 2007; Thun et al., 2008; Longo et al., 2011). Other risk factors including exposure to asbestos (O'Reilly et al., 2007), radon gas and other forms of air pollution including second-hand smoke are also causes of lung cancer (U.S. Department of Health and Human Services, 2006; Mason et al., 2010). Although tobacco smoking is the major risk factor for lung cancer, genetic diversity also plays a big role in the etiology of lung cancer. A

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combination of genetic and environmental factors plays a critical role in determining the cause and development of lung cancer (Jin et al., 2009).

Various studies showed that age, gender and ethnicity affect the incidence of lung cancer as well. Lung cancer does not typically occur before the age of 40 but after, and lung cancer rate increase with age until 70 (Stewart and Kleihues, 2003). Over 30% of males are smokers while only 6% of females smoke (Ng et al., 2014). However, 17% of males with cancer are lung cancer patients and 9% of all female cancer patients have lung cancer (Ferlay et al., 2010). This indicates that a large cohort of female lung cancer patients have a never-smoking history. Moreover, lung cancer with never-smoking history is much more common in females, especially among East Asians and East Asian immigrants (McCredie et al., 1999; Stewart and Kleihues, 2003; McCracken et al., 2007). Evidence indicates that female lung cancer patients with never-smoking history are influenced more by genetic/familial history rather than second-hand smoking or radon gas (Subramanian and Govindan, 2007). Therefore, understanding the interactions between genetic alleles, and physiological and environmental factors involved in lung cancer carcinogenesis is crucial for the early diagnosis, and prevention of lung cancer and also for the development of therapeutic strategies.

GENETIC RISK FACTORS FOR NSCLC

Genome-wide association studies (GWASs)

GWAS is a population-level examination to identify genetic alleles associated with disease status or clinical phenotypes throughout the entire genome, rather than focusing on a specific gene. GWAS is based on a large sample size and independent replications. Since 2005, GWAS has been widely used in discovering risk alleles for various cancers in human populations. A cornucopia of GWAS focuses on lung cancer in particular. These studies have identified a number of factors that are significantly correlated with the onset and progression of lung cancer.

Table 1 summarizes the single-nucleotide polymorphisms (SNPs) in different genetic loci related to the susceptibility of NSCLC. Of the 20 GWAS studies in NSCLC, a total of 28 SNPs have been significantly associated with NSCLC risk, among which three major susceptible loci in the human genome are related to lung cancer risk. Loci 15q24-25 (Amos et al., 2008; Hung et al., 2008; Thorgeirsson et al., 2008; Wu et al., 2009), 5p15 (McKay et al., 2008; Rafnar et al., 2009; Zienolddiny et al., 2009), and 6p21 (Wang et al., 2008; Zienolddiny et al., 2009) are consistently associated with lung cancer in multiple populations. Interestingly, some of these loci have been associated with lung cancer risk only in specific ethnic groups. For instance, the 15p24-25 locus harboring nicotinic acetylcholine receptor subunit genes *CHRNA3*, *CHRNA4*, and *CHRNB5*, is only associated with lung cancer risk in Caucasians. These nicotinic acetylcholine receptor subunits are related to nicotine dependence in smokers. While present in Caucasian populations, no association between nicotinic acetylcholine receptor-encoding loci and lung cancer has been found in Asian patients. This may be partly due to the low frequency of the minor allele of these particular SNPs in East Asian populations (Shiraishi et al., 2009; Wu et al., 2009). Loci 5p15 and 6p21 have been associated with lung cancer in both East Asian (e.g., Chinese, Korean and Japanese) (Jin et al., 2009; Kohno et al., 2010; Yoon et al., 2010; Bae et al., 2012; Lan et al., 2012; Ke et al., 2013; Lu et al., 2013) and Caucasian populations (Wang et al., 2008; Zienolddiny et al., 2009; Truong et al., 2010). Beyond these three well-established loci, other regions including 3q28-29, 13q12.12, 22q12.2 and 18p11.22 have also been found to be associated with lung cancer particularly in Asian populations (Yoon et al., 2010; Hu et al., 2011; Ahn et al., 2012; Lan et al., 2012; Hu et al., 2014).

Two genes encoding telomerase reverse transcriptase (TERT) and cleft lip and palate transmembrane protein 1-like protein (CLPTM1L) located in the 5p15 locus have received the most attention and are deemed to mediate observed genotype-phenotype correlations. Of all 20 GWAS-identified SNPs, rs402710 in the *CLPTM1L* gene was identified in eight studies in both Caucasian and Asian populations, rs401681 (in *CLPTM1L* gene) was identified in five studies, while rs2736100 in the *TERT* gene was identified in 13 studies. Interestingly, the rs2736100 locus was also associated with increased risk for common cancers in many other organs, including bladder, colorectal, pancreatic cancers and glioma (Shete et al., 2009; Gago-Dominguez et al., 2011; Kinnersley et al., 2012; Campa et al., 2015), suggesting that this locus is involved in genetic susceptibility to cancer in general.

Association between *TERT* polymorphism and lung cancer

Beyond the association with lung cancer as a combined phenotype, *TERT* polymorphisms are specifically associated with a few subtypes of lung cancer. These subtypes include lung adenocarcinoma, lung cancers in women, lung cancers related to East Asian ethnicity and lung cancers related to nonsmokers. Table 2 lists studies relating the rs2736100 to each of these subpopulations.

Lung adenocarcinoma

About 50% of lung cancers are adenocarcinomas and the incidence of adenocarcinomas is increasing (Jee et al., 1998; Janssen-Heijnen and Coebergh, 2003; Liam et al., 2006; Toyoda et al., 2008; Lortet-Tieulent et al., 2014). Although all major histological types of lung cancer are associated with tobacco smoking, adenocarcinoma is the most common type in never smokers (Subramanian and Govindan, 2007; Stewart and Wild, 2014). Published data have reported that *TERT* polymorphism rs2736100 C allele is associated with an increased risk of NSCLC. A case-control study has revealed that genotype frequencies of both AC and CC were significantly elevated with NSCLC (OR = 1.18; 95% CI, 1.01−1.39; $P = 0.040$ and OR = 1.46; 95% CI, 1.19−1.78; $P < 0.001$, respectively) (Wang et al., 2014). Notably, the association was

Table 1
GWAS-identified genetic loci associated with NSCLC risk

Chromosome	Gene	SNP	Ethnicity	Number of samples (case/control)	Reference
5p15.33	*CLPTM1L*	rs402710	Caucasian	3259/4159	McKay et al., 2008
5p15.33	*TERT*	rs2736100			
15q25.1	*LOC123688*	rs8034191	Caucasian	1154/1137	Amos et al., 2008
15q25.1	*CHRNA3*	rs1051730			
6p21.33	*BAT3*	rs3117582	Caucasian	1952/1438	Wang et al., 2008
6p21.33	*MSH5*	rs3131379			
5p15.33	*CLPTM1L*	rs401681			
15q25.1	*CHRNA3*	rs1051730	Caucasian	369/440	Zienolddiny et al., 2009
15q25.1	*CHRNA3*	rs16969968			
5p15.33	*CLPTM1L*	rs402710			
6p21.33		rs4324798			
5p15.33	*TERT*	rs2736100	Asian	1121/1344	Jin et al., 2009
5p15.33	*CLPTM1L*	rs402710			
5p15.33	*TERT*	rs2736100	Caucasian	13300/19666	Landi et al., 2009
5p15.33	*TERT*	rs2736100	Asian	1004/1900	Miki et al., 2010
3q28	*TP63*	rs10937405			
3q28	*TP63*	rs4488809			
3q29	*C3orf21*	rs2131877	Asian	1425/3011	Yoon et al., 2010
3q29	*C3orf21*	rs10433328			
3q29	*C3orf21*	rs952481			
3q29	*C3orf21*	rs4677657			
5p15.33	*TERT*	rs2736100			
5p15.33	*CLPTM1L*	rs402710			
5p15.33	*CLPTM1L*	rs401681			
15q25.1	*CHRNA3*	rs16969968	Caucasian, Asian	10812/13913	Truong et al., 2010
5p15.33	*TERT*	rs2736100			
5p15.33	*CLPTM1L*	rs402710			
5p15.33	*TERT*	Rs2736100	Asian	584/585	Hsiung et al., 2010
6p21.31	*HLA-DQA1*	DQA1*03	Asian	1656/1173	Kohno et al., 2010
3q28	*TP63*	rs4488809	Asian	8559/9378	Hu et al., 2011
5p15.33	*TERT*	rs2736100			
5p15.33	*CLPTM1L*	rs4488809			
22q12.2	*MIPEP-TNFRSF19*	rs753955			
22q12.2	*MTMR3-HORMAD2-LIF*	rs17728461			
5p15.33	*TERT*	rs2736100	Asian	195/228	Chen et al., 2012
5p15.33	*CLPTM1L*	rs402710			
5p15.33	*CLPTM1L*	rs401681			
5p15.33	*TERT*	rs2736100	Asian	1094/1100	Bae et al., 2012
5p15.33	*CLPTM1L*	rs402710			
5p15.33	*CLPTM1L*	rs401681			
15q25.1		rs2036534			
15q25.1		rs6495309			
10q25.2	*VTI1A*	rs7086803	Asian	4543/5505	Lan et al., 2012

(*continued on next page*)

Table 1 (*continued*)

Chromosome	Gene	SNP	Ethnicity	Number of samples (case/control)	Reference
6q22.2	*DCBLD1-ROS1*	rs9387478			
6p21.32	*HLA-DRA*	rs2395185			
3q28	*TP63*	rs4488809			
5p15.33	*TERT*	rs2736100			
18p11.22	*FAM38B*	rs11080466	Asian	446/497	Ahn et al., 2012
18p11.22	*FAM38B*	rs11663246			
5p15.33	*TERT*				
5p15.33		rs2853677	Asian	6029/13535	Shiraishi et al., 2012
5p15.33	*TERT*	rs2736100			
3q28	*TP63*	rs10937405			
17q24.3	*BPTF*	rs7216064			
6q21.3	*BTNL2*	rs3817963			
5p15.33	*CLPTM1L*	rs401681	Asian	611/1062	Ke et al., 2013
5p15.33	*TERT*	rs2736100	Asian	784/782	Zhao et al., 2013
5p15.33	*CLPTM1L*	rs402710	Asian	31811/36333	Lu et al., 2013

more prominent in adenocarcinoma (AC genotype, OR = 0.94; 95% CI, 0.60−1.47; $P = 0.78$ and AA, OR = 0.39; 95% CI, 0.20−0.76; $P = 5.40 \times 10^{3}$) than in squamous cell carcinoma (AC: OR = 0.83; 95% CI, 0.43−1.59; $P = 0.57$ and AA: OR = 0.55; 95% CI, 0.23−1.31; $P = 0.17$) (Wang et al., 2010). Different types of lung cancer have different pathophysiological and clinical features, suggesting that the carcinogenesis mechanisms are different (Daigo and Nakamura, 2008). Other previous studies have also shown that rs2736100 is strongly related to adenocarcinoma in different populations (Jin et al., 2009; Truong et al., 2010). Because rs2736100 is strongly linked to lung adenocarcinoma, there may be specific regulation mechanisms important to this histologic type, although other possibilities may not be excluded (e.g., relatively smaller sample sizes of non-adenocarcinoma subtypes in these studies might lead to an insufficient statistical power).

Table 2
Classification of studies related to *TERT* polymorphism rs2736100

Adenocarcinoma	Asian	Never smoking	Women
McKay et al., 2008	Jin et al., 2009	Jin et al., 2009	Jin et al., 2009
Broderick et al., 2009	McKay et al., 2008	Hsiung et al., 2010	Hsiung et al., 2010
Jin et al., 2009	Broderick et al., 2009	Truong et al., 2010	Truong et al., 2010
Landi et al., 2009	Jin et al., 2009	Zhao et al., 2013	Zhao et al., 2013
Hsiung et al., 2010	Landi et al., 2009		
Miki et al., 2010	Hsiung et al., 2010		
Truong et al., 2010	Miki et al., 2010		
Yoon et al., 2010	Truong et al., 2010		
Bae et al., 2012	Yoon et al., 2010		
Chen et al., 2012			
Zhao et al., 2013			
Wang et al., 2014			

East Asian populations

Based on previous GWAS, a meta-analysis has demonstrated that the association between SNP rs2736100 and the risk of lung cancer is higher in East Asian populations (C allele, OR = 1.26; 95% CI, 1.23−1.30; $P < 10^{-5}$) than in Caucasians (C allele, OR = 1.13; 95% CI, 1.10−1.16; $P < 10^{-5}$) (Yuan et al., 2014). SNP rs401681 is located in intron 13 of *CLPTM1L*, and has linkage disequilibrium (LD) with rs402710. Rs401681 is also in the region which includes a putative promoter region of *TERT*. Both rs402710 and rs401681 polymorphisms have been established as risk factors in the development of lung cancer. A recent study has shown that these two polymorphisms are significantly associated with Caucasian and East Asian populations. The strength of associations between polymorphisms rs402710 and rs401681, and the risk of lung cancer are similar between Caucasian populations (C allele: OR = 1.17; 95% CI, 1.13−1.20; $P < 10^{-5}$ and OR = 1.14; 95% CI, 1.11−1.17; $P < 10^{-5}$) and East Asians (C allele: OR = 1.12; 95% CI, 1.07−1.18; $P < 10^{-5}$ and OR = 1.13; 95% CI, 1.09−1.17; $P < 10^{-5}$) (Zhao et al., 2014). As such, it is evident that the risk of lung cancer associated with different polymorphisms in the 5p15.33 locus varies by ethnicity. The rs2736100 polymorphism is likely to affect East Asians more than Caucasians.

Non-smoking females

Although long-term exposure to tobacco smoke is the major risk factor of lung cancer, there are still 10%−15% of lung cancer patients which are never smokers. Never smokers are characterized as patients who have smoked fewer than 100 cigarettes in their lifetimes (Thun et al., 2008). Global cancer statistics show that 15% of males and 53% of females lung cancer patients have cancer that is not caused by cigarette smoking (Parkin et al., 2005; Torre et al., 2015). To find the risk factors in never smokers, an association study has provided evidence to support the notion that *TERT* polymorphism rs2736100 is a risk factor for the development of lung cancer in never smokers, primarily for adenocarcinoma (Wang et al., 2010). In Asian countries, most female lung cancer patients are never smokers. Also among never smokers, the portion of females afflicted by adenocarcinoma is five times higher than males (Wakelee et al., 2007). As a combined subpopulation in lung cancer, lung adenocarcinoma in Asian never-smoking females has been studied for its genetic susceptibility with the rs2736100 polymorphism as a strong candidate factor. A GWAS of never-smoking females from a Han Chinese population (584 cases and 585 controls) found that rs2736100 was the most significant polymorphism associated with lung adenocarcinoma. Patients harboring AC or CC genotypes of rs2736100 have a higher risk of adenocarcinoma than those with the AA genotype (OR = 1.62; 95% CI, 1.40−1.87 and OR = 2.35; 95% CI, 1.95−2.83, respectively) (Hsiung et al., 2010). The same research group who carried out this study also conducted a multistage GWAS of lung cancer in Asian women who never smoked (5510 cases and 4544 controls). They established that the locus rs2736100 at 5p15.33 is one of the most significant loci associated with NSCLC (C allele, OR = 1.38; 95% CI, 1.30−1.47, $P = 4.24 \times 10^{-27}$) (Lan et al., 2012). Another hospital-based case-control study in never-smoking Chinese female populations (524 cases and 524 controls) revealed that individuals who carry AC or CC genotypes of rs2736100 have an increased risk in comparison with patients who carry the AA genotype (OR = 1.44; 95% CI, 1.09−1.90; $P = 0.01$ and OR = 1.85; 95% CI, 1.29−2.65; $P = 0.001$, respectively) (Yin et al., 2014). Furthermore, smoking status analysis revealed that rs2736100 is significantly associated with risk of lung cancer in both never smokers and smokers. SNP rs2736100 C allele has been shown to have a higher risk of lung cancer in never smokers than current smokers (OR = 1.34; 95% CI, 1.29−1.38; $P < 10^{-5}$ *vs.* OR = 1.16; 95% CI, 1.09−1.24; $P < 10^{-5}$) (Yuan et al., 2014), suggesting that rs2736100 is more likely associated with lung cancer in never smokers.

As mentioned above, beyond genetic factors, environmental variables can also contribute to an increased risk of lung cancer. These typical environmental risk factors of non-smoking women in East Asia are related to indoor air pollution. For example, second-hand smoking has been established as a risk factor for lung cancer (Vineis et al., 2005; Gorlova et al., 2006). Also, cooking oil vapors stemming from high temperature cooking and indoor coal burning for heating are common environmental risk factors in non-smoking Asian women, especially in rural areas in China (Lam, 2005). The epidemiologic characteristics of never-smoking lung cancer patients show some unknown genetic or environmental susceptibility factors. It is likely that the combination of genetic and environmental factors drive lung adenocarcinoma risk in populations of never-smoking lung cancer patients. On the other hand, one study investigated the interaction between cooking oil fume exposure and SNP rs2736100 on the risk of lung cancer and showed that there are no significant links between these two factors (Yin et al., 2014). Further exploration of the interaction between environmental factors and *TERT* polymorphisms is merited.

TELOMERE BIOLOGY, RS2736100 AND NSCLC RISK

TERT, telomere and NSCLC

The telomere is an important factor for the maintenance of normal chromosomal structure and function, and has been demonstrated to influence cell proliferation and senescence (Autexier and Lue, 2006). It has been well established that TERT is crucial for telomere sequence replication and stabilization by controlling telomere length. The *hTERT* gene, encoding human telomerase reverse transcriptase, is a catalytic subunit of telomerase. Human telomerase is a ribonucleoprotein enzyme which has a RNA component, hTERC, that provides the template for the synthesis of human telomeric repeats of the form $(TTAGGG)_n$ to the end of the chromosome's telomere. TERT is not only essential for maintaining telomeres through the protection of chromosomal ends from degradation, but also prevents inappropriate DNA fusion and rearrangement (Feng et al., 1995; Weinrich et al., 1997; Poole et al., 2001). The expression of *TERT* is found at very low levels for most types of normal cells. In germ cells, however, the expression is much higher, and up to 90% of human tumors show increased telomerase activity (Lantuejoul et al., 2007).

As for the role of TERT in cancers, some studies have found that longer telomere lengths are correlated with an increased risk for some types of cancers, such as melanoma, lymphoma, lung cancer and liver cancer in blood cell or serum DNA (Han et al., 2009; Lan et al., 2009; Shen et al., 2011; Fu et al., 2012). Conversely, there are other studies which have reported that shorter telomere length is also associated with an increased risk of cancer in blood and buccal cells (Wentzensen et al., 2011). In lung cancer biology, ectopic expression of *TERT* in primary lung epithelial cells immortalizes but does not transforms the cells, suggesting that increased TERT activity may confer increased cell proliferation capacity to normal cells (Lundberg et al., 2002; Sato et al., 2013). To gauge the influence of germline background of telomere length, real-time PCR (qRT-PCR) has been used to measure the mean leukocyte telomere length (LTL). As a germline genetic indication for cancer risk, a previous study has measured LTL of lung cancer patients in a population of smoking males in Finland. These LTL results indicate that longer LTL is associated with an increased risk of lung cancer (Shen et al., 2011). Other research consisting of a case-control

study in a Chinese population shows a similar result (Lan et al., 2013).

On the other hand, TRAP (Telomeric Repeat Amplification Protocol) assays have been used to evaluate telomerase activity in lung cancer cells. According to reports, almost all small cell lung cancer (SCLC) and the majority of NSCLC display altered telomerase activity in 62%−96% of cases. Of NSCLC, 69%−92% of adenocarcinomas, 60%−91% of squamous carcinomas and 67%−100% of large cell carcinoma are telomerase positive and display a change in telomerase activity (Lantuejoul et al., 2007). Multiple studies have verified *TERT* expression level in tumor tissues of NSCLC by qRT-PCR. The results of these studies indicate that *TERT* expression is significantly higher in NSCLC tumor tissues (Counter et al., 1998; Hsu et al., 2003; Wu et al., 2003; Brennan et al., 2011). Moreover, *TERT* DNA copy number gains in the early stage of NSCLC (Kang et al., 2008).

A variety of mechanisms are relevant to TERT's role in adenocarcinoma. In a functional study, TERT has been established to promote epithelial proliferation through transcriptional pathways Myc and Wnt (Choi et al., 2008). An *in vivo* study has shown that down-regulation of *TERT* and subsequently reduced telomerase activity induces lung adenocarcinoma cells to go through apoptosis, causing tumor size to be reduced (Xie et al., 2011). In mouse models, telomere maintenance was involved in the progression from KRAS-activated adenoma to adenocarcinoma (Sweet-Cordero et al., 2006).

Telomere length and rs2736100

The *TERT* polymorphism rs2736100 has been associated with LTL in different populations through multiple GWASs (Codd et al., 2013; Lan et al., 2013; Machiela et al., 2015). Age-adjusted relative telomere length research has demonstrated associations between telomere length and rs2736100 in cancer patients (Melin et al., 2012). While multiple SNPs (including rs2736100) have been identified to determine the LTL in general populations in GWAS, recent studies in East Asian populations have demonstrated that those associated with longer LTL are also associated with increased lung cancer risk among never-smoking East Asian women (Machiela et al., 2015). More importantly, a prospective study of lung cancer risk among never-smoking Chinese women demonstrates a dose−response relationship between LTL and risk of lung cancer (Lan et al., 2013). Meanwhile, LTL is known to be significantly longer in females than in males in general populations (Needham et al., 2014). It was also demonstrated that smokers have shorter telomere length in both leukocytes as well as in lung cells as compared to nonsmokers (Walters et al., 2014; Verde et al., 2015). While younger age is also associated with longer telomeres, Asian lung cancer patients are, in general, younger at their age of onset as compared to the Caucasian population, especially in never-smoker patients (Kawaguchi et al., 2010). These studies together support the concept that longer telomeres are a strong risk factor for NSCLC, and that longer telomeres can be attributed to genetic (rs2736100 and other SNPs), environmental (non-smoking status, etc.) and physiological factors (female gender, age, etc.), although whether East Asians have intrinsically longer telomeres remains unclear.

The elucidation of the mechanisms that underlie the modified function of rs2736100 will be important for developing a more comprehensive understanding of this SNP's function in carcinogenesis. However, the possible mechanism driving the association between rs2736100 C and longer telomeres still remains largely unknown. A previous *in silico* study suggests that this polymorphism may be located in a regulatory region of the *TERT* gene (Zou et al., 2012). Our recent studies observe that the rs2736100 A allele has a significantly higher affinity to nuclear proteins of human lung epithelial cells as compared to the C allele. Meanwhile, rs2736100 C is significantly associated with increased *TERT* transcription in both lung cancer tissue and in their adjacent normal tissues (Wei et al., 2015). Of course, other SNPs in LD with rs2736100 may also contribute to the increased *TERT* expression or function. Showing this will require a large population-based investigation. In addition, our recent study observes that cancer cells carrying the rs2736100 C allele are more likely to harbor TP53 mutations, indicating that either longer telomere length may increase the susceptibility to TP53 mutagenesis, or simply the combination of increased telomere length and TP53 mutations serve as an etiological condition for cancers (Kim et al., 2013).

With all of the above in mind, it can be concluded that the inherited capacity to maintain a longer telomere is a risk factor for NSCLC, while rs2736100 or other LTL-associated SNPs may mediate this linkage.

LONGER TELOMERES: A RISK FACTOR FOR *EGFR* MUTATION-DRIVING NSCLC

NSCLC and *EGFR* mutations

Epidermal growth factor receptor (*EGFR*) is an important oncogene in lung cancer, particularly in NSCLC. The discovery of driver somatic mutations in *EGFR* is one of the most important findings related to NSCLC etiology and the targeted treatment of NSCLC patients (Lynch et al., 2004; Paez et al., 2004).

Somatic mutation of *EGFR* in lung cancer was first recognized in 2004 (Lynch et al., 2004; Paez et al., 2004). Following these initial studies, numerous genetic epidemiological studies have surveyed *EGFR* mutations among common tumors and cancer patient populations worldwide. Based on these studies, somatic *EGFR* mutations are identified predominantly in lung cancer. These mutations are located in *EGFR* TK domain (exons 18−21), with 94% of mutations occurring in exons 19, 20 and 21 (Shigematsu et al., 2005). The three most common mutation types of *EGFR* are in-frame deletions, single-nucleotide substitutions and in-frame duplications and/or insertions. Among all TK domain mutations, 85%−90% have been shown to be exon 19 deletions and exon 21 L858R mutations (Sakurada et al., 2006). The majority of

these mutations are found to be gain-of-function which are mainly located around the ATP binding cleft of EGFR which controls the ATP binding pocket (Kim et al., 2004). Cells bearing deletions in exon 19 and L858R missense mutations have higher sensitivity to EGFR TK inhibitors (TKI) (Lynch et al., 2004), while the rare exon 22 mutation (E884K) may confer varying sensitivity to different EGFR inhibitors (Choong et al., 2006). On the other hand, mutations in exon 20 (T790M) are associated with resistance to EGFR TKIs (Sharma et al., 2007).

Association between *EGFR* mutations and clinical and demographic factors

EGFR mutations in lung cancer are associated with unique clinical and demographic features, including adenocarcinoma histology, non-smoking history, younger age, female gender and East Asian ethnicity. Studies have shown a strong linkage between all of these features and mutations in the *EGFR* TK domain by comparing them with lung cancers classified to other histology and patient subpopulations (Shigematsu et al., 2005).

A study has screened *EGFR* mutations in lung cancer patients to compare Japanese and American populations. The results of this study show that *EGFR* mutations occur more frequently in adenocarcinomas than in other histologic types, more in women than in men, and more in Japanese than in Americans (Paez et al., 2004). The incidence of *EGFR* mutations in Chinese populations has been observed in over 40% of adenocarcinomas (Song et al., 2013). In Americans, however, *EGFR* mutations have only been detected in about 20% of lung adenocarcinomas (Villa et al., 2014). Previous studies also observe that one of the major predictors for *EGFR* mutations is never-smoking history in lung cancer patients (Kosaka et al., 2004; Shigematsu et al., 2005; Tokumo et al., 2005). It has been shown in one population test that in a set of adenocarcinoma patients, 73% of the never smokers also had *EGFR* mutations (Tokumo et al., 2005). In another sample set of NSCLC patients, it has been shown that *EGFR* mutations occur much more frequently in never smokers than in ever smokers with an incidence rate of 51% compared to 10% (Shigematsu et al., 2005). One possible reason is that *EGFR* mutations may take a longer time to occur than other mutations in smokers that are caused by carcinogens, which further indicates the more important role of genetic background in *EGFR* mutagenesis.

Although lung cancer is generally diagnosed in elderly populations, previous studies show that patients carrying *EGFR* mutations are significantly younger than those who have wild-type *EGFR* (Nishii et al., 2014; Wei et al., 2015). Gender is another predictive factor significantly correlated with *EGFR* mutations (Shigematsu et al., 2005; Sharma et al., 2007). Gender is related to smoking behavior as most women are never smokers, which, in part, explains the reason that the incidence of *EGFR* mutations is higher in women than in men. The other explanation for *EGFR* mutations related to women is that expression of estrogen receptors is associated with NSCLC; however, a recent study has shown that *EGFR* mutations occurring in females are not significantly related to estrogen (Bell et al., 2008).

Based on these epidemiologic and clinical characteristics, never-smoking Asian women may represent a distinct subpopulation, which has a higher risk to develop NSCLC, especially adenocarcinoma. At the molecular level, this population is more likely to develop *EGFR* mutations (Thatcher et al., 2005). A better understanding of the molecular mechanisms that underlie lung carcinogenesis related to these geographic factors and population specific features could greatly benefit the early diagnosis, prevention, and targeted therapy of lung cancer.

Telomere length, rs2736100 and *EGFR* mutation-driving NSCLC

As reviewed above, given the associations among telomere length and lung adenocarcinoma, smoking status and gender, it is very possible that longer telomeres are actually a risk factor specifically for *EGFR* mutation-driving NSCLC.

The intersection of NSCLC patients who have these features shows the connection between *TERT* polymorphism rs2736100 and *EGFR* mutations, which is highlighted in Fig. 1. This prompts us to hypothesize that SNP rs2736100 may be a risk factor for patients with *EGFR* mutations. Using a population of over 700 samples collected in China, we recently demonstrated that patients with *EGFR* mutations in their tumors have significantly longer LTL than patients whose tumor does not harbor *EGFR* mutations. This result remains significant after adjusting for age, gender, smoking status and tumor histology. Moreover, rs2736100 C is significantly associated with *EGFR* mutation-positive NSCLC but not with *EGFR* mutation-negative NSCLC (Wei et al., 2015). This observation indicates that the previously reported association between rs2736100 and lung cancer subtypes (i.e., that in East Asian females, never smokers and adenocarcinoma) is actually due to its association with *EGFR* mutation-driving NSCLC as a unique disease. Taken together, these findings clearly depict that longer intrinsic telomere length is a risk factor for *EGFR* mutation-driving lung cancer subtypes, while the long telomere length can be attributed to genetic alleles (rs2736100), non-smoking history, female gender and East Asian background. This is illustrated in Fig. 2.

This population-based linkage implicates a strong interaction between TERT and EGFR signaling at the molecular level

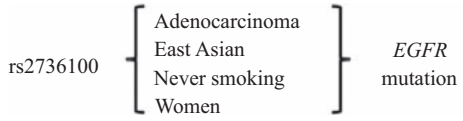

Fig. 1. Common features connecting polymorphism rs2736100 and *EGFR* mutations.

Depiction of the overlap in various features (adenocarcinoma, East Asian, never smoking and female) that are associated with both *TERT* polymorphism rs2736100 C allele and *EGFR* mutations.

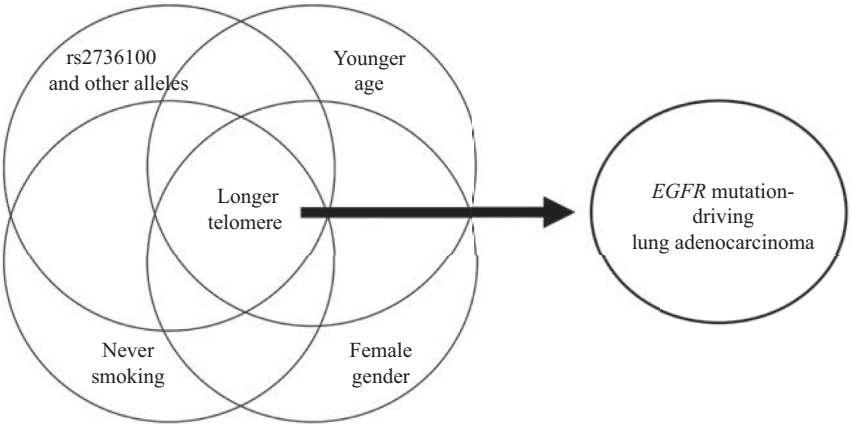

Fig. 2. Longer telomeres and *EGFR* mutation-driving adenocarcinoma.
A variety of factors including genetic (rs2736100 and other alleles), physiological (age and gender) and environmental (nonsmoking) lead to longer telomeres in lung epithelial cells and give context to *EGFR* mutation-driving adenocarcinoma.

in the etiology of NSCLC. Some studies have shown a preliminary understanding of the functional relationship between TERT and EGFR. One study has shown a linkage between EGFR and TERT through various signaling factors including ERK, Src and Akt by demonstrating that EGFR inhibition shows a dose-dependent decrease in *TERT* expression (Budiyanto et al., 2003). Another study has shown that the signaling response between EGFR and TERT is disrupted by MEK inhibitors (Maida et al., 2002). This may further support the conclusion that the Ras/MEK/ERK pathway plays a role in the crosstalk between EGFR and TERT. The NF-κB pathway is also stimulated by EGFR which has been associated with *TERT* expression (Aravindan et al., 2013). While some mechanisms have related *TERT* expression to *EGFR* expression, the detailed crosstalk between these two pathways in lung cancer is still largely unclear. Previous studies have shown that ectopic expression of *TERT* in airway epithelial cells leads to the immortalization of these epithelial cells and serves as useful model for studying carcinogenesis (Piao et al., 2005). Other somatic cells like endothelial cells and fibroblasts can also be immortalized by increases in TERT activity (Bodnar et al., 1998; Yang et al., 1999). Moreover, the expression of mutant *EGFR* in TERT immortalized lung epithelial cells helps to boost cellular transformation (Greulich et al., 2005). These lines of evidence indicate a basic role of TERT in prolonging the cellular life span, promoting epithelium renewal, as well as enhancing proliferation of lung epithelial cells at the pre-cancer stage. Therefore, while a capacity for maintaining a longer telomere may serve as the "first hit" for an intrinsic potential, somatic *EGFR* mutations may serve as the "second hit", which initiates and promotes cell transformation and cancer development (Fig. 3). Additional investigation is obviously required to further elucidate the interrelationships among *TERT* polymorphism, telomerase activity, telomere function and *EGFR* mutations in lung adenocarcinoma.

CONCLUSION AND PERSPECTIVE

Integrating current evidence about genetic polymorphism, somatic mutations as well as environmental and physiological factors outlines a portrait of the etiology of NSCLC, especially for adenocarcinoma. More succinctly, the co-presence of both *EGFR* mutations and the rs2736100 C allele in

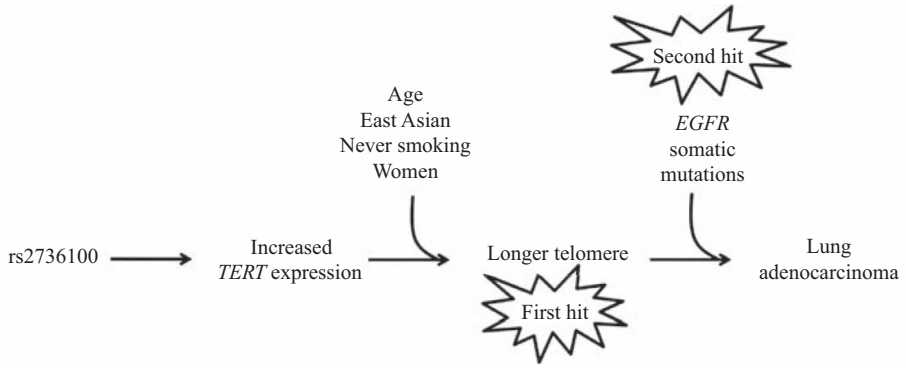

Fig. 3. A "two-hit" hypothesis for lung adenocarcinoma development in patients with *TERT* polymorphism rs2736100.
The higher capacity of epithelial cells for maintaining telomere length attributed to genetic alleles (e.g., rs2736100) as well as environmental and physiological factors provides these cells with a potential for enhanced proliferation and self-renewal ability (first hit), while the occurrence of *EGFR* mutations (second hit) later on triggers the carcinogenesis.

adenocarcinomas in never-smoking East Asian women is both distinct and significant. Further understanding of the interaction between these factors may significantly increase our power to identify patients who are likely to develop lung adenocarcinoma. Meanwhile, the preceding evidence strongly suggests the interaction of TERT and EGFR pathways in the etiology of NSCLC. Understanding the detailed molecular mechanisms underlying this interaction may lead to the establishment of new strategies for prevention and targeted treatment of lung adenocarcinoma.

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