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The two single nucleotide polymorphisms in the *H37/RBM5* tumour suppressor gene at 3p21.3 correlated with different subtypes of non-small cell lung cancers

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Summary Allele loss and genetic alteration in chromosome 3p, particularly in 3p21.3 region, are the most frequent and the earliest genomic abnormalities found in lung cancer. Multiple 3p21.3 genes exhibit various degrees of tumour suppression activity suggesting that 3p21.3 genes may function as an integrated tumour suppressor region through their diverse biological activities. We have previously demonstrated growth inhibitory effects and tumour suppression mechanism of the *H37/RBM5* gene which is one of the 19 genes residing in the 370 kb minimal overlap region at 3p21.3. In the current study, in an attempt to find, if any, mutations in the *H37* coding region in lung cancer cells, we compared nucleotide sequences of the entire *H37* gene in tumour versus adjacent normal tissues from 17 non-small cell lung cancer (NSCLC) patients. No mutations were detected; instead, we found the two silent single nucleotide polymorphisms (SNPs), C1138T and C2185T, within the coding region of the *H37* gene. In addition, we found that specific allele types at these SNP positions are correlated with different histological subtypes of NSCLC; tumours containing heterozygous alleles (C + T) at these SNP positions are more likely to be associated with adenocarcinoma (AC), whereas, homozygous alleles (either C or T) are associated with squamous cell carcinoma (SCC) ($p = 0.0098$). We postulate that, these two silent polymorphisms may be in linkage disequilibrium (LD) with a disease causative allele in the 3p21.3 tumour suppressor region which is packed with a large number of important genes affecting lung cancer development. In addition, because of prevalent loss of heterozygosity (LOH) detected at 3p21.3 which precedes lung cancer initiation, these SNPs may be developed into a marker screening for the high risk individuals.

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1. Introduction

Lung cancer is the leading cause of cancer death in the United States resulting in nearly 1.2 million deaths worldwide and over 150,000 deaths in the United States each year [1,2]. The lung cancer 5-year survival rate has remained 13—15% throughout the past three decades despite innovations in diagnostic testing, surgical technique, and development of new chemotherapeutic agents [3]. In contrast, the survival rates for other common cancers such as breast, prostate, and colon cancers, which are managed by similar principles of diagnosis, staging, resection, and chemotherapy, have improved dramatically. Although survival improvements in these cancers may be attributed to availability of effective early detection screening, it is possible that the poorer outcomes of lung cancer may be due to fundamental differences in its tumour biology; whereas, breast, colon, and prostate malignancies are predominantly adenocarcinomas (ACs), lung cancer histology is heterogeneous. Small cell lung carcinomas (SCLCs) account for 20% of all lung cancers, and the rest 80% consists of non-small cell lung carcinomas (NSCLCs) which, in turn, is further sub-classified into 40% AC, 40% squamous cell carcinoma (SCC) and 20% large cell carcinoma [4]. Growing biological and epidemiological data suggest that different NSCLC histological subtypes, in particular the two most common, AC and SCC, have distinct etiologies, therefore, they should be treated differently. For example, SCC, originated in the tracheobronchial tree, tends to stay localized while growing slowly to large sizes and cavitating, whereas AC, developed in the glandular tissue, metastasizes much earlier to the lymph nodes and brain [5,6]. However, current clinical regimens are the same for both types of lung cancer because the presently available treatment options make no different outcomes in either type. Delineating genetic alterations specific to each subtype of lung cancer may be the most effective way of improving molecular markers for early detection and prediction of response to chemoprevention/chemotherapy as well as developing better individualized treatment.

Deletion and genetic alteration in chromosome 3p, particularly in 3p21.3 region, are the most frequent and the earliest genomic abnormalities found in lung cancer [7]. For instance, loss of heterozygosity (LOH, i.e. heterozygous deletion) in 3p21.3 region occurs in more than 65% of NSCLCs and 95% of SCLCs [8]. Genomic aberrations can be found as early as in preneoplastic lesions of smoker's lung [9], suggesting one or more 3p21.3 genes may function as preventing tumour initiation. In addition, smokers whose peripheral blood lymphocytes showed greater damage in this 3p21.3 region after treatment with the benzo-*a*-pyrene diol epoxide carcinogen *in vitro* had an increased lung cancer risk, suggesting the potential for genetic polymorphisms in this region which predispose cells to lung cancer development [10]. Multiple 3p21.3 genes, in particular ~19 genes contained in the 370 kb smallest region of overlap, exhibit various characteristics of tumour suppressor activity including growth inhibition, apoptosis, and cell cycle arrest [8]. Therefore, it is believed that 3p21.3 genes may cooperate as an integrated 'tumour suppressor region' either to promote tumourigenesis through loss of expression, or to suppress tumour growth through activation of their tumour suppressing pathways. Since the genetic abnormalities in the 3p region occur in the earliest stage of lung cancer development, the 3p21.3 tumour suppressor genes (TSGs) hold particular promises to be developed as biomarkers for early cancer detection, screening and chemoprevention. In addition, their potent and multifunctional tumour suppressor functions as well as their direct protein—protein interactions with many important cellular target, confer these 3p21.3 genes great opportunities for gene replacement therapeutics in the future [8].

H37/*RBM5* is among the 19 genes located within the 370 kb minimal overlapping deletion regions at 3p21.3. The growing literatures on *H37* strongly suggest its involvement in apoptosis and cell cycle regulation, with all results converging on a role for *H37* as a TSG. Using the lung cancer cell model, we showed that H37 clearly inhibits tumour growth both *in vitro* and *in vivo* with antitumour mechanisms involving cell cycle (G1) arrest and apoptosis [11]. H37 also mediates growth inhibition by eliciting reduced expression of cyclin A and pRB as well as by increased expression of pro-apoptotic protein Bax [11]. Consistently, H37 triggers mitochondrial apoptotic pathways downstream of Bax, in a p53-independent manner, which include breakdown in the mitochondrial membrane potential, cytochrome *c* release into cytosol, and enhanced caspase-9 and caspase-3 activities [11]. Further, as compared with adjacent normal tissue, our findings show reduced expression in primary lung cancers of *H37* transcript and protein in 9 of 11 (82%) and 46 of 62 (73%) samples, respectively [12], although the *H37*/3p21.3 LOH genomic status for these samples was unknown. The involvement of *H37* in malignancy has been also reported by other investigators for a variety of cancer types. For example, *H37* was identified as a serum antigen reacting with autologous antibody in renal cancer [13]. *H37* is down-regulated in human schwannomas [14] and in *ras*-transformed Rat-1 embryonic fibroblasts [15]. Ectopic expression of *H37* also suppresses growth of HT 1080 fibrosarcoma cells [15]. Alternatively spliced transcripts from the *H37* locus and antisense fragments also serve as apoptotic regulators in hematopoetic cells [16—20]. Most significantly, the *H37*/*RBM5* gene was included in the 17 common gene signature associated with metastasis (one of the nine genes down-regulated in metastases) identified in multiple solid tumour types [21]. Various types of solid tumours including lung cancer carrying this gene expression signature had high rates of metastasis and poor clinical outcome.

In the current study, to further confirm *H37*'s direct functional role in lung tumour development, we compared the *H37* coding DNA sequences in primary lung tumour versus adjacent normal tissues. Our initial aim was to find, if any, mutations in the *H37* coding region in tumour. No mutations were detected, instead what we found in the *H37* coding region was the two silent single nucleotide polymorphisms (SNPs) which appear to be associated with different histological subtypes of NSCLCs. Given the important attributes of the 3p21.3 region for lung cancer development, we discuss potential value of the found *H37* SNPs which may be in linkage disequilibrium (LD) with disease causative polymorphisms yet to be discovered in the vicinity of the *H37* gene. Further, the *H37* SNPs may be useful as a diagnostic tool to detect LOH at 3p21.3 which is suggestive of higher risk for developing lung cancer.