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***KRAS* mutation, *KRAS-LCS6* polymorphism, and non-small cell lung cancer**

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Abstract

The *let-7* family of microRNAs are important regulatory molecules in lung cancer. One downstream target of *let-7* is the *RAS* gene family, including *KRAS*, an important oncogene in the etiology and clinical outcome of lung adenocarcinoma. Recently, a SNP in the *let-7* binding region of the *KRAS* 3' UTR was identified (termed *LCS6*). This functional polymorphism alters *let-7* binding, resulting in both increased *KRAS* expression and decreased *let-7* exposure. Further, this SNP has been reported as a risk trait for lung cancer risk among low-moderate smokers. Given the functionality of *LCS6*, we tested the hypothesis that this SNP is associated with the occurrence of *KRAS* mutation as well as patient survival. Here, we report there is no association between the *LCS6* *KRAS* polymorphism and *KRAS* mutation. Further, we find no association between the *LCS6* polymorphism and lung cancer survival. These unexpected findings imply that this newly reported *KRAS-LCS6* polymorphism will have limited clinical utility for NSCLC.

Keywords

LCS6; *let-7*; *kras*; lung cancer

Introduction

Altered expression of the *let-7* family of microRNAs (miRNA) is implicated in many human cancers, including non-small cell lung cancer [1–3]. miRNAs hybridize to the 3'UTR of target mRNA altering the stability and expression of target sequences. The human *RAS* genes contain *let-7* complementary sites in their 3'UTR, and *let-7* has been shown to repress *RAS* expression

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[4]. Further, *let-7* is down-regulated in lung cancer [4]. Mechanistically, this would lead to enhanced expression of the lung oncogene *KRAS*, also successfully demonstrated by Johnson et al [4].

We, and others, have reported a strong association between *KRAS* mutation and survival in non-small cell lung cancer [5]. Consistent with these findings, reduced *let-7* expression is also associated with poor prognosis in lung cancer [3,6]. Mitigating factors (i.e. exposures or polymorphisms) that alter expression of *let-7* or *KRAS* might be expected to have a significant impact on patient survival.

Recently, a report by Chin et al [7] described a novel SNP in the 3'UTR of the *KRAS* gene that alters binding of *let-7*. This variant, the 6th *let-7* complementary site in the *KRAS* 3'UTR (*LCS6*), results in up-regulation of the *KRAS* gene and concomitant down-regulation of *let-7*. In addition, Chin et al [7] report that this polymorphism is associated with a modest increase in lung cancer risk, particularly among low-dose smokers, suggesting that this variant is a biomarker of susceptibility to the carcinogenic effects of tobacco smoke. Presumably, the enhanced risk is related to the combined effects of up-regulated expression of *KRAS*, and down-regulation of the *let-7* miRNA among those with the *LCS6* variant genotype.

To further understand this model of cancer susceptibility, we have evaluated whether the *LCS6* polymorphism is associated with *KRAS* mutation in tumors (previously described for this population [5]). Given the up-regulation of *KRAS* expression associated with the variant allele, our *a priori* hypothesis was that *KRAS* mutations would be preferentially selected for among those who were constitutively wild type for the *LCS6* SNP. Further, given prior evidence for lower *let-7* levels to correlate with poor prognosis [3], we hypothesize that the *LCS6* variant (with accompanying low level *let-7*), is associated with reduced survival time.

Materials and methods

Details on the study population have been previously described [5]. Briefly, cases consisted of all newly diagnosed patients with resectable lung cancer who received treatment at the Massachusetts General Hospital (MGH) Thoracic Surgery, Oncology, and Pulmonary Services from November 1992 through December 1996. Data on case demographics and exposures were derived from an administered questionnaire, clinical data were obtained from pathology reports and clinical record, and patient outcome data were obtained from the Massachusetts General Hospital cancer registry supplemented with SSDI query. *KRAS* codon 12 mutation was determined by RFLP (n=365) [5].

From this surgical case series, a subset of cases were evaluated for the *LCS6* genotype (n=218); inclusion criteria was determined by availability of blood-derived DNA. Compared to those without available DNA, the subset of cases studied were slightly younger (65.9 years vs 66.8 years), had smoked more (60.3 vs 52.3 packyears), tended to be male (57% vs 47%), and had a lower proportion of adenocarcinomas (62% vs 69%). None of these differences were statistically significant. DNA was extracted from whole blood with the QIAmp DNA mini kit according to the manufacturer's protocol (Qiagen, Valencia, CA). Genotyping of the *KRAS-LCS6* SNP was done using Taqman® allelic discrimination (Applied Biosystems, Foster City, CA) with a custom designed primer probe set. Genotyping was performed in a blinded fashion, appropriate controls were included in each run, and approximately 10% of samples were embedded duplicates. There was over >95% concordance observed between replicates.

Statistical analysis of *LCS6* polymorphism and mutation status included χ^2 and unconditional logistic regression. Survival time was defined as the time from surgery to the patient's death, or the last time that the patient was known to be alive. There were 200 deaths, and the mean follow-up time for survivors was 4.94 years. Kaplan-Meier survival probability curves were

constructed, and differences between groups were tested with the use of the logrank method. In addition, a Cox proportional hazards model was used to evaluate the association between genotype and outcome controlling for age, sex, and stage. All *P* values represent two-sided statistical tests.

Results

The overall allele prevalence was 6.6%, consistent with the report by Chin et al [7]. In this case series, there was no significant difference in the variant allele frequency comparing adenocarcinoma (14.7%) and squamous cell carcinoma (11.8%). Further, there was no significant association of the *LCS6* polymorphism and *KRAS* mutation overall, nor in the adenocarcinoma subgroup (Table 1). Given the modifying effects of smoking reported by Chin [7], we further examined the data stratified by 40 packyears smoked, and there remained no evidence of an association between *KRAS* mutation and the polymorphism (data not shown). Finally, as Chin et al suggested that the *LCS6* polymorphism may impact patient outcome, we evaluated survival time as a function of genotype, and found no association between the *LCS6* polymorphism and survival among all histologies (figure 1), or restricted to histologic subgroups (data not shown).

Discussion

We tested the hypothesis i) that there is negative selection pressure in lung cancer for *KRAS* mutation to occur in individuals who do not have the *KRAS-LCS6* polymorphism and, ii), that the *LCS6* variant would be associated with poor survival. In both instances, we observed no evidence of any association. These findings are unexpected given the recent report by Chin et al [7] suggesting that the polymorphism is associated with susceptibility to the carcinogenic effects of tobacco. As the *KRAS* mutation prevalence was the same in both genotype groups, it seems clear that, despite the upregulation in *KRAS* that occurs with the *LCS6* variant, this does not result in any selective pressure for, or against, *KRAS* mutations.

Let-7 expression levels are notably decreased in lung tumors [3,4], and this has been associated with a worse clinical outcome [3,6]. However, in our case series, we find that the *LCS6* polymorphism does not correlate with outcome, despite its reported association with reduced *let-7* [7]. The reason for this is unclear, although it is possible, for example, that the variant is not associated with a sufficient decrease in *let-7* expression to alter outcome. Other possibilities for our null observations may lie with study design. Our analysis was restricted to a subset of cases from a larger case-series study from a single institution. However, we have demonstrated that the subset available for study was similar to those cases not studied. Further, while our statistical power is limited, within this subset of cases there remains a clear association between *k-ras* mutation and outcome.

In the case-control risk context, there is marked lung cancer risk estimates attributed to this polymorphism (2.5-fold) and this was most evident among those who were low to moderate smokers. Restricting our case-only analysis in this way did not alter any of our observations; *LCS6* polymorphism and *KRAS* mutation status remained independent events, and there was no impact of *LCS6* polymorphism on survival. The *LCS6* variant genotype may predispose individuals to a somatic event that is ubiquitous in tumors, allowing an observation of risk in the case-control framework, but undetectable in the tumor-series. For instance, *let-7* is known to down-regulate Dicer, a critical component of the miRNA processing machinery; and reduced *let-7* levels lead to increased levels of Dicer and other mature miRNAs [8]. Although an altered balance of miRNAs associated with the *LCS6* variant appears to not be associated with prognosis of NSCLC, the same alterations in miRNA expression levels may have important clinical implications for other tumor types. In fact, a recent paper demonstrated that the *LCS6*

variant is associated with poor prognosis in head and neck cancer [9]. Hence, while our data suggest that this newly reported *KRAS-LCS6* polymorphism will have limited (if any) clinical utility for NSCLC, additional studies in other cancer sites remain of interest.

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Figure 1.
Lack of association between *LCS6* genotype and NMSCLC survival

Table 1

LCS6 genotype and *KRAS* mutation

	All Histologies		Adenocarcinoma		Squamous Cell Carcinoma	
	Wild type	Any variant	Wild type	Any variant	Wild type	Any variant
Prevalence	191 (87.6%)	27 (12.4%)	93 (85.3%)	16 (14.7%)	60 (88.2%)	8 (11.8%)
<i>KRAS</i> codon 12 mutation						
No	161	24	71	13	58	8
Yes	27 (14.4%)	3 (11.1%)	21 (22.6%)	3 (18.8%)	2 (3.3%)	0