

# KRAS Mutation in Small Cell Lung Carcinoma and Extrapulmonary Small Cell Cancer

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**Background:** Lung cancer is one of the most lethal cancers. It is mainly classified into 2 groups: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Extrapulmonary small cell carcinomas (EPSCC) are very rare. The Ras oncogene controls most of the cellular functions in the cell. Overall, 21.6% of human cancers contain a Kirsten Ras (KRAS) mutation. SCLC and EPSCC have several similar features but their clinical course is different.

**Aims:** We investigated the KRAS mutation status in SCLC and EPSCC.

**Study design:** Mutation research.

**Methods:** Thirty-seven SCLC and 15 EPSCC patients were included in the study. The pathological diagnoses were confirmed by a second pathologist. KRAS analysis was performed in our medical genetic department. DNA isolation was performed with primary tumor tissue using the QIAamp DNA FFPE Tissue kit (Qiagen; Hilden, Germany) in all patients. The therascreen KRAS Pyro Kit 24 V1 (Qia-

gen; Hilden, Germany) was used for KRAS analyses.

**Results:** Thirty-four (91.9%) of the SCLC patients were male, while 11 (73.3%) of the EPSCC 1 patients were female. SCLC was more common in males, and EPSCC in females ( $p=0.001$ ). A KRAS mutation was found in 6 (16.2%) of SCLC patients. The most common mutation was Q61R (CAA>CGA). Among the 15 EPSCC patients, 2 had a KRAS mutation (13.3%). When KRAS mutant and wild type patients were compared in the SCLC group, no difference was found for overall survival ( $p=0.6$ ).

**Conclusion:** In previous studies, the incidence of KRAS mutation in SCLC was 1-3%; however, it was 16.2% in our study. Therefore, there may be ethnic and geographical differences in the KRAS mutations of SCLC. As a result, KRAS mutation should not be excluded in SCLC.

**Keywords:** Small cell lung cancer, extrapulmonary small cell carcinoma, KRAS mutation

Lung cancer is one of the most lethal cancers (1). The clinicopathological features of lung cancer are heterogeneous. It is classified into 2 groups as non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). The genetic biomarkers are used to identify a targeted treatment in NSCLC (2,3). Small cell lung cancer accounts for about 15-20% of all lung cancers, and is the most aggressive type (4,5). The specific genetic alterations that take place in small-cell lung cancer development are still unclear (6). Extrapul-

monary small cell carcinomas (EPSCCs) are very rare. The most frequently involved regions are the genitourinary and gastrointestinal systems (7). The Ras oncogene controls several cellular functions including cell proliferation, apoptosis, migration and differentiation (8). Kirsten RAS (KRAS) mutations are present in over 20% of all cancers (9). SCLC and EPSCC have several similar features but their clinical course is different (10, 11). Therefore, we investigated the KRAS mutation status in SCLC and EPSCC.

## MATERIALS AND METHODS

We retrospectively reviewed the records of patients diagnosed with SCLC and EPSCC between 2005 and 2014. This study was approved by the Institutional Review Board. Thirty-seven SCLC and 15 EPSCC patients were included in the study. We collected paraffin-embedded tissue blocks of patients from the archive of our pathology department. The pathological diagnoses were confirmed by the second pathologist.

KRAS analyses were performed in our certified medical genetics department. The KRAS mutations were studied: CAA>CTA (Q61L), CAA>CAT (Q61H), CAA>CGA (Q61R), GGT>GTT (G12V), GGC>GAC (G13D), GGT>GAT (G12D), GGT>TGT (G12C), GGT>AGT (G12S), GGT>GCT (G12A), GGT>CGT (G12R), and GGT>c.34\_35GG>TT (G12F). DNA isolation was performed with primary tumor tissue using the QIAamp DNA FFPE Tissue kit (Qiagen; Hilden, Germany) in all patients. The therascreen KRAS Pyro Kit 24 V1 (Qiagen; Hilden, Germany) was used for KRAS analyses. KRAS point mutations were analyzed with Pyro Mark Q24 software system (Qiagen; Hilden, Germany). This study was approved by the Institutional ethics board. Informed consent was obtained from all individual participants (or the legal representative) included in the study.

### Statistical analysis

Statistical analysis was used for Statistical Package for the Social Sciences (SPSS) software version 18.0 (SPSS Corp.; Chicago, IL, USA). Overall survival was calculated from the date of diagnosis to the date of death from disease or last follow-up. The relationship between nonparametric variables was studied by Chi-square test. Parametric variables were compared with independent sample t test. P values below 0.05 were considered to be significant. Survival estimates were calculated by using the Kaplan-Meier method.

## RESULTS

Thirty-four (91.9%) of the SCLC patients were male, and eleven (73.3%) of the EPSCC patients were female. When genders were compared in SCLC and EPSCC patients, SCLC was more common in males, however EPSCC was more common in females ( $p=0.001$ ). A KRAS mutation was found in 6 (16.2%) SCLC patients, with the most common mutation being the Exon 3 mutation. Only one patient had an Exon 2 mutation. The most common mutation was the Q61R (CAA>CGA) mutation found in 4 patients with an Exon 3 mutation. One patient carried Q61L (CAA>CTA), and 1 patient carried G12C (GGT>TGT). Among the 15 EPSCC patients, 2 had a KRAS mutation (13.3%). One

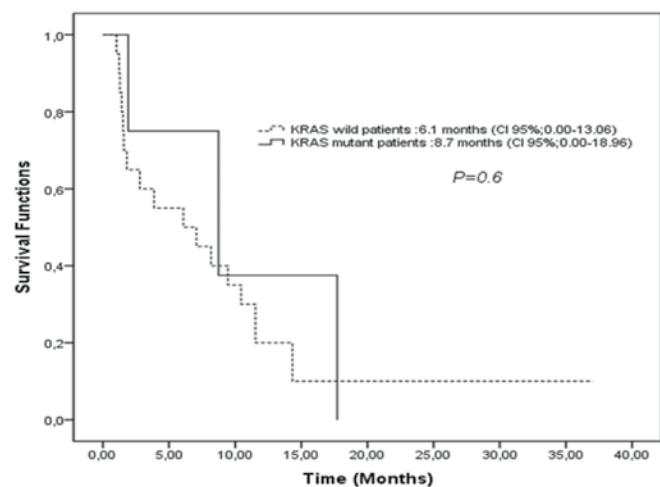


FIG. 1. SCLC overall survival

patient with pancreatic EPSCC carried the G12D (GGT>GAT) mutation. One patient with prostatic EPSCC carried the Q61L (CAA>CTA) mutation (Table 1). No difference in KRAS mutation status was observed between SCLC and EPSCC patients ( $p=0.8$ ). An Exon 3 mutation was found in 80% of SCLC and 50% of EPSCC patients. Overall survival was 6.1 months (CI 95%; 0.00-13.06) in KRAS wild type SCLC and 8.7 months (CI 95%; 0.00-18.96) in KRAS mutant SCLC cases. When KRAS mutant and wild type SCLC patients were compared, no difference was found in overall survival ( $p=0.6$ ) (Figure 1).

## DISCUSSION

KRAS mutations are present in 12-36% of lung adenocarcinoma, but are rare in squamous cell lung cancer. In lung adenocarcinoma, KRAS mutations are more common in smokers (12-16). The prognostic significance of KRAS mutations in NSCLC is controversial (17-19). Maitra et al. (20) identified KRAS mutations in 2 (17%) of 12 gallbladder EPSCC patients. In our study, 2 of 15 EPSCC patients had KRAS mutations (13%). In their genetic profile study, Wakuda et al. (21) found that 1 (1.6%) of 60 SCLC patients had KRAS mutations; this patient also had adenocarcinoma. In their study on 43 SCLC and 3 EPSCC patients, Yamamoto et al. (22) found that none of the patients had KRAS mutations. In our study, 6 (16.2%) of 37 SCLC patients had KRAS mutations. There were ethnic and geographical differences in the KRAS mutation status of patients with colorectal cancer and NSCLC (19,23,24). Genetic, dietary and environmental factors play a role in the development of KRAS mutations. More than 80% of KRAS mutations are exon 2 mutations in NSCLC and colorectal cancers (19,23). In our study, 80% of KRAS mutations in SCLC were in exon 3.

**TABLE 1.** Patient characteristics

	<b>Small cell lung carcinoma (SCLC) (n: 37)</b>		<b>Extrapulmonary small cell carcinoma (EPSCC) (n: 15)</b>	<b>p</b>
Age	56	(42-72)	60	(46-85)
KRAS Status				
Wild 31	(83.8%)	13	(86.7%)	0.8
Mutant	6	(16.2%)	2	(13.3%)
Gender				
Male	34	(91.9%)	4	(26.7%)
Female	3	(8.1%)	11	(73.3%)
Primary tumor				
Lung	37	(100%)	-	-
GIS	-		5	(33.3%)
GUS	-		8	(53.3%)
Breast	-		2	(13.3%)
<b>KRAS mutations SCLC</b>			<b>KRAS mutations EPSCC</b>	
Codon 61 Q61L (CAA>CTA)	1	(2.7%)	Codon 61 Q61L (CAA>CTA)	1 (6.6%)
Codon 61 Q61R (CAA>CGA)	4	(10.8%)	Codon 12 p.G12D (GGT>GAT)	1 (6.6%)
Codon 12 p.G12C (GGT>TGT)	1	(2.7%)	KRAS wild type	13 (86.6%)
KRAS Wild type	31	(83.7%)		
<b>Extrapulmonary small cell carcinoma tumor location and Kras status</b>				
<b>Patients</b>		<b>Tumor location</b>		<b>KRAS status</b>
1.		Pancreas		Codon 12 p.G12D (GGT>GAT)
2.		Prostate		Codon 61 Q61L (CAA>CTA)
3.		Pancreas		Wild type
4.		Esophagus		Wild type
5.		Esophagus		Wild type
6.		Bladder		Wild type
7.		Bladder		Wild type
8.		Bladder		Wild type
9.		Cervix		Wild type
10.		Cervix		Wild type
11.		Ovary		Wild type
12.		Gallbladder		Wild type
13.		Endometrium		Wild type
14.		Breast		Wild type
15.		Breast		Wild type

SCLC: small cell lung carcinoma; EPSCC: extrapulmonary small cell carcinoma; KRAS: Kirsten ras; GIS: gastrointestinal system; GUS: genitourinary system

The main limitation of our study is the small number of patients; however, EPSCC are rare tumors. There is no targeted therapy approved for the treatment of small cell carcinoma to date. Knowing KRAS mutational status in these tumors may provide further treatment options. There is also a need for other studies examining the genetic profile of SCLC and EPSCC.

In conclusion, there may be ethnical and geographical differences in KRAS mutations of SCLC as in NSCLC and colorectal cancers. A comparison of the incidence of KRAS mutations in different populations may guide the studies on targeted treatment. KRAS mutations should not be excluded in SCLC.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Trakya University School of Medicine.

**Informed Consent:** N/A.

**Peer-review:** Externally peer-reviewed.

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## REFERENCES

1. Siegel RL, Sahar L, Portier KM, Ward EM, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015;65:5-29. [\[Crossref\]](#)
2. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* 2012;13:239-46. [\[Crossref\]](#)
3. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. *N Engl J Med* 2010;363:1693-703. [\[Crossref\]](#)
4. Chute JP, Chen T, Feigal E, Simon R, Johnson BE. Twenty years of phase III trials for patients with extensive-stage small-cell lung cancer: perceptible progress. *J Clin Oncol* 1999;17:1794-801.
5. Sher T, Dy GK, Adjei AA. Small cell lung cancer. *Mayo Clin Proc* 2008;83:355-67. [\[Crossref\]](#)
6. Jackman DM, Johnson BE. Small-cell lung cancer. *Lancet* 2005;366:1385-96. [\[Crossref\]](#)
7. Wong YN, Jack RH, Mak V, Henrik M, Davies EA. The epidemiology and survival of extrapulmonary small cell carcinoma in South East England, 1970-2004. *BMC Cancer* 2009;9:209. [\[Crossref\]](#)
8. Arrington AK, Heinrich EL, Lee W, Duldulao M, Patel S, Sanchez J, et al. Prognostic and predictive roles of KRAS mutation in colorectal cancer. *Int J Mol Sci* 2012;13:12153-68. [\[Crossref\]](#)
9. Bamford S, Dawson E, Forbes S, Clements J, Pettett R, Dogan A, et al. The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website. *Br J Cancer* 2004;91:355-8. [\[Crossref\]](#)
10. Cicin I, Usta U, Karagol H, Uzunoglu S, Kocak Z. Extrapulmonary small cell carcinoma localized in lymph nodes: is it a different clinical entity? *Acta Oncol* 2009;48:354-60. [\[Crossref\]](#)
11. Cicin I, Karagol H, Uzunoglu S, Uygun K, Usta U, Kocak Z, et al. Extrapulmonary small-cell carcinoma compared with small-cell lung carcinoma: a retrospective single-center study. *Cancer* 2007;110:1068-76. [\[Crossref\]](#)
12. Riely GJ, Kris MG, Rosenbaum D, Marks J, Li A, Chitale DA, et al. Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. *Clin Cancer Res* 2008;14:5731-4. [\[Crossref\]](#)
13. Seo JS, Ju YS, Lee WC, Shin JY, Lee JK, Bleazard T, et al. The transcriptional landscape and mutational profile of lung adenocarcinoma. *Genome Res* 2012;22:2109-19. [\[Crossref\]](#)
14. Sos ML, Thomas RK. Genetic insight and therapeutic targets in squamous-cell lung cancer. *Oncogene* 2012;31:4811-4. [\[Crossref\]](#)
15. Brose MS, Volpe P, Feldman M, Kumar M, Rishi I, Herrero R, et al. BRAF and RAS mutations in human lung cancer and melanoma. *Cancer Res* 2002;62:6997-7000.
16. Couraud S, Zalcman G, Milleron B, Morin F, Souquet PJ. Lung cancer in never smokers--a review. *Eur J Cancer* 2012;48:1299-311. [\[Crossref\]](#)
17. Karachalio N, Mayo C, Costa C, Magri I, Gimenez-Capitan A, Molina-Vila MA, et al. *Clin Lung Cancer* 2013;14:205-14. [\[Crossref\]](#)
18. Shepherd FA, Domerg C, Hainaut P, Janne PA, Pignon JP, Graziano S, et al. Pooled analysis of the prognostic and predictive effects of KRAS mutation status and KRAS mutation subtype in early-stage resected non-small-cell lung cancer in four trials of adjuvant chemotherapy. *J Clin Oncol* 2013;31:2173-81. [\[Crossref\]](#)
19. Smits AJ, Kummer JA, Hinrichs JW, Herder GJ, Scheidel-Jacobse KC, Jiwa NM, et al. EGFR and KRAS mutations in lung carcinomas in the Dutch population: increased EGFR mutation frequency in malignant pleural effusion of lung adenocarcinoma. *Cell Oncol (Dordr)* 2012;35:189-96. [\[Crossref\]](#)
20. Maitra A, Tascilar M, Hruban RH, Offerhaus GJ, Albores-Saavedra J. Small cell carcinoma of the gallbladder: a clinicopathologic, immunohistochemical, and molecular pathology study of 12 cases. *Am J Surg Pathol* 2001;25:595-601. [\[Crossref\]](#)
21. Wakuda K, Kenmotsu H, Serizawa M, Koh Y, Isaka M, Takahashi S, et al. Molecular profiling of small cell lung cancer in a Japanese cohort. *Lung Cancer* 2014;84:139-44. [\[Crossref\]](#)
22. Yamamoto H, Shigematsu H, Nomura M, Lockwood WW, Sato M, Okumura N, et al. PIK3CA mutations and copy number gains in human lung cancers. *Cancer Res* 2008;68:6913-21. [\[Crossref\]](#)
23. Kodaz H, Hacibekiroglu I, Erdogan B, Turkmen E, Tozkir H, Alabayrak D, et al. Association between specific KRAS mutations and the clinicopathological characteristics of colorectal tumors. *Mol Clin Oncol* 2015;3:179-84.
24. Murtaza BN, Bibi A, Rashid MU, Khan YI, Chaudri MS, Shaikoori AR. Spectrum of K ras mutations in Pakistani colorectal cancer patients. *Braz J Med Biol Res* 2014;47:35-41. [\[Crossref\]](#)