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Pulmonary Adenocarcinoma With Signet Ring Cell Features:

A Comprehensive Study From 3 Distinct Patient Cohorts

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Abstract

Comprehensive biological characteristics of pulmonary adenocarcinomas with signet ring cell features (SRC⁺) are not well known. Herein, we systematically evaluated clinical and molecular features of SRC⁺ cases with particular attention to smoking status. Surgically treated lung adenocarcinomas (n=763) with follow-up 5 years in 3 cohorts were reviewed: all patients in 2006 to 2007 (“all-comers,” n=222; 168 ever-smokers), a never-smoker cohort (n=266), and a cohort of ever-smokers (n=275). SRC⁺ tumors had 10% of SRCs agreed by 2 pathologists. SRC⁺ cases were tested for rearrangement of *ALK* and *ROS1*, as well as 187 known mutations in 10 oncogenes including *EGFR*, *KRAS*, *BRAF*, *ERBB2*, *JAK2*, *AKT1*, *AKT2*, *KIT*, *MET*, and *PIK3CA*. Overall, 53 of 763 cases (7%) were SRC⁺. In the 2006 to 2007 “all comer” cohort, 9% were SRC⁺. In the never-smoker cohort, 9% were SRC⁺. In the smoker cohort, 3% were SRC⁺. Univariable analysis showed that SRC⁺ never-smokers had shorter overall and disease-free survival ($P=0.006$ and 0.0004 , respectively), but the significance faded in the multivariable analysis. For the other 2 cohorts, crude 5-year survival was decreased by 6% to 27% in SRC⁺ cases without reaching statistical significance. In SRC⁺ tumors, *KRAS* mutation was most common (29%), followed by *ALK* (26%), *EGFR* (18%), *ROS1* (6%), *BRAF* (6%), and *PIK3CA* (3%). In summary, SRC⁺ tumors in never-smokers had a worse survival by univariable analysis only. SRC⁺ cases seemed enriched for *ALK*⁺ and *ROS1*⁺, and other mutations were generally in keeping with the patient’s smoking status.

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Keywords

signet ring cell; *ALK*; *ROS1*; lung; adenocarcinoma; smoking status; mutations; survival

Signet ring cells (SRCs) are best known in the setting of poorly differentiated adenocarcinomas of the gastrointestinal tract, especially the stomach, colon, and appendix.¹ SRCs are characterized by singly dispersed tumor cells with intracytoplasmic mucin and an eccentrically displaced nucleus.¹ SRCs are a rare feature of primary pulmonary adenocarcinoma.^{2–6} Because of their rarity in primary tumors of the lung, metastatic disease is often considered in the differential diagnosis. If the differential diagnosis cannot be resolved on careful histologic examination, immunostains are often helpful, as primary pulmonary adenocarcinomas with SRCs frequently express thyroid transcription factor-1 (TTF-1) and CK7 but are usually negative for CK20 and CDX-2.^{2,7–9}

Several studies have suggested that pulmonary adenocarcinomas with SRC features (SRC⁺) may have a more aggressive clinical course and may occur more frequently in nonsmoking patients.^{6,8,10,11} However, their comprehensive clinical and molecular characteristics are not well known because of a relative paucity of large series on pulmonary adenocarcinomas with SRC⁺. In addition, the diagnosis of SRC⁺ can be difficult, given the subjectivity in separating true SRC from morphologic mimics including cytoplasmic vacuolization, clear cell change, and degenerative changes such as tumor cell “ballooning.” Thus, one may have to question the findings of some previous studies that did not undergo a central review to confirm the diagnosis by careful histologic evaluation.

SRC⁺ primary pulmonary adenocarcinomas have been associated with *ALK* gene rearrangement (*ALK*⁺).^{12–14} The *EML4-ALK* fusion was described in 2007 and occurs in 3% to 7% of pulmonary adenocarcinomas.^{15–17} More recently, chromosomal rearrangements involving the *ROS1* receptor tyrosine kinase gene have been described as a rare (<3%) driver mutation in pulmonary adenocarcinomas, and the tumors with *ROS1* rearrangement (*ROS1*⁺) have been shown to respond to crizotinib.^{18–20} Patients with *ROS1*⁺ tend to be younger and more likely to be never-smokers, similar to those with *ALK* rearranged tumors (*ALK*⁺).^{18,21} Whereas *ALK*⁺ lung adenocarcinomas with SRC⁺ have been widely reported, the incidence of *ROS1*⁺ in SRC⁺ lung adenocarcinomas is not well known.

The main goal of our present study is to better characterize the clinicopathologic features of pulmonary adenocarcinomas with SRC⁺, including relationship to smoking status and clinical outcome, on the basis of a large number SRC⁺ cases confirmed by a careful histopathologic evaluation. In addition, we also examined the genetic abnormalities in SRC⁺ tumors including *ALK*, *ROS1*, and other genes known to be mutated in pulmonary adenocarcinomas, aligned with the patients' smoking status.

MATERIALS AND METHODS

The study protocol was approved by the Mayo Foundation Institutional Review Board. Surgically treated lung adenocarcinomas (n=763) in 3 non-overlapping cohorts representing distinct patient groups were reviewed by 2 pulmonary pathologists: a cohort including all

pulmonary adenocarcinoma patients who underwent definitive surgical management in 2006 to 2007 with available 5-year follow-up (“all-comers,” n=222); a never-smoker cohort (“never-smokers,” 1997 to 2009, n=266); and a cohort of current and former smokers (“ever-smokers,” 1997 to 2009, n=275). It is noteworthy that the original pathology database search for the ever-smoker cohort was enriched for cases having the key word “bronchioloalveolar features” in the original diagnosis. The decision to design the cohorts in this manner has several justifications. On the basis of current literature, it seems possible that the incidence, clinical behavior, and mutation profile of SRC⁺ pulmonary adenocarcinomas may be different between smokers and never-smokers. Thus it was important to look at large cohorts of both smokers and never-smokers. As never-smokers represent a minority of nonselected cohorts, we specifically selected 300 never-smokers to possibly enrich for SRC⁺ cases and reach the desired cohort size of never-smokers to compare with a similarly sized group of smokers. The “all-comers” cohort was included also to look at overall incidence and to look at patients presenting in a narrow time period (2006 to 2007) in an attempt to control for any changes in incidence or treatment strategies over time.

Two thoracic pathologists reviewed all available diagnostic pathology slides and assessed tumors for SRC features. A tumor was classified as having SRC features if 10% of the tumor cells showed classic SRC morphologic features, including single cell pattern, loss of basal nuclear orientation, relative discohesion, intracytoplasmic mucin, and margination/compression of the nucleus. The minority of cases in which there was disagreement between the 2 pathologists were reconciled by double scoping with consensus decision. Mucicarmine staining and immunohistochemical (IHC) analyses using antibodies directed against TTF-1 (Clone SPT24; Leica Novocastra, New Castle, UK) and CDX-2 (Cell Marque, Rocklin, CA) were performed on all cases with SRC features.

IHC for ALK and ALK fluorescence in situ hybridization (FISH) were performed on all SRC⁺ cases as previously described.²² ALK status was confirmed by FISH for cases with any degree of IHC positivity given the fact that completely negative IHC always correlated with FISH-negative status in our previous studies. Inter-phase molecular cytogenetic studies using commercially available ALK probe (Vysis, Des Plaines, IL) were performed on formalin-fixed paraffin-embedded (FFPE) sections. ROS1 FISH was performed on FFPE slides using a dual-color, single-fusion probe set composed of Vysis’ ROS (Cen) Spectrum Green probe and ROS1 (Tel) Spectrum Orange probe (Abbott Molecular).

Lung Cancer Mutation Screening Panel (Lu- CaMSP) analysis was performed on all SRC⁺ cases with sufficient tissue as previously described.²² DNA was extracted from FFPE tumor samples. The unstained FFPE tissues were deparaffinized using xylene. After the ethanol series, DNA was extracted with the AllPrep DNA/RNA FFPE kit (Qiagen Inc., Valencia, CA) following manufacturer’s protocol. Samples were run for 187 individual mutations in 10 genes including *EGFR*, *KRAS*, *BRAF*, *ERBB2*, *JAK2*, *AKT1*, *AKT2*, *KIT*, *MET*, and *PIK3CA*. All positive results were confirmed by gene sequencing.

Clinical and demographic information was obtained from the Mayo Clinic Lung Cancer Epidemiology Database. Descriptive statistics were used. In addition, 4 multivariable

models were created for each of the 3 cohorts: (a) overall survival (OS) using a 95% cutoff for lepidic growth as a possible covariate; (b) OS using a 90% cutoff for lepidic growth as a possible covariate; (c) disease-free survival (DFS) using a 95% cutoff for lepidic growth as a possible covariate; and (d) DFS using a 90% cutoff for lepidic growth as a possible covariate. The multivariable models were constructed by a stepwise procedure with a 0.1 significance level for variable entry and removal. Ten covariates were considered in the model selection process: age at diagnosis; sex; smoking status (never, former, and current)—not included in the never-smokers cohort; stage (I, II to IV); invasion type (invasive adenocarcinoma vs. lepidic predominant tumor); tumor grade (well differentiated, moderately differentiated, and poorly/undifferentiated); lung cancer history; surgery; chemotherapy; and radiation. For assigning histologic grade of tumor, we used the grade appearing in the original report of diagnosis, which has been shown to be an independent prognostic factor for survival in an analysis of 5018 hospital-based and 712 population-based non-small cell lung carcinoma cases in our previous study.²³

RESULTS

Clinical and pathologic features of all the patients are summarized by different cohorts in Table 1. The entire study group had an average age of 68 years (range, 17 to 91 y), with a female predominance (61%). Most patients were white (93%). When comparing the 3 cohorts to one another, differences included lower stage ($P<0.0001$) and grade ($P<0.0001$) in the ever-smoker cohort (likely due to enrichment for lepidic tumors, as original database search included preference for tumors including the diagnostic term “bronchioloalveolar features”). The never-smokers had a higher number of female patients (73%, $P<0.0001$), as would be expected.

Overall, 53 of 763 cases (7%) were SRC⁺. The percentage of SRC⁺ cases varied by cohort, comprising 9% of both the all-comers cohort and the never-smokers cohort but only 3% of the ever-smoker cohort. Strict definition of SRC morphology was used to ensure a homogenous study group, with consensus agreement by 2 pathologists (Fig. 1A). Several mimics of SRCs were noted, including “ballooning”-type degenerative change (Fig. 1B), clear cell change (Fig. 1C), and intracytoplasmic mucin in the absence of the other morphologic features. Tumors with these mimics were not included in the SRC group, to ensure a homogenous study population of patients with tumors showing classic SRC features. All 53 SRC⁺ cases were positive for TTF-1 (Fig. 1D). Although not strictly required for inclusion in the study, mucicarmine nicely highlighted intracytoplasmic mucin in most cases (87%, Fig. 1E). Only a single case (2%) showed focal CDX-2 staining.

Clinical Characteristics

All-Comers Cohort—Seventy-six percent of patients were former or current smokers, whereas 24% were never-smokers. Twenty SRC⁺ cases were found in this cohort (9%). Between SRC[−] and SRC⁺ patients, there was no significant difference in age at diagnosis, sex, race, or smoking status, despite a trend toward male predominance ($P=0.06$) in the patients with SRC⁺ tumors. SRC⁺ cases were higher stage ($P=0.003$) and displayed a trend toward increased need for adjuvant therapy ($P=0.058$). However, no difference in OS or

DFS was seen when comparing SRC⁺ with SRC⁻ cases in univariable and multivariable analyses (Fig. 2). Overall features of the all-comers cohort (n=222) based on SRC status are summarized in Supplement Table 1 (Supplemental Digital Content 1, <http://links.lww.com/PAS/A220>).

Never-Smokers Cohort—Twenty-four SRC⁺ cases were found in this cohort (9%). Between SRC⁻ and SRC⁺ patients, there was no significant difference in age at diagnosis, sex, or race in this cohort. However, SRC⁺ cases were higher grade ($P=0.0003$), higher stage ($P=0.004$), more likely to experience lung cancer progression at 5 years (58% vs. 26% in the SRC⁻ group, $P=0.0007$), and had worse OS ($P=0.006$) and DFS ($P=0.0004$) when compared with SRC⁻ cases by univariable analysis. When multivariable analysis was performed, SRC status was not an independent predictor of survival (Fig. 3), indicating that the adverse outcome seen in these patients is likely due to the higher grade and stage seen in tumors with SRCs. Overall features of the never-smokers cohort (n=266) based on SRC status are summarized in Supplement Table 2 (Supplemental Digital Content 2, <http://links.lww.com/PAS/A221>).

Ever-Smokers Cohort—Nine SRC⁺ cases were found in this cohort (3%). No significant difference in age at diagnosis, sex, race, or stage was observed when comparing SRC⁺ with SRC⁻ cases. There was no significant difference in DFS between SRC⁺ and SRC⁻ cases by univariable or multivariable analysis (Fig. 4). It is noteworthy, however, that multivariable analysis for OS revealed higher mortality (hazard ratio=2.3, 95% confidence interval=1.01–5.27, $P=0.048$) in SRC⁺ cases than in SRC⁻ cases (Fig. 4), whereas univariable analysis for OS did not show a significant difference by SRC status (hazard ratio=1.8, 95% confidence interval =0.81–4.19, $P=0.146$). Overall features of the ever-smokers cohort (n=275) based on SRC status is summarized in Supplement Table 3 (Supplemental Digital Content 3, <http://links.lww.com/PAS/A222>).

Molecular Results

Of the 53 SRC⁺ cases, 14 cases (26%) showed *ALK* rearrangement (*ALK*⁺) by FISH. *ALK* immunostain results in these 14 cases included 11 with 3+ staining, 2 with 2+, and 1 with 1+. Fourteen other SRC⁺ cases showed 2+ (4 cases) and 1+ (10 cases) IHC but were negative for *ALK* FISH. The number of *ALK*⁺ SRC⁺ cases varied by cohort, but this was not statistically significant ($P=0.1385$): 6 of 20 all-comers (30%), 8 of 24 never-smokers (33%), and 0 of 9 ever-smokers were *ALK*⁺.

Forty-seven of 53 SRC⁺ cases had sufficient tissue for *ROS1* FISH testing, and 3 of these 47 SRC⁺ cases (6%) showed *ROS1* gene rearrangement (*ROS1*⁺). All 3 *ROS1*⁺ patients were never-smokers and were negative for other driver mutations tested in the present study. Among the 47 SRC⁺ cases tested for *ROS1* FISH, *ROS1*⁺ tumors comprised 3 of 34 (9%) *ALK*-negative tumors, 3 of 21 (14%) tumors in never-smokers, 3 of 13 (23%) *ALK*-negative tumors in never-smokers, 3 of 11 (27%) tumors that were “pan negative” (negative for *ALK* rearrangement and for *EGFR* and *KRAS* mutations), and 3 of 5 (60%) pan-negative tumors in never-smokers.

Molecular testing with the full LuCaMSP panel was successful in 49 SRC⁺ cases. Results are summarized in Table 2. Fourteen cases (29%) had *KRAS* mutations, 13 of which were codon 12 missense mutations. Nine *EGFR* mutations were detected (18%), including 4 exon 19 deletions, 4 L858R mutations, and 1 novel exon 20 insertion. Nine *MET* mutations were detected; but on the basis of the literature and our previous experience with this mutation panel,²² these likely represent germline polymorphisms and not true pathogenic mutations. These *MET* polymorphisms occurred with a number of other pathogenic mutations, including *ALK* rearrangement and *EGFR* and *KRAS* mutations. Three *BRAF* V600E mutations were detected, as well as a single case of mutation in *PIK3CA* E545K. The molecular results based on smoking status (from all cohorts, all-comers were divided according to smoking status) are shown in Table 2. The expected profile was seen, with *KRAS* mutations predominantly in smokers and *EGFR* mutations mostly in never-smokers. The *BRAF* mutations occurred in smokers. There was 1 tumor that had *BRAF* mutation and *ALK* rearrangement and 1 tumor that had *KRAS* and *PIK3CA* mutations.

DISCUSSION

In the present study, we reviewed a large number of pulmonary adenocarcinomas to identify SRC⁺ cases, to characterize their clinical and molecular features. Comprehensive clinicopathologic characteristics of SRC⁺ pulmonary adenocarcinomas have not been widely reported. Moreover, there has been no study closely looking into the smoking status of SRC⁺ patients as a potential parameter for their clinical and molecular manifestations. Therefore, we carefully selected 3 different cohorts mainly according to their smoking status: all-comers during a 2- year period encompassing both smokers and nonsmokers, never-smokers, and ever-smokers.

Determination of SRC⁺ tumors can be quite challenging in practice, as pulmonary adenocarcinoma is so morphologically diverse, and many mimics of SRCs were encountered in our review. These mimics included balloon- type degeneration of tumor cells, tumor cells with clear cytoplasm, and intracytoplasmic mucin without true SRC morphology as shown in Figures 1B and C. In an attempt to ensure a homogenous study population, we used stringent criteria with central review and consensus decision between 2 pathologists to determine SRC⁺ status, with an emphasis on “classic” SRC features. These features included discohesive growth with intracytoplasmic mucin that compressed the nucleus as illustrated in Figure 1A.

The results of our study support that pulmonary adenocarcinomas with SRC⁺ in never-smokers are more likely to show *ALK* gene rearrangements than conventional pulmonary adenocarcinomas, as has been suggested by other authors.^{12,13} We detected these alterations using IHC for *ALK* and *ALK* gene rearrangement by FISH. SRC features may be a morphologic clue in never-smokers that a tumor has the *EML4-ALK* rearrangement. However, the correlation does not seem to be sufficient to capture all cases—that is, not all cases with SRCs will show *ALK* gene rearrangement, and not all cases with *ALK* gene rearrangement are SRC⁺.

Rearrangement of the *ROS1* gene in pulmonary adenocarcinoma, which has been associated with response to crizotinib, has very recently been associated with similar morphology to *ALK*⁺ tumors, including SRC⁺ and mucinous/cribriform morphology.^{18,21,24} In the present study, we found *ROS1*⁺ in 3 of 47 (6%) SRC cases tested. All of our *ROS1*⁺ and *ALK*⁺ tumors were mutually exclusive and negative for other driver mutations including *KRAS*, *EGFR*, and *BRAF* albeit rare exceptions. The *ROS1*⁺ rate in SRC⁺ tumors (6%) seems to be higher than the reported incidence (1% to 2%) among nonselected lung adenocarcinoma patients,^{18,19} although it is lower than the observed *ALK*⁺ rate (28%) in our SRC⁺ cases. Moreover, further enrichment for *ROS1*⁺ tumors could be achieved by considering never-smokers combined with negative status of other driver mutations: 14% of SRC⁺/never-smokers, 23% of *ALK*⁻/SRC⁺/never-smokers, 27% of SRC⁺/“pan-negative” tumors (*ALK*⁻, negative for *EGFR* and *KRAS* mutations), and 60% of SRC⁺/“pan-negative”/never-smokers.

Our findings suggest that SRC⁺ tumors are associated with shorter DFS and OS in never-smokers but that this does not have independent prognostic significance when considering multivariable analysis. This supports the notion proposed by others that these tumors are associated with more aggressive behavior and poor clinical outcome,^{6,8,10,11} but this is likely related to the higher stage in SRC⁺ tumors. Although a previous study has shown that SRCs have prognostic significance independent of stage and grade,⁶ SRCs in our study did not have independent prognostic significance in multivariable analysis in any cohort. It is interesting to note that the shorter OS and DFS for SRC⁺ cases in the never-smokers by univariable analysis were not apparent in the other 2 cohorts. It might be due to the fact that adenocarcinomas arising in never-smokers represent a more genetically homogenous group than those in smokers; adenocarcinomas in never-smokers would have known enrichment of tumors with *ALK* or *ROS1* rearrangement, as well as *EGFR* mutation. It could also be that when considering never-smokers, the separation of the SRC⁺ cases (which would be enriched for *ALK*⁺) from the SRC⁻ cases (which would be enriched for *EGFR* mutation) may have been responsible for this finding; *EGFR* mutations are associated with lepidic predominant growth and better prognosis,^{25,26} whereas *ALK*⁺ tumors have been associated with a poorer prognosis.²⁷ This factor may underlie the difference in prognosis observed on univariable analysis for nonsmokers with SRC⁺ in this and other studies. It is noteworthy that SRC⁺ patients in the ever-smoker cohort seem to have an approximately 2-fold greater hazard of death than SRC⁻ patients, both by unadjusted and adjusted (the latter reaching statistical significance) analyses. These results need further investigation, but there are too few SRC⁺ cases to check interactions between SRC status and the other covariates in our present study.

As expected, the vast majority of the driver mutations observed in our study were mutually exclusive. However, there was a single case with both *KRAS* G12V and *PIK3CA* E545K mutations. *PIK3CA* mutations have been previously described to occur with other driver mutations, including *EGFR* mutations.^{28,29} Another case had both *ALK* rearrangement and *BRAF* V600E, but this case showed a low percentage of *BRAF*-mutated cells (20% by LuCaMSP, 5% by Sanger sequencing). This may have represented a small tumor subclone with *BRAF* mutation. However, it is impossible to determine whether the *ALK*

rearrangement and *BRAF* mutation occurred in the same tumor cells, or whether these represented distinct subclones within the tumor.

In summary, SRC⁺ tumors were seen in 7% of our adenocarcinoma cases and associated with more aggressive clinical course in the never-smoker cohort, likely explained by higher tumor stage, as SRC status is not an independent predictor of survival on multivariable analysis. SRC⁺ is a morphologic clue that a tumor may have rearrangement of the *ALK* gene, although not entirely sensitive or specific for such change. The incidence of *ROS1*⁺ rearrangement might be quite high among pan mutation–negative, SRC⁺ tumors occurring in never-smokers. Pulmonary adenocarcinomas with SRC⁺ showed mutations in other genes (namely *KRAS* and *EGFR*) in a pattern that would be expected on the basis of the patients' smoking status.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Pulmonary adenocarcinoma with SRC features (A), which should be distinguished from morphologic mimics including tumors with “balloon” degeneration (arrows) (B) and clear cell change (C) on hematoxylin and eosin staining. Positivity for TTF-1 (D) was observed in all adenocarcinoma cases with SRC features, supporting a lung primary site, and intracytoplasmic mucin could be highlighted using mucicarmine (E).

FIGURE 2.
 All-comers (2006 to 2007) cohort: Adjusted survival curves for OS (A) and DFS (B).

FIGURE 3.
Never-smokers cohort: Adjusted survival curves for OS (A) and DFS (B).

FIGURE 4.
Ever-smokers cohort: Adjusted survival curves for OS (A) and DFS (B).

TABLE 1

Comparison of 3 Study Cohorts

	2006–2007 All-Comers (N=222)	Never-Smokers (N=266)	Lepidic-enriched Ever-Smokers (N=275)	Total (N=763)	P
SRC (n [%])					0.0112
SRC <10%	202 (91.0)	242 (91.0)	266 (96.7)	710 (93.1)	
SRC 10%	20 (9.0)	24 (9.0)	9 (3.3)	53 (6.9)	
Age at diagnosis					0.1758
N	222	266	275	763	
Mean (SD)	67.0 (11.1)	66.8 (12.2)	69.0 (9.3)	67.7 (11.0)	
Sex (n [%])					<0.0001
Female	122 (55.0)	195 (73.3)	149 (54.2)	466 (61.1)	
Male	100 (45.0)	71 (26.7)	126 (45.8)	297 (38.9)	
Race (n [%])					0.0811
Missing	5	7	6	18	
White	199 (91.7)	237 (91.5)	258 (95.9)	694 (93.2)	
Other	18 (8.3)	22 (8.5)	11 (4.1)	51 (6.8)	
Smoking status (n [%])					<0.0001
Never	50 (22.5)	266 (100.0)	0 (0.0)	316 (41.4)	
Former	117 (52.7)	0 (0.0)	204 (74.2)	321 (42.1)	
Current	55 (24.8)	0 (0.0)	71 (25.8)	126 (16.5)	
Stage (n [%])					<0.0001
I	150 (67.6)	154 (57.9)	218 (79.3)	522 (68.4)	
II	15 (6.8)	13 (4.9)	17 (6.2)	45 (5.9)	
III	37 (16.7)	56 (21.1)	22 (8.0)	115 (15.1)	
IV	20 (9.0)	43 (16.2)	18 (6.5)	81 (10.6)	
Grade (n [%])					<0.0001
Well differentiated	114 (51.4)	147 (55.3)	202 (73.5)	463 (60.7)	
Moderately differentiated	86 (38.7)	95 (35.7)	57 (20.7)	238 (31.2)	
Poorly/undifferentiated	22 (9.9)	24 (9.0)	6 (2.2)	52 (6.8)	

TABLE 2Molecular Changes in SRC⁺ Cases by Smoking Status (All Cohorts)

Mutation Gene	Never-Smokers (n=27)	Ever-Smokers (n=22)
<i>ALK</i>	9	5
<i>ROS1</i>	3	0
<i>EGFR</i>	8	1
<i>KRAS</i>	3	11
<i>BRAF</i>	0	3
<i>PIC3CA</i>	0	1
<i>MET</i> (SNPs)	4	5