4-1-2023

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Brief Report: Prognostic Relevance of 3q Amplification in Squamous Cell Carcinoma of the Lung

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Received 3 October 2022; revised 9 February 2023; accepted 19 February 2023
Available online - 28 February 2023

ABSTRACT

Introduction: Amplification of 3q is the most common genetic alteration identified in squamous cell carcinoma of the lung (LUSC), with the most frequent amplified region being 3q26 to 3q28.

Methods: In this analysis, we aim to describe the prognostic relevance of 3q amplification by focusing on a minimal common region (MCR) of amplification constituted of 25 genes. We analyzed 511 cases of LUSC from The Cancer Genome Atlas and included 476 in the final analysis.

Results: We identified a 25-gene MCR that was amplified in 221 (44.3%) cases and was associated with better disease-specific survival (not reported [NR] versus 9.25 y, 95% confidence interval [CI]: 5.24–NR, log-rank $p = 0.011$) and a progression-free interval of 8 years (95% CI: 5.1–NR) versus 4.9 years (95% CI: 3.5–NR, log-rank $p = 0.020$). Multivariable analysis revealed that MCR amplification was associated with improved disease-specific survival and progression-free interval.

Conclusions: Amplification of the 25-gene MCR within 3q was present in 44% of this cohort, consisting mainly of Caucasian patients with early stage LUSC. This analysis strongly indicates the prognostic relevance of the 25-gene MCR within 3q. We are further evaluating its prognostic and predictive relevance in a racially diverse patient population with advanced LUSC.

Keywords: Squamous cell carcinoma of the lung; 3q; Amplification; Biomarker; NSCLC

Introduction

Lung cancer is the second most common cancer and the leading cause of cancer-related deaths worldwide. The most common type of lung cancer is lung cancer adenocarcinoma (LUAD), followed by lung cancer squamous cell carcinoma (LUSC), both of which comprise most of NSCLC.[1] The outcomes of patients with lung cancer have changed substantially since the identification of driver mutations and the introduction of targeted

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Dr. Gadgeel contributed as a senior author.

Disclosure: Dr. Gadgeel reports receiving compensation for participating in advisory boards from AstraZeneca, Genentech/Roche, AbbVie, Takeda, Bristol Myers-Squibb, Blueprint, Lilly, Merck, GlaxoSmithKline, Daichii, Esai, Pfizer, Janssen, and Mirati; and travel support from Merati. He is a member of the Data Safety Monitoring Board of two trials sponsored by AstraZeneca. Dr. Palanisamy serves as a member of an advisory board for AstraZeneca. Dr. Abu Rous received honoraria from MJH Life Sciences and IntrinsiQ Specialty Solutions. The remaining authors declares no conflict of interest.

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Cite this article as: Abu Rous F, Li P, Carskadon S, et al. Brief report: prognostic relevance of 3q amplification in squamous cell carcinoma of the lung. *JTO Clin Res Rep*. 2023;4:100486.

ISSN: 2666-3643

https://doi.org/10.1016/j.jtocrr.2023.100486

therapies and immune checkpoint inhibitors.[2] Driver mutations that can be therapeutically targeted are more often detected in LUAD and rarely detected in patients with LUSC. Hence, patients with advanced LUSC are typically treated with platinum doublet chemotherapy and immune checkpoint inhibitors without the benefit of targeted therapies.[2] Despite this combination's unprecedented response rate in metastatic LUSC, approximately 40% of the patients had no response[2]; therefore, identifying molecular biomarkers in LUSC for prognostic and predictive purposes is urgently needed.

Amplification of 3q is the most common genetic alteration observed only in LUSC, with the most frequently amplified region being 3q26 to 3q28.[3] It has been described in preinvasive and invasive LUSC and represents one of the most striking differences between LUSC and LUAD.[4] Interestingly, 3q amplification is prevalent and carries prognostic relevance in head and neck, cervical, and esophageal squamous cell cancers.[5] The distal amplified area of 3q includes genes that are key in squamous differentiation, such as SOX-2, p63, and PIK3CA, which explains the prevalence and importance of this genetic alteration in squamous cell carcinomas.[5] Many studies that reported the significance of 3q amplification in LUSC investigated the entire 3q region (approximately 40 genes) or focused on one to two genes of known biological relevance across tumor types such as SOX-2, p63, PI3KCA, and FGFR1.[6–9] In our study, we have identified a minimal common region (MCR) of amplification constituted of 25 genes within 3q using The Cancer Genome Atlas (TCGA) LUSC data set and evaluated its prognostic relevance.

Materials and Methods

We identified 511 patients with LUSC in the TCGA database; five patients with synchronous disease were excluded. Outcome data were available for 499 patients and extracted from Clinical Data Resources. Copy number variation (CNV) data (amplification or deletion) were downloaded for these patients from CBioPortal; three patients had missing CNV data and were excluded from the analysis.

We detected a MCR of amplification (chr3: 181,711,924–183,428,101) approximately 1.7 MB within a large, amplified region between (chr3: 170,169,718–187,736,569) approximately 17.5 MB on chromosome 3q (Fig. 1). MCR was estimated on the basis of the smallest genomic interval amplified in most cases and consisted of 25 frequently amplified genes. Patients with partial MCR amplification (n = 20) were excluded. The final analysis was completed using clinical and CNV data for 476 patients. Information on MCR genes and statistical analysis are provided in the Supplementary Materials. Data used in this study were from the National Cancer Institute TCGA database. Informed consent was obtained from each patient before tissue collection.

Results

Patient Characteristics

In the overall cohort, the mean age was 67.4 years, 73.9% were males, and 26.1% were females. Race information was available for 367 cases; 332 (69.7%) identified as white, 26 (5.5%) identified as African American, and nine (1.9%) identified as Asian. Only six patients (1.3%) had stage IV disease, whereas 48.5% had stage I, 33.1% had stage II, and 17.2% had stage III. Regarding smoking status, 26.9% were current smokers, 67.4% were former smokers, and 3.4% were never smokers (Table 1).

MCR Amplification

MCR is constituted of 25 genes that were frequently amplified and signaled through the PI3K downstream pathway, which was altered in 71% of the cases (Supplementary Fig. 1). Full 25-gene MCR amplification was found in 221 (46.4%) patients, whereas 255 (53.6%) patients had no amplification. MCR amplification was associated with more CDKN2A homozygous deletion which was detected in 73 patients (33.2%) with MCR amplification and 59 (23.2%) without amplification (OR = 1.64, $p = 0.021$, 95% confidence interval [CI]: 1.07–2.54). No difference in TP53 alteration was detected between MCR-amplified and non-amplified cases (OR = 0.707, $p = 0.079$, 95% CI: 0.473–1.056). MCR amplification was strongly associated with PIK3CA amplification which was detected in 215 (97.3%) MCR-amplified cases compared with two (0.8%) non-amplified cases (OR = 4469, $p < 0.001$).

In MCR-amplified cases, all 25 genes within MCR were amplified but not necessarily highly expressed at the mRNA level. We identified four genes that were amplified and highly expressed, which are as follows: ABCC5, AP2M1, EIF4G1, and PSMD2.

Outcomes

The median disease-specific survival (DSS) of MCR-amplified cases was significantly longer than non-amplified cases (not reported [NR] versus 9.25 y, 95% CI: 5.24–NR, log-rank $p = 0.011$). The median progression-free interval (PFI) for amplified cases was 8 years (95% CI: 5.1–NR) versus 4.9 years (95% CI: 3.5–NR) for non-amplified cases (log-rank $p = 0.020$) (Fig. 2A and B). In multivariable analysis, stage III was associated with worse DSS (hazard ratio [HR] = 3.29, 95% CI: 1.93–5.60, $p < 0.001$) and PFI (HR = 2.40, 95% CI: 1.56–3.70, $p < 0.001$), and MCR amplification was

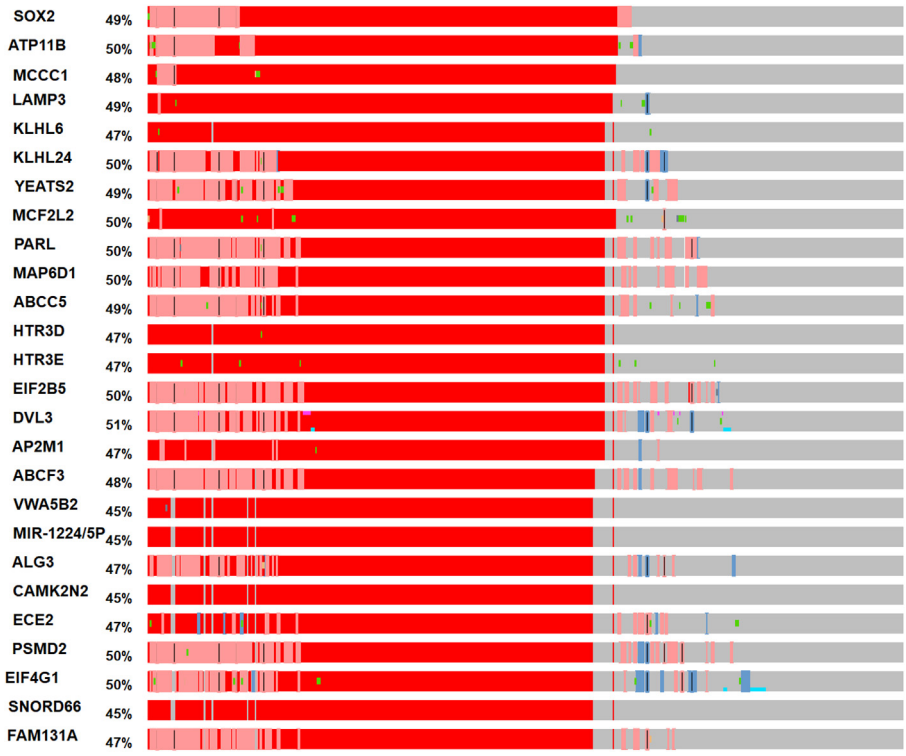

Figure 1. MCR of amplification, analyzed 17.5 MB region on 3q (chr3: 170,169,718-187,736,569) between PHC3 and BCL6. MCR was identified as 1.7 MB region (chr3: 181,711,924-183,428,101) from SOX2 to MCF2L2. MCR, minimal common region.

associated with improved DSS (HR = 0.63, 95% CI: 0.40–1.00, $p = 0.050$) and PFI (HR = 0.70, 95% CI: 0.50–1.00, $p = 0.049$). Compared with current smokers, former smokers had a lower risk of death (HR = 0.62, 95% CI: 0.38–0.99, $p = 0.044$) and progression (HR = 0.68, 95% CI: 0.47–0.99, $p = 0.045$) (Supplementary Table 1). We compared with the model of PIK3CA, and the association of PIK3CA amplification with DSS (HR = 0.71, 95% CI: 0.50–1.00, $p = 0.050$) and PFI (HR = 0.66, 95% CI: 0.42–1.04, $p = 0.075$) was less significant compared with the association with MCR amplification.

Discussion

Unlike patients with LUAD, patients with LUSC are rarely found to have single-driver genetic alterations that can be therapeutically targeted. In one study, 13% of LUSC samples were found to have at least one potentially targetable genetic alteration compared with 66% of LUAD samples.[10] Smoking as a risk factor plays an important role in the diverse genomic landscape of LUSC, making the task of identifying a single-driver genetic alteration very challenging. LUSC is characterized by a marked genomic complexity and a high rate of mutations (8.1 mutations per megabase), as revealed by a comprehensive genomic analysis of LUSC by TCGA. This analysis found recurrent mutations in 11 genes, including *TP53,* in almost all analyzed tumors. It also reported selective 3q amplification as the most common genetic alteration in LUSC, and it is the most notable difference between LUSC and LUAD.[11]

Many genes in this region have been evaluated as potential therapeutic targets, and their role as biomarkers was investigated as well. *SOX-2* is a "lineage-survival oncogene" that promotes squamous identity and encodes a transcription factor that has an important role in cellular proliferation and expansion. SOX-2 was reported to be altered in 17.7% of the samples in one cohort, and its overexpression was associated with a better median overall survival (68 versus 35 mo, $p = 0.036$).[6]

P63 is another gene within the 3q amplicon encoding a transcription factor that leads to cellular apoptosis after activating *TP53* genes. The *P63* gene encodes six splicing variants, ΔNp63 being the most common. ΔNp63 lacks the NH2-terminal domain altering its function to promote growth rather than apoptosis. Patients with tumors exhibiting genomic amplification of p63 had significantly prolonged survival ($P<0.05$) compared with P63 non-amplified patients.[7] PI3CKA amplification, which promotes cancer growth and migration, was detected in 37% of LUSC and was reported to be associated with worse survival in a Japanese cohort of 92 patients.[8] FGFR1 is a known oncogene of the FGFR family, and it was found to be amplified in

Table 1. Characteristics of the Study Cohort

Characteristics	Overall	MCR Amplified	MCR Nonamplified
Total, n (%)	476	221 (46.4)	255 (53.6)
Age at diagnosis, mean (SD)	67.4 (8.6)	66.6 (8.0)	68.1 (9.1)
Sex, n (%)			
Female	124 (26.1)	58 (26.2)	66 (25.9)
Male	352 (73.9)	163 (73.8)	189 (74.1)
Race, n (%)			
White	332 (69.7)	154 (69.7)	178 (69.8)
African American	26 (5.5)	12 (5.4)	14 (5.5)
Asian	9 (1.9)	3 (1.4)	6 (2.4)
Unknown	109 (22.9)	52 (23.5)	57 (22.4)
Stage, n (%)			
I	229 (48.5)	109 (49.8)	120 (47.4)
II	156 (33.1)	74 (33.8)	82 (32.4)
III	81 (17.2)	34 (15.5)	47 (18.6)
IV	6 (1.3)	2 (0.9)	4 (1.6)
Smoking, n (%)			
Current	128 (26.9)	62 (28.1)	66 (25.9)
Former	321 (67.4)	150 (67.9)	171 (67.1)
Never	16 (3.4)	4 (1.8)	12 (4.7)
Unknown	11 (2.3)	5 (2.3)	6 (2.4)
CDKN2A status, n (%)			
No alteration	342 (72.2)	147 (66.8)	195 (76.8)
Homozygous deletion	132 (27.8)	73 (33.2)	59 (23.2)
TP53 status, n (%)			
No alteration	319 (67)	139 (62.9)	180 (70.6)
Alteration	157 (33)	82 (37.1)	75 (29.4)
PIK3CA status, n (%)			
No amplification	259 (54.4)	6 (2.7)	253 (99.2)
Amplification	217 (45.6)	215 (97.3)	2 (0.8)

MCR, minimal common region.

16% of the samples in 226 patients; however, no association with survival was detected.[9] FXR1 was identified as a potential driver in the 3q amplicon by Qian et al.[12] They also reported that FXR1 overexpression is a poor prognostic factor in multiple solid malignancies, including NSCLC.[12]

It has been proposed that multiple genes in this area of amplification may have a synergistic effect on the progression of LUSC. Qian et al. identified a 12-gene signature within 3q from the LUSC data set in TCGA and reported a negative correlation between their 12-gene signature and gene groups involved in immune checkpoints and immune-related processes, indicating a suppressed immune pathway in their patient population.[13]

Evaluating a single gene within the 3q region has led to contradicting results, as described previously. Hence, we evaluated the region and identified MCR within 3q where all 25 genes were frequently amplified. We found this region to be amplified in 44.3% of LUSC in a cohort composed mainly of Caucasians (90.2%) with early stage diseases I to III (98.5%) and lacked adequate representation of the minority patient population and patients with advanced lung cancer. MCR amplification was strongly associated with PIK3CA amplification. Although PIK3CA is not located within the MCR (located on 3q26.32), its proximity with the MCR (starts from 3q26.33) can explain this strong association. Despite this strong association, MCR amplification is the primary driver of improved outcomes in this patient population because the results were less significant when modeling for PIK3CA amplification, and it has been found in previous studies that PIK3CA amplification predicts worse outcomes.[8] We identified four amplified and highly expressed genes within MCR, which are as follows: ABCC5, AP2M1, EIF4G1, and PSMD2. Our group has found EIF4G1 as a poor prognostic marker and a potential therapeutic target in multiple cancers.[14] ABCC5 is involved in recurrent gene rearrangement in LUSC, on the basis of our observations from the TCGA database, which requires further validation and characterization. The function of these amplified and highly expressed genes will be investigated in future studies.

This approach has been used by Qian et al.[13] as described previously; however, the 25-gene MCR not only carries a prognostic value but also has a potential

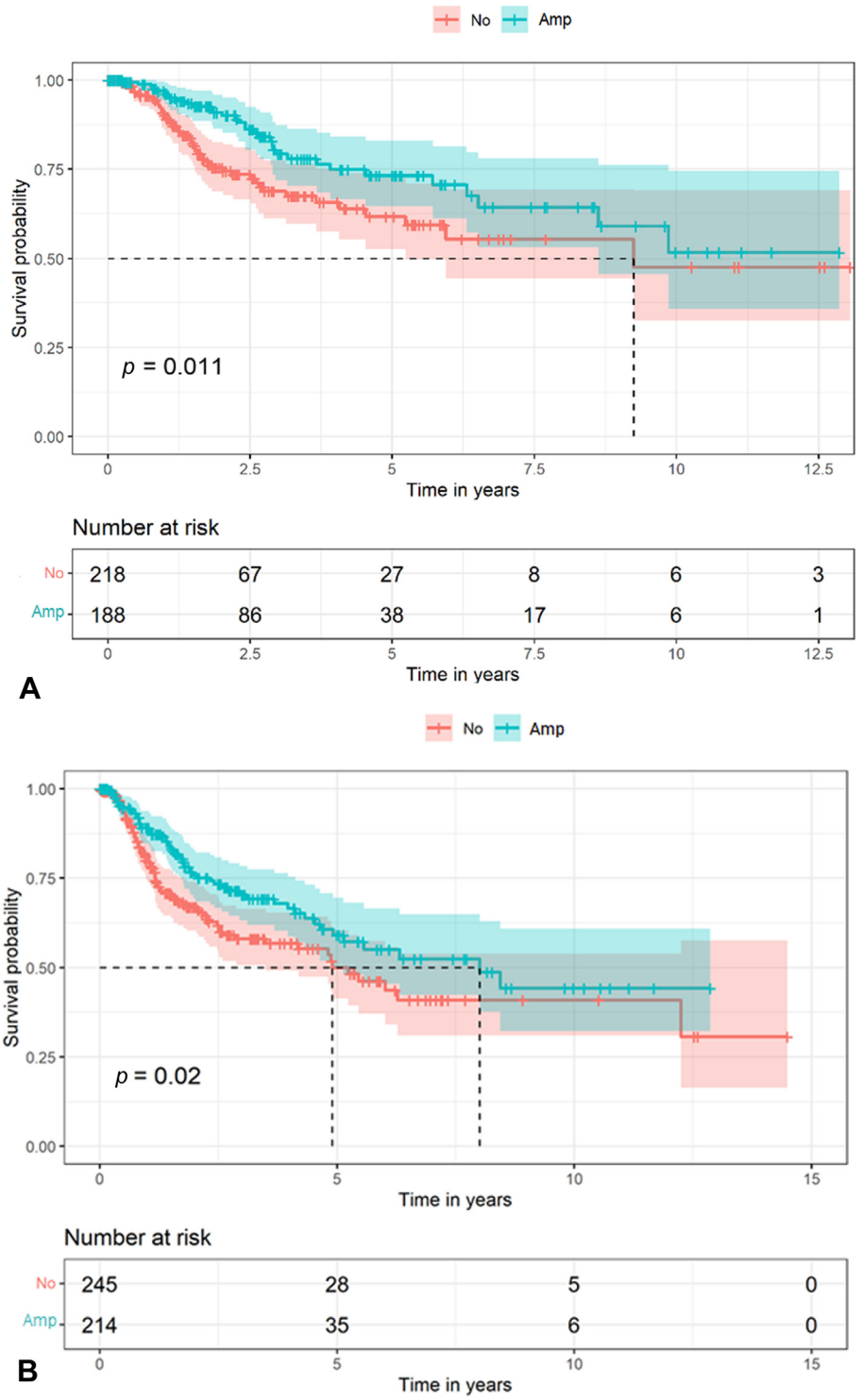

Figure 2. (A) Disease-specific survival, (B) progression-free interval for MCR-amplified and non-amplified patients. Amp, MCR amplified; MCR, minimal common region; No, MCR nonamplified.

therapeutic significance as PI3K is the most altered downstream pathway among MCR-amplified cases. The PI3K pathway is a known target and can potentially offer new therapeutic options for patients with LUSC; PI3Kinase inhibitors are currently used in patients with breast cancer and certain hematologic malignancies. These agents have been evaluated in patients with NSCLC with limited efficacy. There is a need to conduct a focused assessment of these agents in patients with LUSC with MCR amplification.

Conclusions

Amplification on 25-gene MCR within 3q is a promising prognostic marker in patients with early stage LUSC. It also offers opportunities for targeted therapeutics, given the common PI3K pathway between MCR genes. We are conducting a validation retrospective study to evaluate the prognostic and predictive value of 3q MCR amplification using tissue samples from a racially diverse patient population with advanced LUSC using a laboratory-developed fluorescence in situ hybridization probe to detect MCR amplification.

CRediT Authorship Contribution Statement

Fawzi Abu Rous: Roles/Writing—original draft, Visualization, Conceptualization.

Pin Li: Formal analysis, Writing—review and editing.

Shannon Carskadon: Data curation, Visualization.

Sunny RK Singh: Writing—review and editing, Visualization.

Rebecca Chacko: Writing—review and editing.

Hassan Abushukair: Writing—review and editing, Data curation.

Shirish Gadgeel: Methodology, Writing—review and editing, Supervision, Visualization, Conceptualization.

Nallasivam Palanisamy: Conceptualization, Data curation, Methodology, Visualization, Writing—review and editing, Supervision.

Acknowledgments

The authors thank Laila Poisson, PhD (Department of Public Health Sciences, Henry Ford Health) for providing assistance with statistical analysis.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2023.100486.

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