Received: 8 April 2022 | Accepted: 18 April 2022

DOI: 10.1111/jop.13303

ORIGINAL ARTICLE

Journal of Oral Pathology & Medicine WILEY

Recurrent copy number alterations involving *EGFR*, *CDKN2A*, and *CCND1* in oral premalignant lesions

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Funding information
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Abstract

Background: A major challenge in the management of patients with oral leukoplakia is the difficulty to identify patients at high risk of developing oral squamous cell carcinoma. Our knowledge about genomic alterations in oral leukoplakia, and in particular those that progress to oral squamous cell carcinoma, is scarce and there are no useful biomarkers that can predict the risk of malignant transformation.

Methods: Using a novel, custom-made tissue microarray including 28 high-risk oral leukoplakias and the corresponding oral squamous cell carcinomas from 14 cases that progressed to cancer, we assayed copy number alterations involving the oral squamous cell carcinoma driver genes *CDKN2A*, *CCND1*, *EGFR*, and *MYC* by fluorescence in situ hybridization. The copy number alterationss were correlated with clinicopathological data from all patients.

Results: Copy number alterations were identified in 14/24 oral leukoplakias, analyzable for one or more of the oral squamous cell carcinoma driver genes. *EGFR* was the most frequently altered gene in oral leukoplakias with amplification/gain in 43.5% followed by loss of *CDKN2A* (26.1%), gains of *CCND1* (26.1%), and *MYC* (8.3%). Losses of *CDKN2A* were more common in oral leukoplakias progressing to oral squamous cell carcinoma compared to those that did not. Copy number alterations were more common in oral squamous cell carcinomas than in oral leukoplakias.

Conclusions: Our findings demonstrate that copy number alterations involving the oral squamous cell carcinoma drivers *CDKN2A*, *EGFR*, and *CCND1* occur in oral leukoplakias and suggest a possible role for these genes in the development and/or progression of subsets of oral leukoplakias.

KEYWORDS

CDKN2A, *EGFR*, fluorescence in situ hybridization, malignant transformation, oral leukoplakia

1 | INTRODUCTION

Patients with oral squamous cell carcinomas (OSCC) usually have a poor prognosis, with a 5-year survival rate of approximately 50–60%.[1–5] Surgical resection of OSCCs often results in significant loss of function and may also severely affect the facial appearance. Post-operative radiotherapy may also accentuate these effects, resulting in a significant impact on the quality of life. Early detection and treatment are therefore of significant importance for the prognosis[1,2,5] and quality of life.[6] Oral leukoplakia (OL) is a potentially malignant disorder that precedes a significant part of OSCCs. Approximately 10% of OLs undergo malignant transformation.[7] So far, there is no clear evidence that therapeutic interventions prevent malignant transformation of OLs.[8–12] However, recent studies have shown that follow-up programs of OL patients result in earlier detection of OSCC and thus in improved survival rates.[5] Various clinical, histopathological, and genomic factors have been suggested to be associated with an increased risk of malignant transformation of OLs.[12–24] However, there are currently no reliable biomarkers that can identify OLs with a high risk of developing into cancer.[17]

Copy number alterations (CNAs), that is, amplifications/gains and losses of genes or chromosomal segments, are common alterations in head and neck squamous cell carcinomas (HNSCC) and include, for example, losses at 3p and 9p, and gains at 3q, 5p, 7p, and 8q.[24–26] Among 517 HNSCCs available in the cBioPortal database (www.cbioportal.org),[27,28] deletion of the tumor suppressor gene *CDKN2A* in 9p21.3 is the most common gene-specific CNA found in 30.9% of HNSCCs. Other common cancer gene-specific CNAs in HNSCC include amplifications of *CCND1* and *FGF3/4* in 11q13.3 (23.2%), *PIC3CA* in 3q26.32 (15.7%), *TP63* in 3q28 (16.1%), *EGFR* in 7p11.2 (10.4%), and *MYC* in 8q24.21 (9.3%).[27,28] Our knowledge about CNAs in OL, and in particular in those that progress to OSCC, is very limited.[18–22] Here, we have analyzed CNAs involving four known oncogenic drivers, *CDKN2A*, *CCND1*, *EGFR*, and *MYC*, in OLs from 28 patients, half of which subsequently developed OSCC. We also analyzed the corresponding OSCCs from the same patients. Copy number profiling revealed recurrent oncogenic events involving these genes not only in OSCCs but also in OLs.

2 | PATIENTS AND METHODS

2.1 | Patients

Patients with clinically confirmed OLs were identified through searches in databases and medical records at the Departments of Oral Medicine and Oral and Maxillofacial Surgery, Public Dental Health Service, Gothenburg, Sweden. All OLs were surgically excised if feasible or incisional biopsies were taken of representative areas. Fourteen patients from whom enough representative formalin-fixed paraffin-embedded (FFPE) material was available and who developed OSCC in the same area as the preceding OL biopsy was taken were selected (Table 1). Only patients developing OSCC later than 6 months after OL diagnosis were included. For comparison, FFPE tissue from a group of 14 OL patients with high-risk characteristics (non-homogenous, dysplastic, and/or located at the tongue) were selected that largely matched the cancer progressing group with regard to gender, age, and anatomical localization (Table 1). These patients had at least 50 months of follow-up after OL diagnosis with no signs of OSCC development. All specimens were histopathologically analyzed and clinically re-assessed using photographs of the lesions. The study was approved by the Regional Ethics Committee in Gothenburg, Sweden (Nos. 739-10 and T613-17).

TABLE 1 Demographic and clinicopathologic characteristics of 28 patients with oral leukoplakias (OL) and the corresponding oral squamous cell carcinomas (OSCC) from 14 patients

Parameter	OSCC development ($n = 14$)	No OSCC development ($n = 14$)
Gender		
Female	5	6
Male	9	8
Age at OL diagnosis		
Median (years)	67	70
Range (years)	41–86	39–93
Follow-up	To OSCC	End of follow-up
Median (months)	35	102
Range (months)	12–150	50–268
Anatomical localization		
Tongue	6	6
Floor of the mouth	1	0
Buccal mucosa	4	5
Gingiva	3	3
Dysplasia (grade)		
No dysplasia	4	2
Mild	3	5
Moderate	5	5
Severe	2	2
Clinical subtype		
Homogenous	1	7
Non-homogenous	13	7
OSCC (grade)		
Well differentiated	8	–
Moderately differentiated	4	–
Poorly differentiated	2	–

2.2 | Construction of an OL/OSCC tissue microarray

Hematoxylin and eosin slides from the selected specimens were re-evaluated by an oral pathologist (JÖ) and representative areas of OL and OSCC were marked. Two 1.0 mm cores were taken from each

donating tissue block and placed in a new recipient block using a semi-automated arraying machine (TMArrayer, Pathology Devices, Westminster, MD). The TMA was constructed at the Tissue Microarray Center, Department of Translational Medicine, Lund University, Sweden.

2.3 | Fluorescence in situ hybridization

Copy number profiling was done by fluorescence in situ hybridization (FISH) on 4-μm-thick FFPE sections of the OL/OSCC TMA. The following locus-specific probes were used: Zyto*Light* SPEC *EGFR*/CEN 7 (Z-2033, ZytoVision, Bremerhaven, Germany), *MYC*/CEN 8 (Z-2092, ZytoVision), *CDKN2A*/CEN 9 (Z-2063, ZytoVision), and *CCND1*/CEN 11 (Z-2071, ZytoVision). The locus-specific probes were labeled with ZyGreen and the CEN probes with ZyOrange. FISH was performed using the Zyto*Light* FISH Tissue Implementation kit (ZytoVision) according to the manufacturers' instructions. Slides were hybridization for 24–40 h followed by four post-hybridization washes in Wash Buffer A for 5 min each. Fluorescence signals were digitized, processed, and analyzed with the Isis FISH imaging system v.5.5 (MetaSystems, Altlussheim, Germany). At least 20 non-overlapping nuclei were scored from each case. A gene was considered amplified, gained, or lost when at least 20% of the nuclei [10% in cases 1 (*CDKN2A*) and 13 (*EGFR*) due to shortage of analyzable tissue] showed a signal pattern consistent with these alterations. Copy number gain was defined as 1–4 extra signals for the gene-specific probe or the gene-specific/CEN probes. Gene amplification was detected as clusters of gene-specific FISH signals.

Fisher's exact test was used to compare CNAs in *CDKN2A*, *EGFR*, *CCND1*, and *MYC* between the different groups. A p-value <0.05 was considered to be statistically significant. SPSS Statistics for Macintosh ver. 26.0 software package (IBM Corp., Armonk, NY) was used for the statistical analysis.

3 | RESULTS

3.1 | Clinicopathological characteristics

The clinicopathological characteristics of the 28 patients (17 men and 11 women) are shown in Table 1 and in Table S1. The median age of the patients at the time of OL diagnosis was 68.5 years (range 39–93) and the median age of the patients when they developed OSCC ($n = 14$) was 70 years (range 50–89).

The OL patients who developed OSCC ($n = 14$) was diagnosed with OL at a median age of 67 years (range 41–86 years). Malignant transformation occurred at a median time of 35 months after the initial OL diagnosis (range 12–150 months). OL patients who did not develop OSCC ($n = 14$) was diagnosed with OL at a median age of 70 years (range 39–93 years), and had a median follow-up time of 102 months (range 50–268 months). Six OLs showed no dysplasia, whereas the remaining 22 cases showed varying degrees of dysplasia. Thirteen of the 14 OL patients who developed OSCC had non-homogenous leukoplakias compared to 7/14 of those who did not ($p = 0.0329$). There were no other significant differences between OLs that progressed to OSCC and those that did not.

3.2 | CNAs in OLs

CNAs were detected in 14 of 24 OLs analyzable for one or more of the four genes *CDKN2A*, *CCND1*, *EGFR*, and *MYC* (Figure 1). Ten cases had no detectable CNAs. *EGFR* was the most frequently altered gene with amplification in one case (Figure 2A–C) and copy number gain in nine cases (43.5%). Six cases (26.1%) showed loss of *CDKN2A* (Figure 2D–F) and six had copy number gain of *CCND1* (26.1%). Two OLs (8.3%) showed copy number gain of *MYC*.

3.3 | CNAs in OSCCs

CNAs were detected in 8 of 11 (72.7%) analyzable OSCC specimens (Figure 1). Also, among the OSCCs, *EGFR* was the most frequently altered gene (63.6%) with amplification in three cases (Figure 2G) and copy number gain in four cases. Six OSCC showed loss of *CDKN2A* (54.5%) (Figure 2H) and five displayed gains of *CCND1* (45.5%), three of which were amplifications (Figure 2I). Only one OSCC (9.1%) showed gain of *MYC*.

3.4 | CNAs in OLs that developed into OSCCs vs. those that did not

Losses of *CDKN2A* were somewhat more common in OLs that progressed to OSCC (4/9; 95% CI 14%–79%) compared to those that did not (2/14; 95% CI 2%–43%) ($p = 0.16$) (Figure 1). Amplification of *EGFR* was seen only in one OL that developed into OSCC 13 months later. In total, 5/9 (95% CI 21%–86%) OLs progressing to OSCC showed amplification/gain of *EGFR*, compared to 5/14 (95% CI 13%–65%) that did not undergo malignant transformation (Figure 1). Gains of *CCND1* were observed in 2/9 (95% CI 3%–60%) OLs that progressed to OSCC and in 4/14 (95% CI 8%–58%) that did not. Gain of *MYC* was found in only two OLs, one of which developed into an OSCC.

Six of the 14 OLs that progressed to OSCC had analyzable material from both the OL and the OSCC (Figure 1). In general, the OSCCs had more CNAs compared to the OLs. In case 1, the OL showed loss of *CDKN2A* and gain of *EGFR*, whereas the corresponding OSCC showed loss of *CDKN2A*, amplification of *EGFR* (Figure 2G), and gains of *CCND1* and *MYC*. In case 3, the OL showed amplification of *EGFR* (Figure 2C) and the OSCC amplifications of both *EGFR* and *CCND1* as well as loss of *CDKN2A*. The OL in case 4 had loss of *CDKN2A*, whereas the OSCC had loss of *CDKN2A* (Figure 2H), amplification of *CCND1* (Figure 2I), and gain of *EGFR*. In case 7, the OL showed gain of *EGFR* and the OSCC amplification of *EGFR* and loss of *CDKN2A*. In

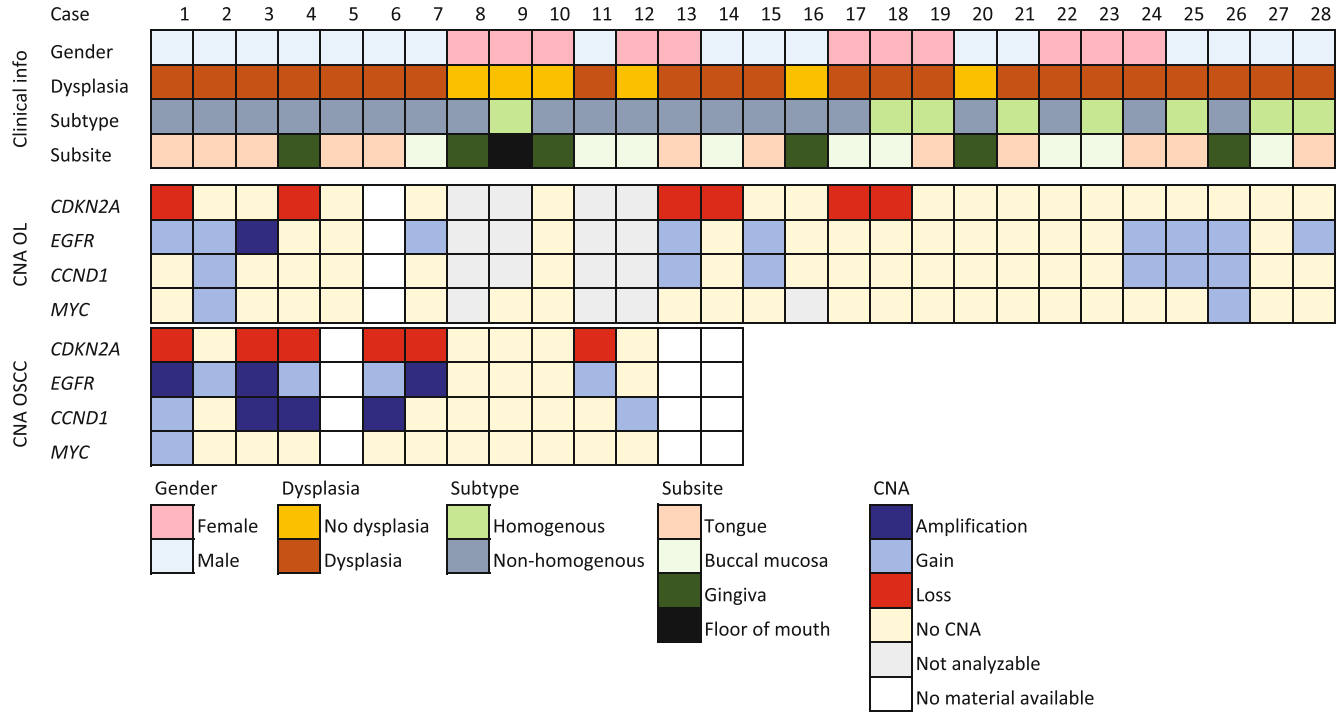

FIGURE 1 Summary of clinicopathological information and copy number alterations (CNA) in 28 oral leukoplakias (OL) and the corresponding oral squamous cell carcinomas (OSCC) from 14 of the patients.

case 2, the OL showed gains of *EGFR*, *CCND1*, and *MYC*, whereas the corresponding OSCC had only gain of *EGFR*. The OL and OSCC in case 10 had no CNAs involving any of the four analyzed genes.

4 | DISCUSSION

A major challenge in the management of OL patients is the difficulty to identify patients at high risk of developing OSCC. Currently, there are no clinically useful biomarkers that can safely predict this risk. Using a newly constructed TMA of high-risk OLs and the corresponding OSCCs we now demonstrate recurrent oncogenic events involving the known OSCC drivers *CDKN2A*, *CCND1*, *EGFR*, and *MYC* also in OLs.

CNAs involving one or more of the analyzed genes were found in 58% of the OLs. *EGFR* was the most frequently altered gene (43.5%), followed by *CDKN2A* (26.1%), *CCND1* (26.1%), and *MYC* (8.3%). As expected, CNAs were more common in OSCCs compared to OLs. Our finding of alterations in these driver genes not only in OSCC but also in OL suggests a possible role for these genes in the development of OL and/or progression of OL into OSCC.

Loss of *CDKN2A* is the most common CNA in HNSCC[27,28] and was also somewhat more common in the present OLs that progressed to cancer compared to those that did not. These findings are supported by a previous loss of heterozygosity (LOH) study showing that LOH involving 9p is more common in oral potentially malignant disorders (OPMD) that progress to carcinoma in situ (CIS) or OSCC compared to those that do not progress to cancer.[23] Seventy-six percent of the OPMD that progressed to cancer showed LOH involving 9p compared to 30% that did not progress. There are also studies showing that inactivation of *CDKN2A* by promoter hypermethylation occurs more frequently in oral dysplastic lesions progressing to cancer compared to those that do not.[29,30] The present and previous studies thus indicate that inactivation of *CDKN2A* is an early event in oral carcinogenesis in a subset of patients.

Interestingly, one of our OLs that progressed to OSCC showed amplification of *EGFR*. Gains of *EGFR* were also found in both OL groups, but were slightly more common in those progressing to OSCC. Notably, we have previously studied a case of recurrent OL that recently progressed to OSCC. Cytogenetic analysis of an OL biopsy from this patient revealed clonal chromosome aberrations with evidence of gene amplification in the form of double-minute chromosomes (unpublished observations). Whether the double minutes in this case contain amplified copies of *EGFR* is not known. Our data are in line with previous FISH/ISH studies, showing increased copy number of *EGFR* in OPMDs progressing to CIS or OSCC.[18,19] Interestingly, the Erlotinib Prevention of Oral Cancer (EPOC) trial showed that patients with oral premalignant lesions and amplification/gain of *EGFR* had significantly shorter oral cancer-free survival compared to those with a normal copy number.[20] However, Erlotinib (an EGFR tyrosine kinase inhibitor) treatment of oral premalignant lesions had no significant impact on the oral cancer-free survival in this study. Taken together, the present and previous studies show that amplification of *EGFR*, although rare, do occur in oral precancerous lesions progressing to cancer and that copy gain of *EGFR* is a rather common finding. As expected, OSCC shows

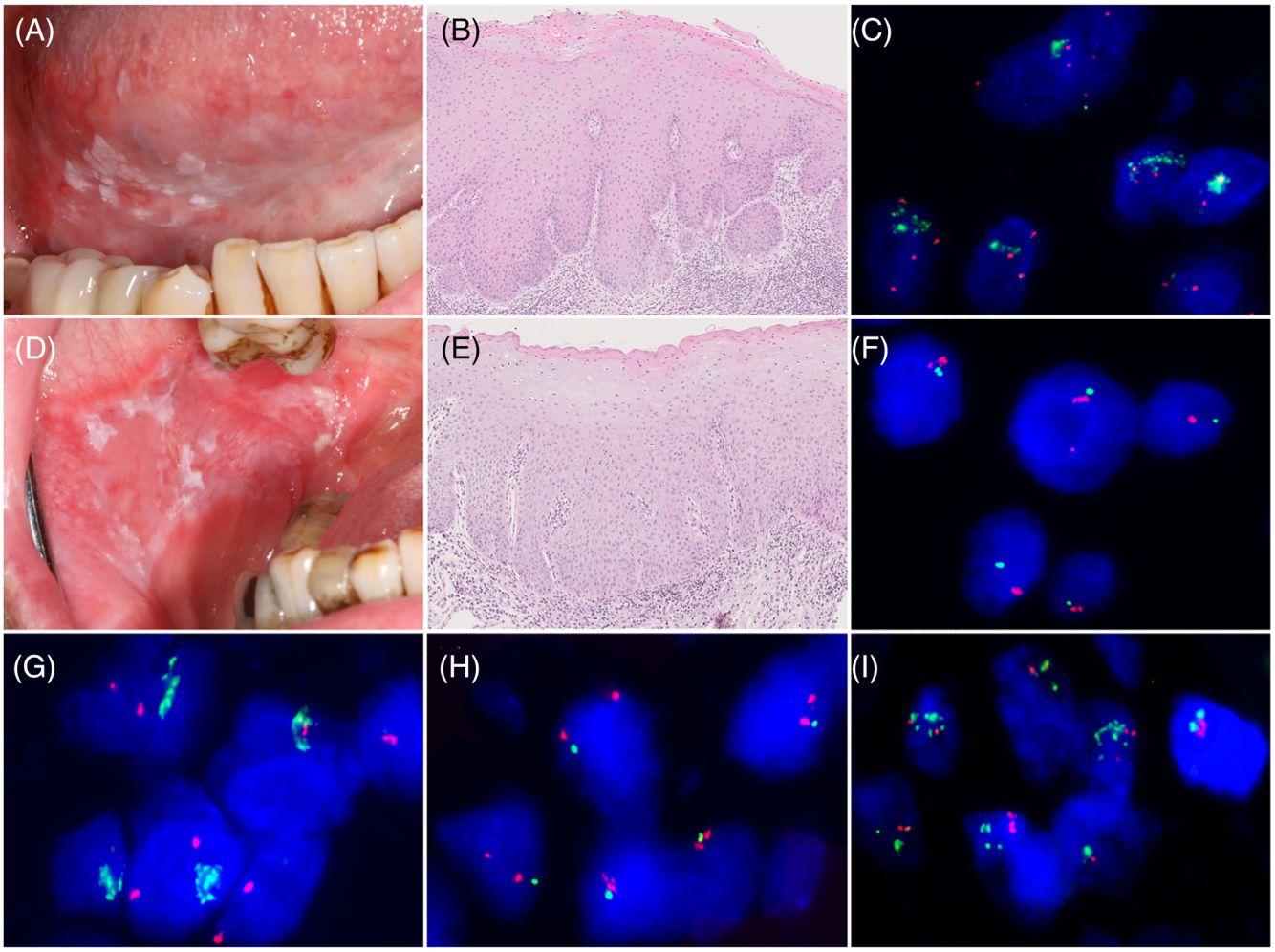

FIGURE 2 Clinical, histopathological, and copy number characteristics of OLs and OSCCs. (A–C) Case 3. (A) Non-homogenous OL at the lateral border/ventral surface of the tongue. (B) Severe epithelial dysplasia with subepithelial inflammation. (C) FISH analysis showing *EGFR* amplification (clustered green signals). (D–F) Case 14. (D) Non-homogenous OL in the buccal mucosa. (E) Moderate dysplasia with subepithelial inflammation. (F) FISH analysis showing loss of one copy of *CDKN2A* (red/green signals). (G) FISH analysis of the OSCC in case 1 showing *EGFR* amplification (clustered green signals). (H) FISH analysis of the OSCC in case 4 showing loss of one *CDKN2A* allele (green signal). (I) FISH analysis of the OSCC in case 4 showing amplification of *CCND1* (clustered green signals). Gene-specific probes are displayed in green and centromere probes in red. Nuclei are counterstained in blue with DAPI.

accumulation of EGFR amplifications/gains. However, the pathogenetic role of *EGFR* in the development of OL and/or progression of OLs into OSCC is still unclear, although there is some evidence to suggest that it has oncogenic activities also early during oral carcinogenesis. Further studies are needed to verify these scanty observations and to determine the role of *EGFR* in the pathogenesis and progression of OLs.

None of our OLs showed amplification of *CCND1* but gains were found in 26.1% of the cases and were slightly more common in OLs that did not progress to cancer. In contrast, Poh et al. (2012) showed that 21/22 oral dysplasias progressing to CIS or OSCC showed gain of *CCND1* compared to only 4/13 that did not progress.[18] The reason for this difference is not known, but may be attributed to factors such as small sample sizes and differences in follow-up time, in particular for the group of non-progressors.

A limitation of our study is the confined number of patients included, and that we only assayed the copy number status of four genes. Another limitation is that we do not know if the OLs at the time of malignant transformation had the same CNAs as the ones that we detected. It is also possible that some of the non-progressing OLs may have been successfully treated by the surgical excision, but did in fact harbor genomic alterations that could have contributed to malignant transformation. For example, the two OLs with loss of *CDKN2A* that did not progress to cancer might have progressed if they had not been surgically removed.

In summary, using a custom-made TMA of high-risk OLs and the corresponding OSCCs, we demonstrate that CNAs involving the OSCC driver genes *CDKN2A*, *EGFR*, and *CCND1* occur also in OLs. Our findings provide new insights into the pathogenesis of OL and suggest a possible role for these genes in the development and/or

progression of subsets of OLs. Further studies of additional cases and other driver genes are needed to get a better understanding of the genetic basis of OLs and to identify new therapeutic targets for patients with these lesions.

AUTHOR CONTRIBUTIONS

Conceptualization: Fredrik Jäwert, Jenny Öhman, Göran Stenman and Göran Kjeller. *Methodology*: Jenny Öhman, André Fehr, and Göran Stenman. *Validation*: André Fehr, and Göran Stenman; *Formal analysis*: Göran Stenman. *Investigation*: André Fehr, Fredrik Jäwert, and Göran Stenman. *Data curation*: André Fehr and Göran Stenman. *Writing—original draft*: Fredrik Jäwert and Göran Stenman. *Writing—review and editing*: Göran Stenman, Göran Kjeller, and Jenny Öhman. *Supervision*: Göran Stenman and Göran Kjeller. *Funding acquisition*: Fredrik Jäwert and Göran Stenman.

ACKNOWLEDGMENTS

This study was supported by grants from the Department of Research and Development, NU Hospital Group, and the Swedish Dental Society. We thank Dr Marta Persson for help with the FISH illustrations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/jop.13303.

DATA AVAILABILITY STATEMENT

Not applicable.

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REFERENCES

1. Zanoni DK, Montero PH, Migliacci JC, et al. Survival outcomes after treatment of cancer of the oral cavity (1985–2015). *Oral Oncol*. 2019;90:115-121. doi:10.1016/j.oraloncology.2019.02.001
2. Rogers SN, Brown JS, Woolgar JA, et al. Survival following primary surgery for oral cancer. *Oral Oncol*. 2009;45(3):201-211. doi:10.1016/j.oraloncology.2008.05.008
3. Listl S, Jansen L, Stenzinger A, et al. GEKID cancer survival working group. Survival of patients with oral cavity cancer in Germany. *PLoS One*. 2013;8(1):e53415. doi:10.1371/journal.pone.0053415
4. Ferreira AK, Carvalho SH, Granville-Garcia AF, et al. Survival and prognostic factors in patients with oral squamous cell carcinoma. *Med Oral Patol Oral Cir Bucal*. 2021;26(3):e387-e392. doi:10.4317/medoral.24242
5. Jäwert F, Nyman J, Olsson E, Adok C, Helmersson M, Öhman J. Regular clinical follow-up of oral potentially malignant disorders results in improved survival for patients who develop oral cancer. *Oral Oncol*. 2021;121:105469. doi:10.1016/j.oraloncology.2021.105469
6. Rogers SN, Hannah L, Lowe D, Magennis P. Quality of life 5-10 years after primary surgery for oral and oro-pharyngeal cancer. *J Craniomaxillofac Surg*. 1999;27(3):187-191. doi:10.1016/s1010-5182(99)80049-3
7. Iocca O, Sollecito TP, Alawi F, et al. Potentially malignant disorders of the oral cavity and oral dysplasia: a systematic review and meta-analysis of malignant transformation rate by subtype. *Head Neck*. 2020;42(3):539-555. doi:10.1002/hed.26006
8. Lodi G, Porter S. Management of potentially malignant disorders: evidence and critique. *J Oral Pathol Med*. 2008;37(2):63-69. doi:10.1111/j.1600-0714.2007.00575.x
9. Kerr AR, Lodi G. Management of oral potentially malignant disorders. *Oral Dis*. 2021;27:2008-2025. doi:10.1111/odi.13980
10. Holmstrup P, Dabelsteen E. Oral leukoplakia-to treat or not to treat. *Oral Dis*. 2016;22(6):494-497. doi:10.1111/odi.12443
11. Lodi G, Franchini R, Warnakulasuriya S, et al. Interventions for treating oral leukoplakia to prevent oral cancer. *Cochrane Database Syst Rev*. 2016;7(7):CD001829. doi:10.1002/14651858.CD001829.pub4
12. Holmstrup P, Vedtofte P, Reibel J, Stoltze K. Long-term treatment outcome of oral premalignant lesions. *Oral Oncol*. 2006;42(5):461-474. doi:10.1016/j.oraloncology.2005.08.011
13. Jäwert F, Pettersson H, Jagefeldt E, Holmberg E, Kjeller G, Öhman J. Clinicopathologic factors associated with malignant transformation of oral leukoplakias: a retrospective cohort study. *Int J Oral Maxillofac Surg*. 2021;50(11):1422-1428. doi:10.1016/j.ijom.2021.01.012
14. Evren I, Brouns ER, Wils LJ, et al. Annual malignant transformation rate of oral leukoplakia remains consistent: a long-term follow-up study. *Oral Oncol*. 2020;110:105014. doi:10.1016/j.oraloncology.2020.105014
15. Alaizari NA, Sperandio M, Odell EW, Peruzzo D, Al-Maweri SA. Meta-analysis of the predictive value of DNA aneuploidy in malignant transformation of oral potentially malignant disorders. *J Oral Pathol Med*. 2018;47(2):97-103. doi:10.1111/jop.12603
16. Farah CS. Molecular, genomic and mutational landscape of oral leukoplakia. *Oral Dis*. 2020;27:803-812. doi:10.1111/odi.13608
17. Monteiro L, Mello FW, Warnakulasuriya S. Tissue biomarkers for predicting the risk of oral cancer in patients diagnosed with oral leukoplakia: a systematic review. *Oral Dis*. 2020;27:1977-1992. doi:10.1111/odi.13747
18. Poh CF, Zhu Y, Chen E, Berean KW, Wu L, Zhang L. Rosin MP. Unique FISH patterns associated with cancer progression of oral dysplasia. *J Dent Res*. 2012;91(1):52-57. doi:10.1177/0022034511425676
19. Bates T, Kennedy M, Diajil A, et al. Changes in epidermal growth factor receptor gene copy number during oral carcinogenesis. *Cancer Epidemiol Biomarkers Prev*. 2016;25(6):927-935. doi:10.1158/1055-9965.EPI-15-0949
20. William WN Jr, Papadimitrakopoulou V, Lee JJ, et al. Erlotinib and the Risk of oral cancer: the erlotinib prevention of oral cancer (EPOC) randomized clinical trial. *JAMA Oncol*. 2016;2(2):209-216. doi:10.1001/jamaoncol.2015.4364
21. Taoudi Benchekroun M, Saintigny P, Thomas SM, et al. Epidermal growth factor receptor expression and gene copy number in the risk of oral cancer. *Cancer Prev Res (Phila)*. 2010;3(7):800-809. doi:10.1158/1940-6207.CAPR-09-0163
22. Tsui IF, Poh CF, Garnis C, Rosin MP, Zhang L, Lam WL. Multiple pathways in the FGF signaling network are frequently deregulated by gene amplification in oral dysplasias. *Int J Cancer*. 2009;125(9):2219-2228. doi:10.1002/ijc.24611
23. Rosin MP, Cheng X, Poh C, et al. Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. *Clin Cancer Res*. 2000;6(2):357-362.
24. Cancer Genome Atlas Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature*. 2015;517(7536):576-582. doi:10.1038/nature14129
25. Cho J, Johnson DE, Grandis JR. Therapeutic implications of the genetic landscape of head and neck cancer. *Semin Radiat Oncol*. 2018;28(1):2-11. doi:10.1016/j.semradonc.2017.08.005
26. Farah CS. Molecular landscape of head and neck cancer and implications for therapy. *Ann Transl Med*. 2021;9(10):915. doi:10.21037/atm-20-6264

27. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012;2(5):401-404. doi:10.1158/2159-8290.CD-12-0095 Erratum in: Cancer Discov 2012 Oct;2(10):960.
28. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*. 2013;6(269):pl1. doi:10.1126/scisignal.2004088
29. Hall GL, Shaw RJ, Field EA, et al. p16 promoter methylation is a potential predictor of malignant transformation in oral epithelial dysplasia. *Cancer Epidemiol Biomarkers Prev*. 2008;17(8):2174-2179. doi:10.1158/1055-9965.EPI-07-2867
30. Cao J, Zhou J, Gao Y, et al. Methylation of p16 CpG Island associated with malignant progression of oral epithelial dysplasia: a prospective cohort study. *Clin Cancer Res*. 2009;15(16):5178-5183. doi:10.1158/1078-0432.CCR-09-0580

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Jäwert F, Fehr A, Öhman J, Stenman G, Kjeller G. Recurrent copy number alterations involving *EGFR*, *CDKN2A*, and *CCND1* in oral premalignant lesions. *J Oral Pathol Med*. 2022;51(6):546-552. doi:10.1111/jop.13303