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Short Communication

Evaluating the diagnostic value and prospects of genomic and mutational sequencing in malignant transformation of oral leukoplakia

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Abstract Genomic and mutational sequencing in malignant transformation of oral leukoplakia (OLK) has emergingly gained momentum. In this short communication, we identified 5 retrospective follow-up studies and 5 cross-section comparative studies on this issue using formalin-fixed paraffin-embedded tissues. Copy number alteration (CNA) was demonstrated to increase with the grade of oral dysplasia. CNA-based algorithms showed better prediction performances than histological grade in assessing the risk of OLK malignant transformation. Importantly, we conducted a pooled-analysis on the mutation frequencies of the common oral cancer driver genes extracted from individual studies. The most common mutation gene was found to be *TP53* (26.26 %; 95 % confidence intervals (CI), 20.61–32.82 %), followed by *NOTCH1* (23.23 %; 95%CI, 17.87–29.61 %), *FAT1* (16.67 %; 95%CI, 12.08–22.52 %), and *CDKN2A* (10.61 %;

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Oral squamous cell carcinoma

95%CI, 6.98—15.73 %). Collectively, it is promising to establish molecular subtyping and risk stratification of OLK patients using genomic and mutational sequencing. We recommend the well-designed studies with a larger OLK patient population with clinical endpoints using fresh or frozen tissues and matched optimal samples as controls in further investigations.

Introduction

Oral leukoplakia (OLK) is the best-known potentially malignant disorder of oral squamous cell carcinomas (OSCC). Although the risk of OLK malignant transformation usually increases along with increasing grade of epithelial dysplasia, it is well-known that oral biopsy with histologic assessment is insufficient and may involve subjectivity.[1,2] Oral carcinogenesis process is recognized to be associated with the accumulation of genetic and molecular alterations, which are generally accepted to be prior to the presence of histopathological morphology, from various grades of dysplasia to malignant change.[3] Furthermore, patient who present with an OLK lesion that harbors no dysplastic morphology but genetic alterations would have a substantial risk of developing an OSCC.[4] Perhaps genetic and molecular alterations have not yet been reflected in histomorphology. In this context, exploring genetic and molecular events before malignant transformation would provide an opportunity for the active intervention in high-risk OLK patients.

Next generation sequencing (NGS) is a massive, high-throughput sequencing technology used to analyze various mutations and genetic changes. Recent years, advances in genomic and mutational analysis using NGS in OLK have the potential for assessing the risk of malignant transformation and may uncover the genetic and molecular profiles of oral carcinogenesis. NGS can enhance the understanding of the complexity of genomic events drive oral carcinogenesis, which is fundamental to advances in new diagnostic and treatment modalities. In 2021, there were 3 reviews focusing on genetic landscape of OLK, encompassing alterations from gross chromosomal alterations to single gene mutation.[5—7] While genomic and mutational sequencing in OLK was just getting started at that time.[8—10] Significantly, several relevant original articles on this issue have been published after 2021.[11—18] However, the current evidence on genomic and mutational sequencing in OLK are not analyzed collectively in detail. Therefore, the aim of this paper was to summarize the available studies on genomic and mutational sequencing in OLK as a comprehensive resource for clinicians and researchers, in addition to addressing future prospects in studies in this field.

Materials and methods

A systematic literature search regarding the original articles on genomic and mutational sequencing in OLK from PubMed and Web of Science databases was conducted on 30 June 2023. In the search strategy, the subject terms "genetic OR genomic OR mutation" and "sequencing" were used along with "oral leukoplakia" OR "oral dysplasia". There was no restriction to language and year of publication, and an additional query was identified from cross-referencing. Inclusion criteria was the articles which addressed the issue of genomic and mutational analysis using NGS in tissue samples of OLK patients. Important references and information derived from background knowledge have also been analyzed. Exclusion criteria were literature reviews, cell and animal experimental studies, the methods that did not use NGS, and sequencing that did not use the tissue samples of OLK patients. Titles and abstracts or full texts of the articles were screened and re-evaluated to confirm the eligible papers. Data search and extraction were undertaken independently by two investigators (Y.Z. and W.L.), and any disagreement was resolved in a consensus symposium. Bibliographical characteristics of the eligible articles were reviewed and recorded the following information: authorship, publication year, country/region of origin, study design, number of subjects, sequencing method, and main results. The frequency of single gene mutation was extracted from individual studies and calculated as a pooled frequency.

Results

Table 1 presents the characteristics of the studies focusing on genomic alteration and mutational signatures by NGS in tissue samples of OLK patients. A total of 10 studies on this issue from 7 countries were retrieved in literature.[9—18] Of these, 5 studies enrolled the OLK patients with clinical endpoints of malignant transformation were of higher quality,[9—13] compared to the others without the clinical endpoints.[14—18] Meanwhile, the numbers of the cases and samples (range, 42—998) enrolled in higher quality studies were more than those in the others (range, 5—14 samples). There were 5 retrospective follow-up studies and 5 cross-section comparative studies. These investigations utilized diverse genomic profiling approaches, including whole-exome sequencing (WES), whole-genome sequencing (WGS), and targeted gene panel sequencing, to comprehensively characterize somatic mutations in OLK. All the sequencing samples were formalin-fixed paraffin-embedded (FFPE) tissues. Blood samples, adjacent normal tissue or tissues distant from the lesion regions were the normal controls in sequencing.

Copy number alteration (CNA) is a type of genetic alteration features and can reflect the evolution of cancer. There

Table 1 Summary of the studies focusing on genomic and mutational sequencing in patients with oral leukoplakia (OLK).

Author, year	Country	Study design	No. of patients	No. of samples	Surrogate sample	Normal control	Sequencing method	Main results
Wood et al., 2017[10]	UK	Comparative	69	256 (38 LGD, 59 HGD and 149 OSCC)	FFPE	Matched blood DNAs	WES	CNAs and point mutations do appear to accumulate as the disease moves from normal tissue to LGD, HGD and SCC. Most of the genomic changes driving oral cancer occur in the pre-cancerous state by way of gradual random accumulation rather than a dramatic single event.
Farah et al., 2019[9]	Australia	Retrospective follow-up	13 (5 progressive, 8 non-progressive)	42 (2 hyperplasia, 7 dysplasia and 5 OSCC)	FFPE	NA	WES	The frequency of exomic mutation variants, particularly in DNA damage repair pathway genes, can be used to differentiate the progressive and non-progressive OLK.
Li et al., 2021[11]	China	Retrospective follow-up	529	26 hyperplasia, 26 dysplasia; 477 hyperplasia with follow-up: 19 progressive and 253 recurrence	FFPE	Tissues distant from the lesion regions	WGS	Dysplasia had a higher CNA rate (86 %) than hyperplasia (46 %). CNA was associated with the histological grade of OLK and may be effective for prognosis prediction in the patients with hyperplasia.
Wils et al., 2023[12]	Netherlands	Retrospective follow-up (12 −258 m)	89	89 (25 progressor, 64 non-progressor)	FFPE	46 normal colorectal tissue samples	A 12-gene panel (*TP53, FAT1, NOTCH1, CASP8, AJUBA, CDKN2A, HRAS, PIK3CA, PTEN,*	CNAs were present in 69 % OLK patients, most commonly gains of chromosome regions 8q24 (46 %) and 20p11(20 %) and

							NSD1, *FBXW7*, *KMT2D*)	loss of 13q12 (19 %). Mutations were present in 66 % OLK patients, most commonly in TP53 (28 %), FAT1(20 %), and NOTCH1 (13 %). These genetic data combined with dysplasia generated a model for the prediction of malignant transformation.
Cai et al., 2024[13]	China	Retrospective follow-up (median: 67 m)	324 [training (n = 226) and validation (n = 98)] and 107 (external cohorts)	431 (324cases including 102 hyperplasia, 129 mild, 61 moderate, and 32 severe dysplasia)	FFPE	Tissues distant from the lesion regions	WGS	CNA events increased with the severity of histological grade of OLK. A CNA score model was developed to accurately predict the prognosis and showed better prediction performances than histological grade in assessing the transformation risk of OLK.
Anne et al., 2022[14]	India	Comparative	5	5 hyperplasia	FFPE	Matched blood DNAs	WES	16 expressed cancer driver genes including FAT1 associated with functionally damaging variants.
Adorno-Farias et al., 2023[15]	Chile	Comparative	10	10 (6 LGD, 4 HGD)	FFPE	NA	WES	13 genes found in OED samples may be related to basal biological functions in OED.
Márquez et al., 2023[16]	Chile	Comparative	10	10 (6 LGD, 4 HGD)	FFPE	NA	A 57-gene panel	HGD cases had significantly more variants, and some LGDs presented a similar mutational landscape to HGD

(*continued on next page*)

Table 1 (*continued*)

Author, year	Country	Study design	No. of patients	No. of samples	Surrogate sample	Normal control	Sequencing method	Main results
Farah et al., 2024[17]	Australia	Comparative	6 PVL	12 (9 dysplasia, 2 hyperplasia, 1 OSCC)	FFPE	NA	WES	after cluster analysis. Genes previously associated with OSCC were mutated in multiple samples. Several DNA damage repair genes were mutated in PVL samples. NOTCH and Hippo pathways were the most frequently impacted by mutation.
Kojima et al., 2025[18]	Japan	Retrospective follow-up (2 −39 m)	14	11 mild, 3 moderate dysplasia	FFPE	Adjacent normal tissue	A 7-gene panel (*TP53, HRAS, PIK3CA, NOTCH1, CDKN2A, FBXW7, BRAF*)	Oral cancer driver gene mutations including TP53, HRAS, PIK3CA, NOTCH1, CDKN2A, FBXW7, and BRAF were detected in 4 OLK cases.

FFPE, formalin-fixed paraffin-embedded; HGD, high grade dysplasia; LGD, low grade dysplasia; OSCC, oral squamous cell carcinoma; PVL, proliferative verrucous leukoplakia; NA, not available; WES, whole exome sequencing; WGS, whole genome sequencing.

were 4 studies on CNA in OLK, demonstrating that CNA events increased with the severity of histological grade.[10–13] Importantly, CNA-based algorithms showed better prediction performances than histological grade in assessing the risk of the malignant transformation.[12,13] The frequency of single-gene mutation in individual studies were incomplete and inconsistent, partly due to the limitations of the relatively small sample size and sampling bias. To derive a more precise estimation of the mutation frequency of the common oral cancer driver genes in OLK, we conducted a pooled-analysis on these frequencies extracted from individual studies (Table 2). The most common mutation was found to be *TP53* (26.26 %; 95 % confidence intervals (CI), 20.61–32.82 %), followed by *NOTCH1* (23.23 %; 95%CI, 17.87–29.61 %), *FAT1* (16.67 %; 95%CI, 12.08–22.52 %), *CDKN2A* (10.61 %; 95%CI, 6.98–15.73 %), *PIK3CA* (7.58 %; 95% CI, 4.56–12.21 %), *HRAS* (2.53 %; 95%CI, 0.92–5.94 %), and *FBXW7* (2.02 %; 95%CI, 0.61–5.26 %).

Discussion

This paper gives us an opportunity to present our discussion, which would help for the consideration of the investigators in further well-designed studies. The etiology of OLK, particularly idiopathic leukoplakia, is not fully understood, and effective biomarkers to predict which lesions will progress to OSCC and by which mechanisms remain unclear.[19] Considering the challenges in the diagnosis and prediction of malignant transformation of OLK, understanding its genetic basis is crucial. Notably, alterations at the molecular level are evidently present before any clinical and histopathologic changes. OLK may be surrounded by oral mucosa that also carry genetic alterations but are clinically/microscopically normal. Detection of those molecular alterations would ideally allow earlier diagnosis and effective specific targets for high-risk OL patients, and therefore help improve prognosis. Among the markers of genomic instability, DNA aneuploidy and loss of heterozygosity (LOH) at certain loci have been reported to be associated with malignant transformation of OLK.[5–7] However, at an individual level, no single marker can predict whether a given lesion will remain indolent or will progress to OSCC.[19,20] Also, a single biomarker may be neither sufficient nor necessary for the development of OSCC due to the molecular spectrum of the disease heterogeneity across populations and individual evolution.

Genomic and mutational sequencing such as WGS and WES can provide a more comprehensive framework for molecular subtyping and risk stratification of malignant transformation in OLK patients.[6] The genomic landscapes of OLK malignant transformation are consistent with the cancer evolution theories of neutral evolution and selection. These genomic insights, especially driver events, provide a foundation for developing personalized treatment and follow-up strategies. Altogether, the studies that assessed CNA in OLK and oral dysplasia showed gains and/or losses where oncogenes or tumor suppressor genes related to the oral carcinogenesis process were mapped. CNA were more evident in high-grade dysplastic and progressive lesions, and significantly associated with the increased risk of malignant progression; whereas it was also detected in non-

Table 2 The common oral cancer driver gene mutations in oral leukoplakia.

Authors, year	Number of cases	Number of mutated cases						
		TP53	*NOTCH1*	*FAT1*	*CDKN2A*	*PIK3CA*	*HRAS*	*FBXW7*
Izumchenko et al., 2015[8]	45	–	27	–	–	–	–	–
Farah et al., 2019[9]	13	5	–	–	3	–	–	–
Wood et al., 2017[10]	16	9	2	–	2	–	–	–
Wils et al., 2023[12]	89	25	12	18	11	10	2	4
Anne et al., 2022[14]	5	1	2	3	–	–	2	–
Márquez et al., 2023[16]	10	10	2	10	2	2	–	–
Farah et al., 2024[17]	6	1	–	2	3	–	–	–
Kojima et al., 2025[18]	14	1	1	0	0	3	1	0
Pooled frequency (95 % confidence intervals)	198	26.26 % (20.61–32.82 %)	23.23 % (17.87–29.61 %)	16.67 % (12.08–22.52 %)	10.61 % (6.98–15.73 %)	7.58 % (4.56–12.21 %)	2.53 % (0.92–5.94 %)	2.02 % (0.61–5.26 %)

dysplastic lesions.[9–12] The studies using NGS in OLK highlighted the role of *TP53* and *NOTCH1* mutations in the early stages of oral carcinogenesis (Table 2). During the process of OLK malignant transformation, genomic instability and mutational signatures accumulate over time. Owing to tumor heterogeneity, the CNA and driver gene mutations in different cases are not completely the same. How to identify the driving factors from complex and diverse driver events remains to be researched further.

Several limitations in available studies should be acknowledged. First, all the studies were conducted mainly using FFPE samples, which have limitations compared with fresh or frozen samples because of degraded DNA in FFPE samples. Second, the cutoff values and algorithms of CNA were different and the mutation frequency of the driver genes varied dramatically. The variability reflects divergent definitions of driver events, which ultimately limits the comparability of these models. The research on establishing a panel of certain driver gene mutations that intensively predict the OLK malignant transformation is warranted. Third, most studies were lack of a favorable normal control, e.g. matched blood DNAs. Adjacent tissues or other known population germline mutation databases as controls were inferior to matched blood samples. Fourth, all studies were not prospective design but retrospective and cross-section comparative nature. In addition, most were single-center studies with the small sample sizes. Given these limitations, we recommend the well-designed studies with a larger OLK patient population with clinical endpoints using fresh or frozen tissue samples and matched blood samples as controls in further investigations.

Taken together, it is promising to provide a comprehensive framework for molecular subtyping and risk stratification of malignant transformation in OLK patients using genomic and mutational sequencing. Large-scale, prospective, multi-center, well-designed studies are essential to validate the available findings and develop robust prognostic models or molecular markers for predicting malignant transformation of OLK. The precise characterization of the genomic and mutational events is of critical importance to translate molecular results into the clinics.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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