Immortalization of epithelial cells in oral carcinogenesis as revealed by genome-wide array comparative genomic hybridization: A meta-analysis

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ABSTRACT: *Background.* This purpose of this meta-analysis study was to identify the most frequent and potentially significant copy number alteration (CNA) in oral carcinogenesis.

Methods. Seven oral squamous cell carcinoma (OSCC)-related publications, corresponding to 312 samples, were identified for this meta-analysis. The data were analyzed in a 4-step process that included the genome assembly coordination of multiple platforms, assignment of chromosomal position anchors, calling gains and losses, and functional annotation analysis.

Results. Gains were more frequent than losses in the entire dataset. High-frequency gains were identified in chromosomes 5p, 14q, 11q, 7p, 17q, 20q, 8q, and 3q, whereas high-frequency losses were identified in chromosomes 3p, 8p, 6p, 18q, and 4q. Ingenuity pathway analysis showed that the top biological function was associated with immortalization of the epithelial cells ($p = 1.93E-04$).

Conclusion. This study has identified multiple recurrent CNAs that are involved in various biological annotations associated with oral carcinogenesis. © 2015 Wiley Periodicals, Inc. *Head Neck* **38**: E783–E797, 2016

KEY WORDS: oral squamous cell carcinoma (OSCC), meta-analysis, copy number alteration, oral carcinogenesis, array comparative genomic hybridization (CGH)

INTRODUCTION

Oral cancer is among the top 10 most common cancers worldwide, with approximately 300,000 new cases and 130,000 deaths worldwide,[1,2] and more than 90% of oral cancers are oral squamous cell carcinoma (OSCC).[3] Despite advances in the treatment and diagnosis of oral cancer, the mortality and morbidity rates have remained high.[4] The heterogeneity and complexity of the genetic basis of oral carcinogenesis could explain why the mortality is so high.[5,6] Therefore, a better understanding of the genetic mechanisms of OSCC progression could pave the way for better treatment and management of the disease.

Genetic instability is one of the hallmarks of oral cancer that leads to uncontrolled cell proliferation, cell morphology alterations, and tumor progression.[7] Oral carcinogenesis is a complex mechanism in which the accumulation of genetic alterations in multiple pathways plays a critical role in the initiation and progression of the disease.[5] Copy number alterations (CNAs), such as amplifications and deletions, are well-known instabilities in cancer that can alter the dosage of oncogenes and tumor suppressor genes, respectively.[5] Chromosomal alterations have been previously detected in the etiology of OSCC through conventional karyotyping and comparative genomic hybridization (CGH).[8,9] With the advent of array-based technologies, array CGH seemed to be the gold standard method to identify CNAs at a higher resolution (<1 Mb) in terms of amplifications and deletions throughout the genome.[10] The data from arrays can be mapped onto genes in the altered regions and give a better understanding of the specific genetic changes in tumorigenesis.[10,11] Genome-wide profiling also provides a clue toward the mechanism of the cellular constituents in cancer cells and how they interact to generate distinct cellular phenotypes.[12]

Over the last 10 years, numerous genome-wide profiling studies have been conducted on OSCC samples using array CGH to identify the CNAs and genetic mechanisms involved in oral carcinogenesis.[13–23] Amplifications within 3q, 6q, 8q, 9p, 9q, 11p, 11q, 14q, 17q, and 20q and deletions within 3p, 4q, 9p, and 18q have been frequently reported among OSCCs.[13–23] These chromosomes harbor multiple cancer-related genes, such as cyclin D1 (CCDN1; 11q13), epidermal growth factor receptor (7p12), V-Myc avian myelocytomatosis (MYC) viral oncogene homolog (8q24), telomerase RNA component (3q24), fragile histidine triad (3p14.2), and p16 (9p21). Cross-analysis of the results of multiple studies using array CGH on a large sample set would be useful to identify

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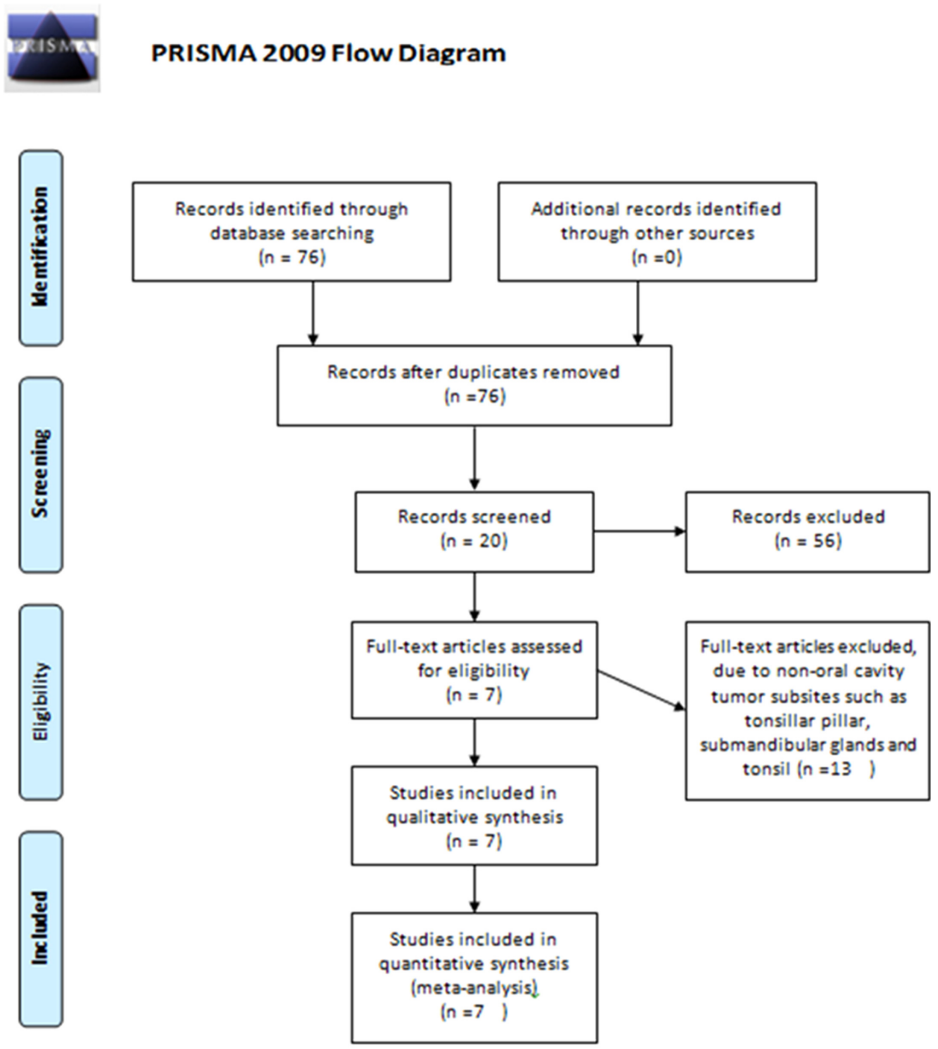

FIGURE 1. The flow diagram depicts the flow of information through the different phases of this meta-analysis study.

the pattern of the CNAs, their frequency, and the basis of any differences across the studies. However, slight variations in the data can be expected because of variation in the array CGH platforms and human genome assemblies that were used. Thus, integrating cross-platform analyzing and interpreting different studies using the same genome assembly may clearly delineate the pattern of associated CNAs. To our knowledge, this is the first such meta-analysis study on OSCC samples. A key purpose of this study was to identify potential genes and associated biological annotations and networks that may play a role in the etiology of OSCC.

MATERIALS AND METHODS

A literature search against the publicly accessible database, PubMed, was performed using keywords such as "array-based comparative genomics hybridization," "copy number alterations," and "oral squamous cell carcinoma." An initial list of 76 studies that met the keyword criteria was compiled. Of these, only 20 studies provided supplementary dataset files. Manual verification was performed on these datasets. Only studies ($n = 7$) that were performed on OSCC samples were included, and the remaining studies were excluded (eg, those related to precancer oral lesions). Hence, subsites, such as tonsillar pillar, submandibular glands, and tonsil, were excluded. The different phases of this meta-analysis study are illustrated in Figure 1. In addition, studies without the position of the genomic alterations and duplicated studies were excluded. Strict criteria were applied to obtain the most representative result for the current OSCC meta-analysis study. In total, 312 OSCC samples from 7 studies met our criteria[22–28] (Table 1).

TABLE 1. List of 7 studies that were included in the current study.

Studies	Number of samples that were recruited in the current study	Platform
Vincent–Chong et al[23] 2013	46	SurePrint G3 Human 1M aCGH, Agilent
Chen et al[24] 2004	60	GenoSensor Array 300, Vysis
Sparano et al[25] 2006	21	4134 BAC clones (Ultra GAPS, Corning, NY)
Ambatipudi et al[22] 2011	60	105K oligo aCGH, Agilent
Yoshioka et al[27] 2013	25	44K oligo aCGH, Agilent
Uchida et al[26] 2011	50	MAC array Karyo 4K, Macrogen
India Project Team of the International Cancer Genome Consortium[28]	50	Illumina Omni Quad DNA microarray
Total	312	

Abbreviation: aCGH, array comparative genomic hybridization.

Data processing

Genome assembly coordination among multiple platforms. Because the various datasets (from the 7 studies[22–28]) were generated using different array CGH platforms and genome assembly coordination, the assembly version between these datasets differed. Therefore, the start and endpoints of the CNAs were converted to match the latest version of the human genome assembly (hg19/GRCh37) using the University of California, Santa Cruz (UCSC) Liftover tool (University of California, Santa Cruz, California, United States).[29] The result was an integrated dataset consisting of a list of cytobands with corrected start and end positions according to the latest human genome assembly database.

Assignment of chromosomal position anchors. Next, redundant cytobands were filtered out, which resulted in a reduction in the number of cytobands from 3350 to 563. Although the redundant cytobands were filtered out, there were still overlapping regions because different studies focused on different aspects or positions in the human genome. To reduce the number of overlapping regions, each cytoband was assigned an anchor, which refers to unique representative start and end positions for a specific cytoband. The representative start position refers to the lowest start position of the overlapping regions, whereas the representative end position refers to the highest end position of the overlapping regions. If 2 different cytobands overlap each other, they are clustered into a single comprehensive cytoband. This conservative approach results in a more comprehensive region and circumvents the issue of missing genes.

Calling gains and losses. We classified CNAs without any limit in size. Therefore, the CNA regions that are gene-rich, most likely would be pathogenic containing a specific region of interest. Overlapping regions were resolved by utilizing the lowest start and highest end position. The numbers of gains and/or losses were calculated by quantifying the number of redundant and overlapping regions for each cytoband. Finally, the frequency was calculated, and cytobands were ranked according to their gain and/loss percentages. An arbitrary threshold of a frequency of 10% or higher was used to call CNAs.

Functional annotation. To explore the possible effects of CNAs in OSCC, we mapped the start and end positions

TABLE 2. Copy number alterations with relevant start and end positions.

No.	Chromosome	Cytoband	Start	End	Size, MB	No. of samples	% ($n = 322$)	CNA, amp/del
1	5	p15.33	22178	50059177	50.04	95	30.45	Amp
2	14	q11.2	19364851	106330010	86.97	87	27.88	Amp
3	11	q13.3	68474153	133951511	65.48	70	22.44	Amp
4	7	p22.3	10704	57558170	57.55	60	19.23	Amp
5	17	q25.3	76282756	81058310	4.78	52	16.67	Amp
6	20	q11.21	29423441	62893159	33.47	47	15.06	Amp
7	8	p11.1	43196185	146279990	103.08	44	14.10	Amp
8	8	q24.13	122660248	146279990	23.62	44	14.10	Amp
9	11	q12.2	60235099	78399879	18.16	44	14.10	Amp
10	3	q27.1	183380180	187479449	4.10	39	12.50	Amp
11	9	q21.11	70224966	141122084	70.90	34	10.90	Amp
12	8	q24.3	141737940	146250965	4.51	32	10.26	Amp
13	3	p26.3	60174	93538496	93.48	104	33.33	Del
14	8	p23.2	2237294	43175454	40.94	69	22.12	Del
15	8	p11.22	39018430	42137557	3.12	55	17.63	Del
16	6	p22.1	27715102	30281087	2.57	41	13.14	Del
17	18	q12.1	26736735	78010002	51.27	37	11.86	Del

Abbreviations: CNA, copy number alteration; Amp, amplification; Del, deletion.

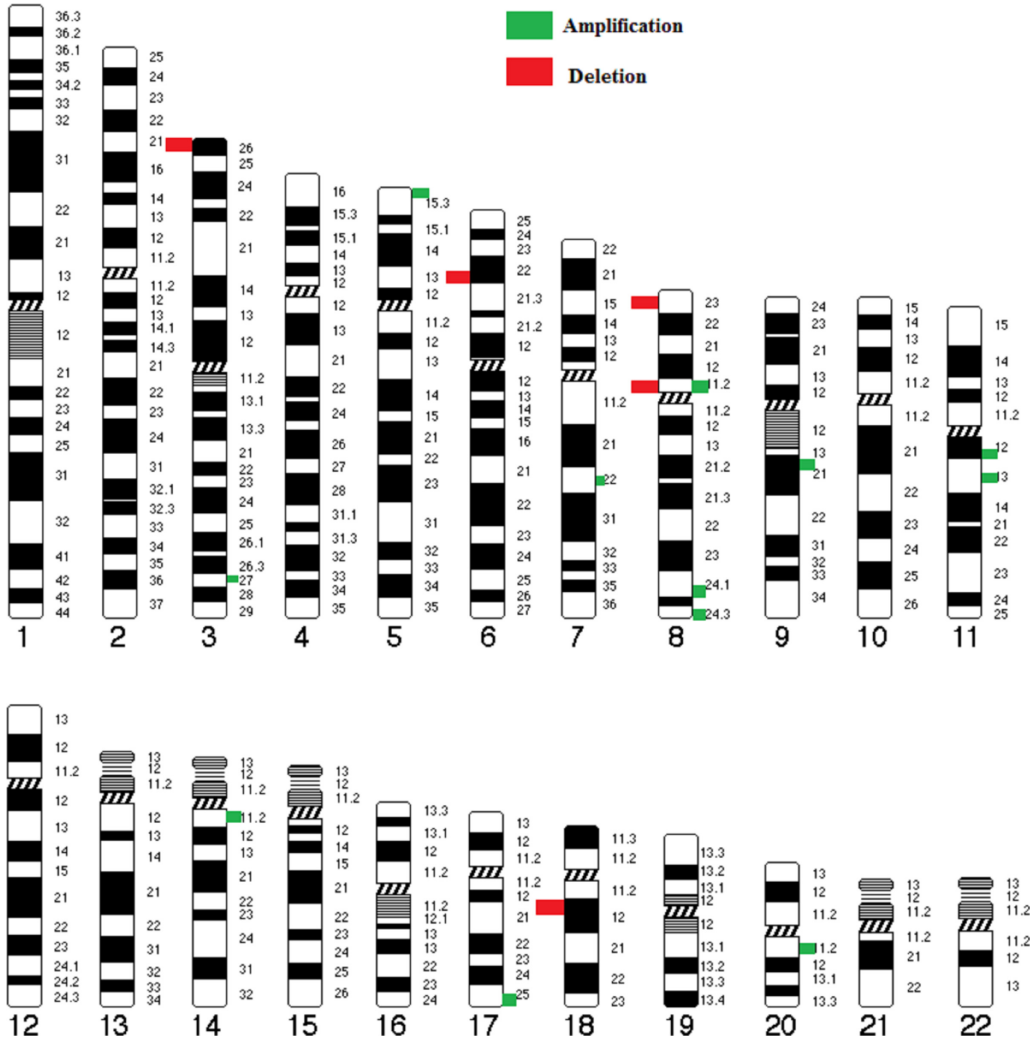

FIGURE 2. The ideogram of the top most frequent copy number alterations (CNAs) in terms of amplifications and deletions identified in this meta-analysis array comparative genomic hybridization (CGH) study in oral squamous cell carcinoma (OSCC).

of each cytoband against the Ensembl genome database (version release 73) to obtain a list of the genes within the cytoband. The RefSeq and UniProt databases were utilized to remove redundancies and filter out non-gene entities, such as RNA and non-coding RNAs. Genes that were not identified in the RefSeq or UniProt databases were filtered out.

A total number of 943 genes that were harbored in the regions with CNAs were functionally analyzed for the significant biological functions and networks using the Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems, Mountain View, CA). The default setting of IPA software was used for mapping those genes to the Knowledge Base that was made as a reference set for both direct and indirect relationships. Then, the molecular networks and biological functions that were most likely relevant to the input gene list were algorithmically generated via the software. The networks that were available in the Ingenuity database were ranked by scores that were defined as a *p* value by which indicates the probability of the genes in a network being generated together because of random chance. A score of 3 shows a 1 in 1000 chance that the genes are in a network as a result of random chance. Therefore, a score of 3 or higher has a 99.9% confidence level of not being generated together because of random chance alone and was used as the cutoff for identifying significant gene networks. The significance of each biological function and canonical pathway was determined based on the *p* value by right-tailed Fisher's exact test with a threshold $< .05$.

RESULTS

Chromosomal profiling of aberrations

From the initial 3349 cytobands, 563 cytobands were identified to be nonredundant (Supplementary Table S1, online only). Copy number changes were observed in 12 and 5 chromosomal regions with gains and losses, respectively (Table 2). The frequency, size, and genomic start and endpoints of the CNAs are shown in Table 2. The

TABLE 3. Top significant molecular and cellular functions given by Ingenuity Pathway Analysis.

Main category	Categories	Function	*p* value	Molecules
Cellular development				
	Cancer, cellular development	Immortalization of epithelial cells	1.94E-04	CCND1, FGF19, ID1, TERT
	Cancer, cellular development	Immortalization of keratinocytes	6.24E-04	CCND1, ID1, TERT
	Cellular development, hematological system development and function, hematopoiesis, and tissue development	Maturation of megakaryocytes	2.44E-03	BIRC5, CCND1, THPO
	Cellular development, connective tissue development and function, embryonic development, hair and skin development and function, organ development, organismal development, and tissue development	Differentiation of dermal fibroblasts	3.06E-03	MAFG, MAFK
	Cell death and survival, cellular development, hair and skin development and function	Lifespan of keratinocytes	3.06E-03	CCND1, TERT
	Cellular development, connective tissue development and function, tissue development	Differentiation of fibroblasts	3.40E-03	HAS2, MAFG, MAFK
	Cellular development	Immortalization of granulosa cells	5.99E-03	CCND1, TERT
	Cellular development, hematological system development and function, hematopoiesis, tissue development	Differentiation of megakaryocytes	1.19E-02	MAFG, MAFK, THPO, ZNF16
	Cell morphology, cellular assembly and organization, cellular development, cellular function and maintenance, nervous system development and function, tissue development	Axonogenesis	1.63E-02	BAI1, BAIAP2, CNTN4
	Cellular development, cellular growth and proliferation, embryonic development, organ development, organismal development, renal and urological system development and function, reproductive system development and function, and tissue development	Proliferation of mammary epithelial cells	1.96E-02	ID1, TERT
Cellular function and maintenance				
	Cellular function and maintenance, and tissue development	Organization of luminal epithelial cells	1.04E-03	DSC2, DSG2
	Cellular function and maintenance, skeletal and muscular system development and function, tissue development	Organization of myoepithelial cells	1.04E-03	DSC2, DSG2
	Cell death and survival, cellular function and maintenance	Colony survival of lymphoma cell lines	3.06E-03	BCL2L1, CCND1
	Cellular assembly and organization, cellular function and maintenance	Growth of microtubules	3.40E-03	BIRC5, CSNK1D, MAPRE1
	Cell morphology, cellular assembly and organization, cellular development, cellular function and maintenance, nervous system development and function, and tissue development	Axonogenesis	1.63E-02	BAI1, BAIAP2, CNTN4
	Cellular assembly and organization, cellular function and maintenance, and tissue development	Polymerization of microtubules	2.90E-02	MAPRE1, TBCD, TPPP
	Cell morphology, cellular function, and maintenance	Autophagy of endothelial cell lines	3.23E-02	CCND1
	Cellular assembly and organization, cellular function and maintenance	Focusing of microtubules	3.23E-02	MAPRE1
	Cellular function and maintenance	Function of hematopoietic progenitor cells	3.23E-02	CBX2
	Cellular assembly and organization, cellular function and maintenance	Function of telomeres	3.23E-02	TERT
Cellular growth and proliferation				
	Cellular growth and proliferation, hematological system development and function	Inhibition of natural killer cells	1.04E-03	HLA-E, HLA-G
	Cellular development, cellular growth and proliferation, embryonic development, organ development, organismal development, renal and urological system development and function, reproductive system development and function, tissue development	Proliferation of mammary epithelial cells	1.96E-02	ID1, TERT
	Cellular development, cellular growth and proliferation, hematological system development and function, hematopoiesis, inflammatory response, lymphoid tissue structure and development, tissue development	Proliferation of megakaryocytes	1.96E-02	BIRC5, THPO
	Cellular development, cellular growth and proliferation, embryonic development, organismal development, tissue development	Proliferation of mesenchymal cells	2.56E-02	CHRD, FGF4
	Cell cycle, cellular growth and proliferation	Antiproliferative response of cells	3.23E-02	ASXL1, TERT

TABLE 3. *Continued*

Main category	Categories	Function	*p* value	Molecules
	Cell cycle, cellular growth and proliferation	Antiproliferative response of cervical cancer cell lines	3.23E-02	ASXL1
	Cellular assembly and organization, cellular development, cellular growth and proliferation, connective tissue development and function, embryonic development, organ development, organismal development, skeletal and muscular system development and function, tissue development	Assembly of podosome rosette	3.23E-02	PTK2
	Cell cycle, cell-to-cell signaling and interaction, cellular development, cellular growth and proliferation, respiratory system development and function	Contact growth inhibition of lung cell lines	3.23E-02	TERT
	Cellular growth and proliferation	Doubling time of colon cancer cell lines	3.23E-02	FXN
	Cellular development, cellular growth and proliferation, hematological system development and function, hematopoiesis, inflammatory response, lymphoid tissue structure and development, tissue development	Growth of colony forming unit-megakaryocytes	3.23E-02	THPO
Lipid metabolism				
	Endocrine system development and function, lipid metabolism, small molecule biochemistry, vitamin and mineral metabolism	Synthesis of corticosterone	1.04E-03	CYP11B1, CYP11B2
	Lipid metabolism, small molecule biochemistry	Synthesis of long chain fatty acid	9.77E-03	DGAT1, FASN
	Lipid metabolism, molecular transport, nucleic acid metabolism, small molecule biochemistry	Concentration of acetyl-coenzyme A	3.23E-02	BCL2L1
	Lipid metabolism, small molecule biochemistry	Conversion of 20:3 (n-6) fatty acids	3.23E-02	FADS1
	Lipid metabolism, small molecule biochemistry	Conversion of 20:4 (n-6) fatty acids	3.23E-02	FADS1
	Lipid metabolism, molecular transport, small molecule biochemistry	Depletion of lactosylceramide	3.23E-02	B4GALT6
	Endocrine system development and function, lipid metabolism, small molecule biochemistry	Hydroxylation of corticosterone	3.23E-02	CYP11B2
	Lipid metabolism, small molecule biochemistry	Metabolism of succinic acid	3.23E-02	SDHA
	Lipid metabolism, nucleic acid metabolism, small molecule biochemistry	Synthesis of malonyl-coenzyme A	3.23E-02	FASN
	Lipid metabolism, small molecule biochemistry	Synthesis of oleic acid	3.23E-02	DGAT1
Small molecule biochemistry				
	Endocrine system development and function, lipid metabolism, small molecule biochemistry, vitamin and mineral metabolism	Synthesis of corticosterone	1.04E-03	CYP11B1, CYP11B2
	Lipid metabolism, small molecule biochemistry	Synthesis of long chain fatty acid	9.77E-03	DGAT1, FASN
	Drug metabolism, molecular transport, nucleic acid metabolism, small molecule biochemistry	Accumulation of 6-mercaptopurine	3.23E-02	ABCC5
	DNA replication, recombination, and repair, nucleic acid metabolism, small molecule biochemistry	Catabolism of dGTP	3.23E-02	NUDT1
	Lipid metabolism, molecular transport, nucleic acid metabolism, small molecule biochemistry	Concentration of acetyl-coenzyme A	3.23E-02	BCL2L1
	Lipid metabolism, small molecule biochemistry	Conversion of 20:3 (n-6) fatty acids	3.23E-02	FADS1
	Lipid metabolism, small molecule biochemistry	Conversion of 20:4 (n-6) fatty acids	3.23E-02	FADS1
	Lipid metabolism, molecular transport, small molecule biochemistry	Depletion of lactosylceramide	3.23E-02	B4GALT6
	Drug metabolism, molecular transport, nucleic acid metabolism, small molecule biochemistry	Efflux of 6-mercaptopurine	3.23E-02	ABCC5
	Nucleic acid metabolism, small molecule biochemistry	Hydrolysis of thiamine triphosphate	3.23E-02	THTPA

number of chromosomal regions with gains was more frequent than losses in the entire dataset. The median size of the gains and losses was 0.36 Mb and 0.15 Mb, respectively. The sizes of the gains were larger than that of the losses in both datasets. Gain in chromosome 5p15.33 was the most common and was found in 30.45% (n = 312), followed by 14q11.2 (n = 87; 27.88%), 11q13.3 (n = 70; 22.44%), 7p22.3 (n = 60; 19.23%), 17q25.3 (n = 52; 16.67%), 20q11.21 (n = 47; 15.06%), 8p11.1 (n = 44; 14.1%), 8q24.3 (n = 44; 14.1%), 11q12.2 (n = 44; 14.1%), 3q27.1 (n = 39; 12.5%), 9q21.11 (n = 34; 10.9%), and 8q24.3 (n = 32; 10.26%). Loss in 3p26.3 was the most common and was found in 33.33% of all samples (104 of 312 samples), followed by 8p23.3 (n = 69; 22.12%), 8p11.22 (n = 55; 17.63%), 6p22.1 (n = 41; 13.14%), and 18q12.1 (n = 37; 11.86%; Figure 2; Table 2). Chromosomes 3 and 8 had the highest number of changes in copy number at different regions across the entire length of the chromosome.

Biological process analysis

To explore the potential effects of the chromosomal alterations implicated in the molecular mechanism of oral carcinogenesis, we further analyzed the biological function annotations and biological network that were associated with CNAs. The significant top molecular and cellular functions were categorized into 5 different groups, including cellular development, cellular function and maintenance, cellular growth and proliferation, lipid metabolism, and small molecule biochemistry. The associated top 10 molecular and cellular functions of these categories are listed in Table 3. The top molecular and cellular function were reported as cellular development, which is mostly associated with immortalization of the epithelial cells (p = 1.93E-04) through contributing of *CCND1*, fibroblast growth factor 19 (*FGF19*), inhibitor of DNA binding 1 (*ID1*), and telomerase reverse transcriptase (*TERT*) genes. The top ranked categories of diseases was reported as related to head and neck neoplasia with 118 genes associated with this cancer. Of 118 genes, 15 of them, namely ADAM metallopeptidase domain 9 (*ADAM9*), anoctamin-1 (*ANO1*), *CCND1*, cortactin (*CTTN*), desmoglein 2 (*DSG2*), Fas-associated protein with death domain (*FADD*), FGF19, FGF3, FGF4, hyaluronan synthase 2 (*HAS2*), metastasis suppressor 1 (*MTSS1*), myeloma overexpressed (*MYEOV*), oral cancer overexpressed 1 (*ORAOV1*), protein tyrosine phosphatase, receptor type, F polypeptide (*PTPRF*), interacting protein (*Liprin*), alpha (*PPFIA1*), and 2 pore segment channel 2 (*TPCN2*), were related to head and neck SCC (Table 4).

Results of network analysis of 943 CNA-associated genes have been summarized in Table 5. The most significant network was linked to cancer, cellular movement, and connective tissue disorders. The 5 most significant functions were associated with growth of tumor (p = 4.33E-06). The top significant network harbors 19 genes, among which the major cores, *CCND1* and *TERT*, were hub nodes in the network, and formed interconnected autoregulatory and feed-forward circuitry (see Figure 3). The associated network function analysis revealed that the core-bound genes are mostly relevant to growth of tumor (p = 4.33E-06) and regulated by angiogenin (ANG), asialoglycoprotein receptor 1 (ASGR1), brain-specific angiogenesis inhibitor 1 (BAI1), Bcl-2-like protein 1 (BCL2L1), baculoviral IAP repeat containing 5 (BIRC5), BTG family member 3 (BTG3), CCND1, fatty acid synthase (FASN), heat shock transcription factor 1 (HSF1), heat shock protein 90kDa (Hsp90), ID1, matrix metallopeptidase 14 (MMP14), plectin (PLEC), protein tyrosine kinase 2 (PTK2), ribosomal protein L22 (RPL22), secreted frizzled-related protein 1 (SFRP1), TERT, TIMP metallopeptidase inhibitor 2 (TIMP2), and TPX2 microtubule-associated (TPX2) genes.

DISCUSSION

Meta-analysis is a systematic and quantitative analytical method that can be used to summarize and cross-analyze the significance of results from multiple studies on the same disease.[30] To date, only a few meta-analysis studies have been conducted using assay CGH data.[31,32] A critical prerequisite for this analysis was the requirement to standardize data of previously published CNAs through cross-platform analysis. Meta-analysis[31] can be a very powerful tool for increasing the robustness and reliability of datasets as well as to identify novel findings that may not be noticeable from a single study. To the best of our knowledge, the present study would be the largest meta-analysis that has been conducted on OSCC samples from the oral cavity using assay CGH data. Because of high intratumoral and genetic heterogeneity of squamous cell carcinoma of the head and neck (SCCHN), only samples from the oral cavity, such as the tongue (excluding the base of tongue), buccal mucosa, alveolar ridge, retromolar trigone, hard palate and floor of mouth, were included.[33,34] Hence, the identified recurrent CNAs from this unique oral cavity SCC could provide new insights into the etiological basis of oral carcinogenesis.

CNAs can lead to disruption of proto-oncogenes or tumor suppressor genes, as they have been determined to be the major determinant of poor prognosis in oral cancer.[22,26] In this study, 12 amplifications and 5 deletions with a frequency $\geq$10% were identified after filtering using our thresholds. Gains were more frequent than losses and were detected in chromosomes 3, 5, 7, 8, 11, 14, 17, and 20, whereas losses were observed in chromosomes 3, 6, 8, and 18. In this study, identifying high-frequency CNAs in chromosomes 3 and 8 reflects the existence of oncogenes and tumor suppressor genes that could be associated with poor prognosis in oral cancer.[35,36] Therefore, further investigation on these chromosomes could provide better prognosis prediction and anticancer strategies for patients with OSCC. In this study, amplification of 5p15.33 was found to be the most frequent event, being present in 30.45% (n = 95) of all OSCCs. Genomic alterations at chromosome 5p have been frequently reported in OSCC, especially amplification of 5p15.33, which was associated with disease-specific survival in human cancers, such as OSCC and bladder cancer.[26,37] This CNA contains various proto-oncogenes, including TERT, programmed cell death protein 6 (PDCD6), and cleft lip and palate transmembrane protein 1 (CLPTM1L). TERT encodes the human telomerase reverse transcriptase (hTERT) and its activation plays an important role in telomere maintenance and cell

TABLE 4. Top 15 significant categories of diseases and the associated diseases or functions annotation given by Ingenuity Pathway Analysis.

Categories	Diseases or functions annotation	*p* value	Molecules	No. of molecules
Cancer	Head and neck neoplasia	4.62E-03	ACIN1, ADAM9, AHRR, ALYREF, ANKRD20A4 (includes others), ANO1, ARC, ASXL1, ASXL3, BAHCC1, BAI1, BAIAP2, BCL2L1, BIRC5, BRD9, C17orf70, CBWD3/CBWD6, CBX8, CCDC40, CCDC57, CCND1, CD6, CD7, CEP131, CHRD, CNTN4, CNTN6, CTTN, CYHR1, CYP11B2, DHRS4, DHRS4L2, DNAH17, DNMT3B, DSG2, DTNA, EIF3B, ELFN1, EPHB3, EPPK1, FADD, FAM20C, FAM83A, FAM83H, FAM91A1, FASN, FBXL6, FER1L6, FGF19, FGF3, FGF4, FN3KRP, FOXD4L4/FOXD4L5, FOXK2, GPX5, HAS2, HEXDC, HLA-A, HLA-E, HLA-G, INTS1, IRX1, KIAA1875, KLHL14, LRRC24, LYNX1, MAFA, MMP14, MOG, MROH5, MRPL21, MS4A5, MS4A7, MTSS1, MYADML2, MYEOV, MYRF, NPTX1, OGFOD3, OPLAH, OR10G2, OR11H12 (includes others), OR2H1, OR4N2, ORAOV1, PARP10, PGA5 (includes others), PLEC, PLEKHG4B, POTEH (includes others), PPFIA1, PRMT5, PTGDR2, RBFOX3, RNF213, RPGRIP1, SCRIB, SCRT1, SLC12A7, SLC16A3, SLC38A10, SLC45A4, SLC6A3, SLC9A3, SOCS3, SUN1, TERT, THEM6, TMC8, TMEM235, TONSL, TOP1MT, TPCN2, TSPAN10, ZC3H3, ZDHHC11, ZDHHC11B, ZNF517	118
Cancer, endocrine system disorders	Endocrine gland tumor	1.73E-02	ACIN1, AHRR, ALYREF, ANKRD20A4 (includes others), ARC, BAHCC1, BAIAP2, BRD9, C17orf70, CBWD3/CBWD6, CCDC40, CCDC57, CCND1, CD6, CEP131, CHRD, CNTN4, CYHR1, CYP11B2, DHRS4L2, DNAH17, DNMT3B, EIF3B, ELFN1, EPHB3, EPPK1, FAM20C, FAM83H, FBXL6, FER1L6, FN3KRP, FOXD4L4/FOXD4L5, FOXK2, HEXDC, HGS, HLA-A, INTS1, IRX1, KIAA1875, KLHL14, LRRC24, MAFA, MMP14, MROH5, MRPL21, MS4A5, MYADML2, OGFOD3, OPLAH, OR10G2, OR11H12 (includes others), PARP10, PDCD6, PGA5 (includes others), PLEC, POTEH (includes others), PTGDR2, RBFOX3, RNF213, RPGRIP1, RPTOR, SCRIB, SCRT1, SDHA, SDHAF2, SLC12A7, SLC16A3, SLC45A4, SLC9A3, SOCS3, SUN1, TERT, THEM6, TMEM235, TONSL, TOP1MT, TSPAN10, ZC3H3, ZDHHC11, ZDHHC11B, ZNF517	81
Cancer	Neck neoplasm	2.60E-02	ACIN1, AHRR, ALYREF, ANKRD20A4 (includes others), ARC, BAHCC1, BAIAP2, BRD9, C17orf70, CBWD3/CBWD6, CCDC40, CCDC57, CCND1, CD6, CEP131, CHRD, CNTN4, CYHR1, CYP11B2, DHRS4L2, DNAH17, DNMT3B, EIF3B, ELFN1, EPHB3, EPPK1, FADD, FAM20C, FAM83H, FBXL6, FER1L6, FN3KRP, FOXD4L4/FOXD4L5, FOXK2, HEXDC, HLA-A, INTS1, IRX1, KIAA1875, KLHL14, LRRC24, MAFA, MROH5, MRPL21, MS4A5, MYADML2, OGFOD3, OPLAH, OR10G2, OR11H12 (includes others), PARP10, PGA5 (includes others), PLEC, POTEH (includes others), PTGDR2, RBFOX3, RNF213, RPGRIP1, SCRIB, SCRT1, SLC12A7, SLC16A3, SLC45A4, SLC9A3, SOCS3, SUN1, TERT, THEM6, TMEM235, TONSL, TSPAN10, ZC3H3, ZDHHC11, ZDHHC11B, ZNF517	75
Cancer, endocrine system disorders	Thyroid gland tumor	3.09E-02	ACIN1, AHRR, ALYREF, ANKRD20A4 (includes others), ARC, BAHCC1, BAIAP2, BRD9, C17orf70, CBWD3/CBWD6, CCDC40, CCDC57, CCND1, CD6, CEP131, CHRD, CNTN4, CYHR1, CYP11B2, DHRS4L2, DNAH17, DNMT3B, EIF3B, ELFN1, EPHB3, EPPK1, FAM20C, FAM83H, FBXL6, FER1L6, FN3KRP, FOXD4L4/FOXD4L5, FOXK2, HEXDC, HLA-A, INTS1, IRX1, KIAA1875, KLHL14, LRRC24, MAFA, MROH5, MRPL21, MS4A5, MYADML2, OGFOD3, OPLAH, OR10G2, OR11H12 (includes others), PARP10, PGA5 (includes others), PLEC, POTEH (includes others), PTGDR2, RBFOX3, RNF213, RPGRIP1, SCRIB, SCRT1, SLC12A7, SLC16A3, SLC45A4, SLC9A3, SOCS3, SUN1, TERT, THEM6, TMEM235, TONSL, TSPAN10, ZC3H3, ZDHHC11, ZDHHC11B, ZNF517	74
Immunological disease	Systemic autoimmune syndrome	4.46E-03	ANG, ARL16, BIRC5, CD5, CD6, GAA, GABBR1, HCG4, HCG9, HCK, HIST1H2BO, HIST1H3I, HIST1H4L, HLA-A, HLA-F, HLA-G, HLA-J, HLA-L, MAPRE1, MOG, MS4A1, NKAPL, OR11A1, OR12D2, OR12D3, OR2B3, PPP1R11, PRSS16, PSMG3-AS1, RNASE2, RNASE3, RNF39, SOCS3, SQLE, TRIM10, TRIM15, TRIM26, TRIM27, TRIM31, TRIM40, VPS37C, ZFP57, ZKSCAN3, ZKSCAN4, ZKSCAN8, ZNF165, ZNF311, ZNRD1, ZNRD1-AS1, ZSCAN12, ZSCAN31	51
Metabolic disease	Glucose metabolism disorder	6.98E-05	BCL2L1, CCNB1IP1, CCND1, CPT1A, CSNK1D, DGAT1, FASN, GAA, GABBR1, GCGR, HCG4, HCG9, HIST1H2BO, HIST1H3I, HIST1H4L, HLA-A, HLA-F, HLA-L, HTR3C, HTR3D, HTR3E, MCF2L2, MOG, MS4A1, NKAPL, OR11A1, OR12D2, OR12D3, OR2B3, PPP1R11, PRSS16, PSMB5, RNF138, RNF39,	50

TABLE 4. *Continued*

Categories	Diseases or functions annotation	*p* value	Molecules	No. of molecules
			SLC6A3, TRIM10, TRIM26, TRIM27, TRIM31, TRIM40, ZFP57, ZKSCAN3, ZKSCAN4, ZKSCAN8, ZNF165, ZNF311, ZNRD1, ZNRD1-AS1, ZSCAN12, ZSCAN31	
Endocrine system disorders, gastrointestinal disease, metabolic disease	Diabetes mellitus	5.14E-05	BCL2L1, CCNB1IP1, CCND1, CSNK1D, DGAT1, GAA, GABBR1, GCGR, HCG4, HCG9, HIST1H2BO, HIST1H3I, HIST1H4L, HLA-A, HLA-F, HLA-L, HTR3C, HTR3D, HTR3E, MCF2L2, MOG, MS4A1, NKAPL, OR11A1, OR12D2, OR12D3, OR2B3, PPP1R11, PRSS16, PSMB5, RNF138, RNF39, SLC6A3, TRIM10, TRIM26, TRIM27, TRIM31, TRIM40, ZFP57, ZKSCAN3, ZKSCAN4, ZKSCAN8, ZNF165, ZNF311, ZNRD1, ZNRD1-AS1, ZSCAN12, ZSCAN31	48
Endocrine system disorders, gastrointestinal disease, immunological disease, metabolic disease	Insulin-dependent diabetes mellitus	6.53E-14	GAA, GABBR1, HCG4, HCG9, HIST1H2BO, HIST1H3I, HIST1H4L, HLA-A, HLA-F, HLA-L, MOG, MS4A1, NKAPL, OR11A1, OR12D2, OR12D3, OR2B3, PPP1R11, PRSS16, RNF39, TRIM10, TRIM26, TRIM27, TRIM31, TRIM40, ZFP57, ZKSCAN3, ZKSCAN4, ZKSCAN8, ZNF165, ZNF311, ZNRD1, ZNRD1-AS1, ZSCAN12, ZSCAN31	35
Cancer, neurological disease	Brain astrocytoma	4.89E-02	ACIN1, ASXL1, ASXL3, CBWD3/CBWD6, CNTN6, DNAH17, DTNA, FAM83A, FER1L6, GPX5, HLA-E, HLA-G, LYNX1, MS4A7, NPTX1, OR11H12 (includes others), OR2H1, OR4N2, PLEC, PLEKHG4B, POTEH (includes others), SLC6A3, TERT, TMC8	24
Cancer, hematological disease, immunological disease	Plasma cell dyscrasia	3.01E-02	ADAM9, CBX2, CCND1, CSMD1, FADD, FASN, FEN1, GPR146, HLA-A, HTR3C, HTR3D, HTR3E, MS4A1, PSMB5, PSMD2, RNF213, SLC6A3, TIMP2, ZKSCAN3	19
Cancer	SCCHN	4.56E-02	ADAM9, ANO1, CCND1, CTTN, DSG2, FADD, FGF19, FGF3, FGF4, HAS2, MTSS1, MYEOV, ORAOV1, PPFIA1, TPCN2	15
Cancer, hematological disease, immunological disease	Multiple myeloma	3.95E-03	CCND1, CSMD1, GPR146, HLA-A, HTR3C, HTR3D, HTR3E, PSMB5, PSMD2, RNF213, SLC6A3, TIMP2, ZKSCAN3	13
Cardiovascular disease, organismal injury and abnormalities	Nonischemic cardiomyopathy	1.56E-02	CYP11B2, DSC2, DSG2, FXN, GAA, mir-28, MYH6, MYH7, MYLK2, SDHA, TTR	11
Cellular development, cellular growth and proliferation, hair and skin development and function	Proliferation of epithelial cell lines	4.49E-02	ABCC5, BIRC5, CCND1, COMMD5, FGF19, NUDT1, PTP4A3, SOCS3, TRIM39, TTR	10
Cellular movement	Invasion of carcinoma cell lines	8.80E-03	ADAM9, CDH2, CTTN, DNMT3B, ID1, NDRG2, PTK2, TOX4, UBD	9

Abbreviation: SCCHN, squamous cell carcinoma of the head and neck.

proliferation in human cancer.[38,39] The study by Sumida et al[40] has demonstrated that overexpression of TERT in chemically induced hamster OSCC increased the tumor cell proliferation, as cell proliferation activity was correlated with the progression of disease. Therefore, this oncogene has been targeted for anticancer therapy in oral cancer. PDCD6 is a proapoptotic calcium-binding protein that involves an apoptosis pathway. It seemed that overexpression of this gene could play a role in the aggressive behavior of cancer by enhancing tumor cell proliferation, invasion, metastasis, and evading apoptosis signal.[41,42] CLPTM1L plays an important role to protect cancer cells from genotoxic stress-induced apoptosis through regulation of B-cell lymphoma extra large (Bcl-xL) in tumorigenesis.[43] These anti-apoptotic characteristics give us a notion of using this gene in cancer gene therapy. Fine-mapping of this region will give a better understanding of the potential oncogenes that play a role in oral carcinogenesis.

Loss in 3p26.3 was found to be the most frequent observation in the CNAs. Deletion of this region has been identified as the prognostic factor in patients with OSCC. This region harbors the potential cancer-related gene, namely cell adhesion molecule with homology to L1CAM1 (CHL1). CHL1 is a member of the family of L1 neural cell adhesion molecules. It has been suggested that loss of this gene in the early stage of cancer contributes in the growth and metastasis of human cancers.[44] Functional studies showed that CHL1 was able to suppress tumor cell proliferation and invasion both in vitro and in vivo in breast tumorigenesis.[45] These observations in combination with our results suggest that CHL1 is a candidate tumor-suppressor gene associated with aggressiveness of OSCC.

Chromosomes 3 and 8 had the highest number of chromosomal aberrations on both arms, and both gains and losses were detected on these chromosomes. The chromosome 8q24 region is the most frequently amplified CNA in various cancers, including OSCC. This current study revealed that single cytoband of 8q24.13 seemed to be the target of amplification on chromosome 8 that harbors several cancer-related genes, such as ATPase family, AAA domain containing 2 (ATAD2), and Derlin 1 (DERL1), which may play prominent roles in tumorigenesis.[46] ATAD2 plays a role as a mediator of MYC and overexpression of this gene contributes to cancer aggressiveness through the MYC-dependent transcription pathway.[47] DERL1 plays a role to regulate the dislocation and retrotranslocation of the misfolded proteins from endoplasmic reticulum and proteins from endoplasmic reticulum lumen into cytosol.[48,49] Overexpression of this gene is reported to play an important role to evade apoptosis and promote cell proliferation, migration, and invasion in tumorigenesis via the extracellular signal-regulated kinase (ERK) signaling pathway in nonsmall cell lung cancer and breast carcinoma.[50,51]

Interestingly, a loss in 8p11.1-p23.3 was one of the most frequent CNAs on the short arm of chromosome 8. The focal region of 8p11.23, which harbors the *ADAM3A* and *ADAM18* genes, could be a region of interest for further investigation. Both genes act as negative regulators of tumor invasion by degrading the extracellular matrix along with MMP genes.[52] Inactivation of tumor suppressor genes at this focal region seemed to play a critical role in lymph node metastasis among OSCC cases.[53] Taken together, it seems that aberration in both arms of chromosomes 8 is associated with invasive behavior of tumors and poor prognosis.[52,54,55]

Copy number changes in both arms of chromosome 3 were common. It seemed that 3p26.3 is the target of deletion and that 3q27.1 is the target of amplification. Loss in 3p and gain in 3q have been frequently found in other cancers as well as OSCC.[56,57] Among these chromosomal regions, 3p26.3 on the short arm and 3q27.1 on the long arm seemed to be regions of interest. In contrast to chromosome 8, it seemed that copy number changes on this chromosome are involved in initiation and progression of a tumor toward malignancy.[58,59] Segmental 3p losses have been detected among oral potentially malignant diseases and early stages of OSCC, whereas multiple segmental or broad 3p deletions have been detected among advanced-stage tumors.[59] Amplification of 3q was reported to be associated with advanced stages of OSCC, indicating that alteration at this region could be an important transition event in tumor progression to invasive OSCC.[60] The focal gain of 3q27.1 that was observed in 12.50% of samples contained various cancer-related genes, including mitogen-activated protein kinase kinase kinase 13, SUMO1/sentrin/SMT3 specific peptidase 2 (SENP2), insulin-like growth factor 2 MRNA binding protein 2 (IGF2BP2), transformer 2 beta homolog (TRA2B), Ets variant 5 (ETV5), and diacylglycerol kinase gamma 90kDa (DGKG).[61–66] Of these genes, overexpression of IGF2BP2 and TRA2B was found to be associated with poor prognosis in SCCHN.[63,64] TRA2B has an interesting role as a splicing regulator of several known oncogenes.TRA2B can also enhance cell proliferation and metastasis in cancer and decrease apoptosis during tumorigenesis in various types of cancers.[64]

It is well known that the long arm of chromosome 11 harbors cancer-related genes. In the present study, 11q13.3, with a size of 65.48 Mb, was amplified in 22.44% of OSCCs. Several studies have identified amplification of this region ranging from 11% to 60% in the SCCHN cohort, suggesting that amplification of this region is dependent on the tumor subsites.[13,67,68] It is well documented that amplification of this region is one of the most frequent CNA in human cancer and considered an early event in head and neck carcinogenesis.[69,70] Several candidate oncogenes, such as *CCND1*, *CTTN*, *ORAOV1*, *ANO1*, and *FADD*, have been reported to promote tumor cell proliferation and evade tumor cell apoptosis, invasion, and migration in tumorigenesis.[71–75] Amplification of 11q13 was correlated with poor prognosis[76] and lymph node metastasis in SCCHN,[77] which implies that these candidate driver genes might be valuable as biomarkers for prognosis and treatment plans in oral cancers.

In downstream IPA analysis of CNA-associated genes, a number of significant biological annotations were identified for genes that might contribute to immortalization and proliferation of epithelial cells and keratinocyte in tumorigenesis. Of these, amplification of *CCND1*, *FGF19*, *ID1*, and *TERT* genes, which are, in turn, located

TABLE 5. Top 5 significant networks and the associated network functions given by Ingenuity Pathway Analysis.

Top diseases and functions (score)	Categories	Functions annotation	*p* value	Molecules	No. of molecules
Cancer, cellular movement, connective tissue disorders (47)					
	Cancer	Growth of tumor	4.33E-06	ANG, ASGR1, BAI1, BCL2L1, BIRC5, BTG3, CCND1, FASN, HSF1, Hsp90, ID1, MMP14, PLEC, PTK2, RPL22, SFRP1, TERT, TIMP2, TPX2	19
	Cancer, cellular movement, connective tissue disorders	Invasion of fibroblasts	4.84E-06	CCND1, MMP14, PTK2, TIMP2	4
	RNA post-transcriptional modification	Cleavage of RNA fragment	2.54E-05	AGO2, ANG, EXOSC8, RNA polymerase II	4
	Dermatological diseases and conditions, hereditary disorder	Advanced stage Netherton syndrome	3.49E-05	DSC1, DSC3, DSG1, DSG3	4
	Cellular development, cellular growth and proliferation	Proliferation of breast cancer cell lines	3.66E-05	BCL2L1, BIRC5, CCND1, FGF4, GPER1, HAS2, HSF1, ID1, MMP14, PTK2, TERT, TIMP2	12
Cellular movement, inflammatory response, cellular development (29)					
	Inflammatory response	Inflammation of body region	2.42E-08	ADIPOQ, AKT1, ARHGDIA, B2M, CALR, CDH1, CEBPE, ESR2, HLA-A, HLA-B27, HLA-E, KCNA1, LIF, miR-100-5p (and other miRNAs w/seed ACCCGUA), MPO, MTOR, PDIA3, PLA2G4A, RPTOR, SMAD2, SMURF2, SOCS3, STAT3, TP53	24
	Cellular development, cellular growth and proliferation	Proliferation of hepatoma cell lines	2.67E-08	ADIPOQ, AKT1, EZH2, miR-100-5p (and other miRNAs w/seed ACCCGUA), MTOR, PLA2G4A, SMAD2, SOCS3, SRC, STAT3, TP53	11
	Cellular movement	Invasion of cells	4.59E-08	ADIPOQ, AKT1, ARHGDIA, CALR, CCDC88A, CDH1, CTTN, DNMT3B, ESR2, EZH2, HIC1, MAP2K5, MTOR, NANOG, NCOA1, NCOR1, NDRG2, SMAD2, SRC, STAT3, TP53, UBD	22
	Cellular development, cellular growth and proliferation	Proliferation of tumor cell lines	5.12E-08	ADIPOQ, AJUBA, AKT1, AKT1S1, CALR, CCDC88A, CDH1, CEBPE, CTTN, DNMT3B, DVL3, ESR2, EZH2, HIC1, LIF, miR-100-5p (and other miRNAs w/seed ACCCGUA), MTOR, NANOG, NCOA1, NCOR1, NDRG2, PDIA3, PLA2G4A, RPTOR, SMAD2, SOCS3, SRC, STAT3, TP53, ZKSCAN3	30
	Cancer	Growth of tumor	1.28E-07	ADIPOQ, AKT1, CALR, CDH1, CTTN, DNMT3B, ESR2, EZH2, HIC1, HLA-G, LIF, MAP2K5, miR-100-5p (and other miRNAs w/seed ACCCGUA), MTOR, NCOA1, NDRG2, PLA2G4A, SOCS3, SRC, STAT3, TP53	21
Organismal injury and abnormalities, tissue development, cellular growth and proliferation (26)					
	Organismal injury and abnormalities	Follicular cyst	1.22E-05	ESR1, NGFR, SAT1	3
	Tissue development	Growth of connective tissue	1.93E-05	CXCL3, EREG, ESR1, FGF1, FOS, FOSB, GABBR1, GPER1, LIF, MED1, MKI67, NGFR, NUPR1, SRF, TACC1, VAV3	16
	Cellular growth and proliferation, tissue development	Proliferation of connective tissue cells	2.63E-05	CXCL3, EREG, ESR1, FGF1, FOS, FOSB, GABBR1, GPER1, LIF, MED1, MKI67, NGFR, NUPR1, TACC1, VAV3	15
		Proliferation of fibroblasts	4.13E-05		11

TABLE 5. *Continued*

Top diseases and functions (score)	Categories	Functions annotation	*p* value	Molecules	No. of molecules
	Cellular development, cellular growth and proliferation, connective tissue development and function, tissue development			CXCL3, EREG, ESR1, FGF1, FOS, FOSB, GPER1, LIF, NGFR, NUPR1, TACC1	
	Cellular development, cellular growth and proliferation, skeletal and muscular system development and function, tissue development	Proliferation of muscle cells	5.84E-05	CAPN2, DGCR8, EREG, ESR1, FGF1, FOS, GPER1, IGFBP2, let-7, LIF, TRIB1	11
RNA post-transcriptional modification, cell cycle, cell death and survival (26)					
	RNA post-transcriptional modification	Processing of mRNA	3.70E-07	ACIN1, BARD1, CSTF1, CTDP1, HBB, PABPC1, PABPN1, POLR2A, SUPT6H	9
	RNA post-transcriptional modification	Polyadenylation of RNA	6.88E-07	BARD1, CSTF1, PABPC1, PABPN1, PAPOLG	5
	RNA post-transcriptional modification	Molecular cleavage of mRNA	7.00E-07	CSTF1, HBB, PABPN1, POLR2A	4
	Cell cycle	Senescence of connective tissue cells	2.31E-06	BRCA1, CASP3, CBX2, CDKN1A, CDKN1B, SREBF1	6
	Cell death and survival	Apoptosis of bone cancer cell lines	2.39E-06	BRAT1, BRCA1, CASP3, CDKN1A, CDKN1B, E2F1, FOXK2, SIRT7, UIMC1	9
Cellular movement, renal and urological system development and function, cell-to-cell signaling and interaction (25)					
	Cellular movement, renal and urological system development and function	Cell movement of kidney cell lines	6.76E-06	CA9, CTNNB1, EPHB3, Fascin, ITGA1, ITGB1, L1CAM, NOX4, TLR2	9
	Cell-to-cell signaling and interaction, embryonic development, tissue development	Adhesion of embryonic cells	2.55E-05	Collagen type IV, CTNNB1, EPAS1, EPHB3, HIF1A, ITGB1	6
	Cellular movement, renal and urological system development and function	Migration of kidney cell lines	3.81E-05	CA9, EPHB3, Fascin, ITGA1, ITGB1, L1CAM, NOX4	7
	Cancer	Tumorigenesis of cells	4.25E-05	CD24, CTNNB1, DAK, EPAS1, HIF1A, ITGA1, L1CAM, NOX4, TLR2	9
	Inflammatory disease, inflammatory response, organismal injury and abnormalities, renal inflammation, renal nephritis, renal and urological disease	Nephritis	4.49E-05	CD151, EPAS1, GADD45B, HIF1A, IFIH1, ITGA1, MS4A1, PKN1, TLR2	9

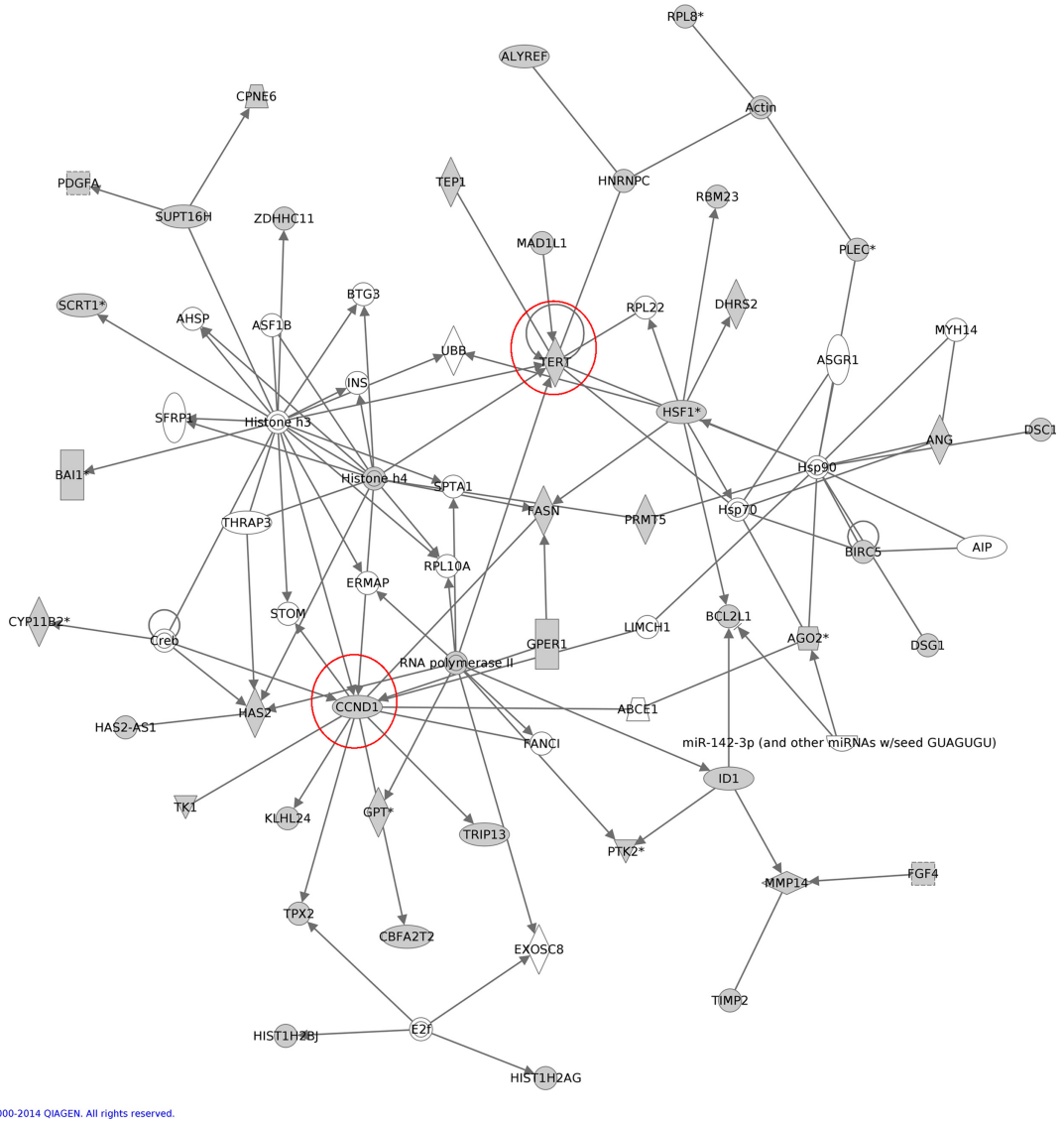

FIGURE 3. The top significant network that was related with cancer, cellular movement, and connective tissue disorders. Major hubs in the network were located in the red circle.

on 11q13.3, 20q11.21, and 5p15.33, were identified to play a central role in tumor cell proliferation and immortalization in oral carcinogenesis. The downstream effects of the recurrent CNAs in cancer cells could be disruption of the oncogenes and tumor suppressor genes in the dysregulated cell signaling pathways/networks. One important network was identified around the *CCND1* and *TERT* genes. Evidence showed that cancer cells are able to maintain their telomeres through the alternative lengthening of telomeres (ALT) mechanism by TERT and to activate the cyclin-dependent kinases (CDKs), CDK4 and CDK6, mechanism for their oncogenic action by CCND1.[39,78] Therefore, these mechanisms enable to promote tumor cells to evade senescence and accelerating cell immortalization and proliferation in tumurigenesis.[39,78] Apart from that, activation of the FGF19-FGFR4 signaling pathway plays an important role in cell proliferation and evades apoptotic signal via the activation of WNT cascade signaling and other stimulation from ligand receptor in tumor progression. As for *ID1*, overexpression of this gene enables to promote tumor growth and cell survival via the Raf-1/mitogen-activated protein kinase (MAPK) pathway.[79] We further ranked the significant biological functions according to the number of the genes and the highest number of the genes that are involved in the significant biological function was associated with head and neck neoplasia ($p = 4.62E-03$), with a total number of 118 genes. Of these genes, 15 genes (*ADAM9*, *ANO1*, *CCND1*, *CTTN*, *DSG2*, *FADD*, *FGF19*, *FGF3*, *FGF4*, *HAS2*, *MTSS1*, *MYEOV*, *ORAOV1*, *PPFIA1*, and *TPCN2*) were associated with SCCHN. Therefore, the current study also highlights the importance of these genes as they may serve as promising targets for therapeutic development efforts and their associated biological functions and networks along with other common oncogenic pathways in tumorigenesis.

CONCLUSION

In conclusion, this study has identified multiple recurrent CNAs that are associated with various biological functions, such as immortalization of epithelial cells by contributing of *CCND1*, *FGF19*, *ID1*, and *TERT* genes in oral carcinogenesis. Apart from that, this study identified critical biological networks that are frequently disrupted in oral carcinogenesis with different oncogenes associated with CNAs. This offers a better understanding of the genomic alterations in OSCC and may be regarded as a potential postulation that the dysregulation of these networks may play a major role in oral carcinogenesis.

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