

**Appendix Table A. Genetic Update of Pro-apoptotic genes**

Gene family	Gene	Other name	Location, size, exons and introns	Studied by	Study design	Methodology	Inference
TNF family Ligands and Receptors	Lymphotoxin alpha (LTA)	TNF $\beta$ , TNFSF1	6p21.3 Size-2271bp Introns -9 Exons-9	Huang Y et al (2013)	Meta-analyses of association between cancer and LTA variants (rs1041981, rs2239704, rs2229094 and rs746868)	30 case-control studies involving 58,649 participants	LTA rs1041981, rs2239704 and rs2229094 polymorphisms exhibited increased risk of cancers.
				Yapjikakis C et al. (2009)	Evaluation of association of TNF- $\alpha$ and TNF- $\beta$ high expression alleles with risk of oral cancer	Functional polymorphisms and gene expression of TNF- $\alpha$ (-308 G/A) and TNF- $\beta$ (252 G/A) were analysed by restriction fragment length polymorphism in Oral squamous cell carcinoma DNA samples	The frequencies of high expression A2 (-308A) TNF- $\alpha$ allele and high expression B1 (252G) TNF- $\beta$ allele were significantly increased in cancer patients and combined TNF- $\alpha$ /TNF- $\beta$ genotypes (A2A2/B1B1, A1A2/B1B2, A1A2/B1B1) were over-represented in cancer patients. High TNF- $\alpha$ levels in serum of patients with oral cancer in comparison to healthy controls was observed.
				Vairaktaris E et al (2008)	Interaction of functional DNA polymorphisms affecting gene expression of serum or saliva levels of interleukins IL-1 beta,-4,-6,-8,-10 and tumor necrosis factors TNF-alpha,-beta with increased risk of development of oral squamous cell carcinoma (OSCC)	162 OSCC cases and 168 healthy controls were assessed to evaluate the contribution of homozygous or heterozygous variant genotypes of polymorphisms IL-1 beta (+3953C/T), IL-4 (-590C/T), IL-6 (-174G/C), IL-8 (-251A/T), IL-10 (-1082A/G), TNF-alpha (-308G/A) and TNF-beta (+252G/A).	Revealed the highly significant contributions of two out of seven studied cytokines (IL-6 and TNF-alpha) in the occurrence of OSCC.
	Tumor Necrosis Factor Alpha (TNFA)	TNF $\alpha$ , Cachectin	6p21.3 Size-2771bp Introns -3 Exons-4	Singh PK et al. (2015)	Evaluated the association of TNF- $\alpha$ gene promoter (-238) G/A and (-308) G/A	272 OSCC and 185 healthy volunteers were genotyped by PCR restriction fragment length polymorphism (RFLP)	TNF- $\alpha$ (-238) G/A polymorphism was significantly associated with OSCC patients as compared to healthy

					polymorphisms with the susceptibility of OSCC patients in North Indian population	for the TNF- $\alpha$ (-238) G/A and (-308) G/A gene polymorphism.	volunteers. No significant association was found in TNF- $\alpha$ (-308) G/A gene polymorphism with OSCC patients and controls.
				Juretić M et al (2013)	Salivary concentrations of TNF- $\alpha$ and IL-6 in patients with premalignant and malignant lesions were evaluated.	Whole saliva of patients with oral premalignant lesions, oral squamous cell carcinoma and healthy control volunteers was collected and investigated for the presence of TNF- $\alpha$ and IL-6 by enzyme immunoassay	All groups had statistically significant differences in values of TNF- $\alpha$ and IL-6 ( $P < 0.001$ ) and these pro-inflammatory cytokines are elevated in the saliva of patients with oral squamous cell carcinoma and oral premalignant lesions as compared to controls, which may have diagnostic and/or prognostic significance
				Song K et al (2012)	Hypothesized that inflammation is one of the main characteristics in tumor microenvironment and serves as a prominent catalyst for fusion events	Immunocytochemistry and flow cytometry analysis for VCAM-1 and VLA-4 expression in 50 oral squamous cell (OSC) specimens and OSC cell lines (OSCC)	Oral cancer cells fused spontaneously with endothelial cells and their fusion with human umbilical vein endothelium cells was increased by 3-fold in vitro by TNF- $\alpha$ . Also OSCC and 70% of OSC express VLA-4. VCAM-1 was also evident on vascular endothelium of OSCC suggesting that anti-VLA-4 or anti-VCAM-1 treatment can decrease cancer-endothelial adhesion significantly.
				Vucicevic-Boras V et al (2012)	Compared salivary and serum concentrations of tumor necrosis factor alpha (TNF- $\alpha$ ) in patients with oral leukoplakia, oral cancer and healthy controls	Cytokine concentrations of 28 patients with oral cancer, 29 leukoplakia, and 31 healthy controls by ELISA	No differences in concentrations of salivary TNF- $\alpha$ was observed between oral cancer patients, in patients with leukoplakia and control group. Serum TNF- $\alpha$ was significantly higher in control subjects than in oral cancer patients.
				Yang CM et al. (2011)	Evaluation of association of TNFA	Genotypes were determined in 205 cancer cases and 198	BQ-chewers carry the G allele or G/G genotype in TNFA -308.

					genetic variants (-308G>A and -238G>A) with the risk and prognosis of betel quid (BQ)-related oral and pharyngeal squamous cell carcinoma	healthy controls who habitually chewed BQ by TaqMan real-time assays.	G allele and G/G genotype were associated with a 1.95-fold and 2.28-fold increased risk of cancer as compared to those with A allele or A/A+A/G genotypes, respectively. Also G allele and G/G genotype were associated with statistically significant increased risk of OPSCC.
				Gupta R et al. (2008)	Assessment of Single Nucleotide Polymorphism (SNP)s in TNF-alpha(-308, -238) and TNF receptor 1 (TNFR1; -609) promoters was done	PCR-RFLP at four sites of TNF receptor 2 gene (TNFR2; exon 9 site 1176; exon 10 sites 1663, 1668 and 1690) by PCR-sequence-specific primers (PCR-SSP) techniques, respectively, in 94 patients and 130 healthy controls.	TNF-alpha-308 G allele was significantly lower, whereas A allele was significantly higher in patients compared with controls. No significant change was observed at 238 promoter site between the two groups. In the case of TNF receptors, both TNFR1 -609 TT and TNFR2 1690 CT genotypes were significantly lower in patients compared with controls. These SNPs may be useful as a marker for high-risk groups among Asian Indians.
				Sundelin K et al (2005)	Studied correlation between intra-tumoral cytokine tumor necrosis factor-a (TNF-a) in stimulation of oral cancer cells to enhance secretion of matrix metalloproteinase (MMP)-1 and -9 that is necessary for tumor progression, intra-tumoral proteolytic activity, promoting angiogenesis and invasion by acting on extracellular matrix	The effects of TNF-a on MMP-1, -2 and -9 expression by two oral squamous cell carcinoma cell lines (UT-SCC-20A and -24A) were studied. ELISA was used to analyse secretion of total MMP protein and gelatin zymography was used for activity analysis.	TNF-a stimulated MMP-9 secretion in both cell lines, but only stimulated MMP-1 secretion in one (UT-SCC-24A). The zymographic results were consistent with the ELISA results, indicating an upregulation of active enzyme when a stimulatory effect on protein expression was detected

					substances.		
				Krishnan R et al (2003)	Efficacy of TNF- $\alpha$ in differentially diagnosing premalignant oral lesions (PMD) and OSCC was studied and also levels of salivary and serum TNF- $\alpha$ were correlated with clinicopathologic factors	Serum and salivary samples from 100 subjects in each group of healthy control, PMD and OSCC were collected and level of TNF- $\alpha$ was analysed by ELISA.	Increased level of both serum and salivary TNF- $\alpha$ was observed in OSCC compared to healthy control and PMD group. Also High specificity and sensitivity was observed for salivary TNF- $\alpha$ in differentiating OSCC from PMD and healthy controls. Significant increase in TNF- $\alpha$ level in moderately and poorly differentiated lesion as compared to well-differentiated lesion and in clinical stage IV.
	TNFSF10	TRAIL, Apo2L, CD253	3q26 Size-18000bp Introns -4 Exons-5	Pavet V et al (2014)	Functional TRAIL signaling in cancer cells demonstrated high mRNA levels of PLAU, which encodes urokinase plasminogen activator (uPA), Also decreasing uPA levels sensitized cancer cells to TRAIL, leading to markedly increased apoptosis.	Mechanistic analyses was done in uPA-depleted cells to demonstrate that reduced basal ERK1/2 prosurvival signaling, decreased preligand decoy receptor 2 (DcR2)-death receptor 5 (DR5) interaction and attenuated recruitment of DcR2 to the death-inducing signaling complex upon TRAIL challenge. Also increased FADD and procaspase-8 recruitment and processing, leads to apoptosis.	PLAU mRNA levels may work as marker for the identification of TRAIL-responsive tumor cells and highlight a key role of uPA signaling in 'apoptosis versus survival' decision-making processes upon TRAIL challenge.
				Chen JJ et al 2013	Apoptotic responses of a panel of seven human OSCC cell lines (HN31, HN30, HN12, HN6, HN4, Cal27, and OSCC3) to rhTRAIL and monoclonal antibodies against DR4 or DR5 .was tested	rhTRAIL induced apoptosis in most of the oral cancer cell lines both in vitro and in vivo. DR5 was expressed on the surface of the tested cell lines that correlated with the cellular susceptibility to apoptosis induced by rhTRAIL and anti-DR5 antibody. By contrast, little or no DR4 was detected on the surface of OSCC3 and HN6	

						<p>cells rendering cellular resistance to DR4 antibody and a reduced sensitivity to rhTRAIL. Expression of a constitutively active Ras mutant (RasV12) in OSCC3 cells selectively upregulated surface expression of DR5, but not DR4, and restored TRAIL sensitivity. Hence TRAIL receptor targeted therapies in human OSCC tumors treatment with active Ras mutant can be done.</p>	
				Yoldas B et al 2011	<p>Demonstrated mapping of the molecular profile of TRAIL and TRAIL receptors (DcR1 and DcR2) for patients with oral cavity squamous cell carcinoma (OCSCC)</p>	<p>Paraffin-embedded tissues from 60 patients with laryngeal SCC and 14 patients with OCSCC were retrospectively analyzed using immunohistochemistry.</p>	<p>An increase in DcR1 but a decrease in DcR2 expression was observed in OCSCC patients compared with control individuals with benign lesions.</p>
				Yeh CC et al (2009)	<p>Reported induction of apoptosis in human oral cancer cells, cell cultures and animal models by suberoylanilide hydroxamic acid and its synergistic anticancer activity with radiation, cisplatin, and TRAIL in cancers.</p>	<p>Western blotting showed that suberoylanilide hydroxamic acid increased Fas, Fas ligand, DR4, and DR5 protein expression and activated caspase-8 and caspase-9. The apoptosis was inhibited by caspase-8 inhibitor Z-IETD-FMK and attenuated by caspase-9 inhibitor Z-LEHD-FMK. Also Human recombinant DR5/Fc chimera protein but not Fas/Fc or DR4/Fc significantly inhibited apoptosis induced by suberoylanilide hydroxamic acid.</p> <p>Suberoylanilide hydroxamic</p>	

						acid induces apoptosis mainly through activation of DR5/TRAIL death pathway. Furthermore, subtoxic concentrations of suberoylanilide hydroxamic acid sensitize two TRAIL resistant human oral cancer cells, SAS and Ca9-22, to exogenous recombinant TRAIL-induced apoptosis in a p53-independent manner. Combined treatment of suberoylanilide hydroxamic acid and TRAIL may be used as a new promising therapy for oral cancer	
				Kok SH et al (2009)	Demonstrated that esculetin significantly suppressed the growth of oral cancer SAS cells in a dose-dependent manner	DNA content flow cytometry, TUNEL assay and Western blotting was performed	Esculetin increases DR5 expression and activates caspase-8 thus inducing cell cycle arrest and apoptosis. Also Esculetin significantly increased TRAIL-induced apoptosis in SAS cells. Thus suggesting that combination of esculetin and TRAIL may be a novel treatment strategy for oral cancers.
				Vigneswaran N et al (2007)	Compared the expression levels of TRAIL and its receptors in normal oral mucosa (NOM), oral premalignancies (OPM), and primary and metastatic oral squamous cell carcinomas (OSCC) in order to characterize the changes in their expression patterns	DNA microarray, immunoblotting and immunohistochemical analyses were used to examine the expression levels of TRAIL and its receptors in oral epithelial cell lines and in archival tissues of NOM, OPM, primary and metastatic OSCC. Apoptotic rates of tumor cells and tumor-infiltrating lymphocytes (TIL) in OSCC specimens were	TRAIL expression was seen in NOM which was progressively lost in OPM and OSCC. DcR2 expression levels was also reduced in OPM and OSCC compared to NOM. OSCC frequently expressed DR4, DR5 and DcR1 but less frequently DcR2. However no such alteration was seen in Expression levels of DR4, DR5 and DcR1 in OPM. Hence loss of TRAIL expression is

					during OSCC initiation and progression.	determined by cleaved caspase 3.	involved in dysregulation of apoptosis leading to carcinogenesis of OSCC.
				Nagaraj NS et al (2006)	Investigated the role of the lysosomal protease cathepsin B (CB) in mediating TRAIL-induced cell death in oral squamous cell carcinoma (OSCC) cells.	OSCC cell lines from primary tumor and lymph node metastasis were examined for expression of apoptosis markers by Western blots, enzyme activity assays, nuclear fragmentation assays, and FACS analysis. Gene-specific ribozymes or chemical inhibitors were used to inhibit CB or caspases in target cells. TRAIL-induced activation of caspase-3, cleavage of Bid and poly-ADP-ribose polymerase, release of cytochrome c, and DNA fragmentation were blocked either by a pan-caspase inhibitor (zVAD-fmk) or a CB inhibitor (CA074Me), consistent with the involvement of TRAIL as well as CB in cell death.	The primary tumor cells were more susceptible to apoptosis than their corresponding lymph node metastatic cells. Stable transfection of ribozyme which inhibited CB expression also decreased the apoptotic process. Thus TRAIL-induced apoptotic cell death in OSCC cells is mediated through CB or through caspase activation. Hence CB causes tumor-suppression as opposed to role for in OSCC which is opposed to invasion- and metastasis-promotion functions of lysosomal proteases
				Vigneswaran N et al (2005)	Examined the TRAIL-mediated cytotoxicity rates of clonally-related primary and metastatic oral cancer (OC) cells and correlated them with the expression levels of TRAIL receptors, cathepsin B and cystatins A, B, C and M.	Two pairs of primary (686Tu and 101A) and metastatic (686Ln and 101B) OC cell lines were treated with various concentrations (5 to 1000 ng/ml) of recombinant human TRAIL protein for 14 h, and cell viability and apoptotic rate were determined.	Primary OC cells revealed greater susceptibility to TRAIL than their metastatic counterparts. Cycloheximide (protein synthesis inhibitor) increased TRAIL sensitivity, whereas CB-specific chemical inhibitor CA-074 reduced the sensitivity of primary OC cells to TRAIL. DNA laddering and M30 CytoDEATH immunodetection assays confirmed that TRAIL-induced OC cell death is an apoptotic process. Expression levels of

							TRAIL death (DR4 and DR5) and decoy (DcR1 and DcR2) receptors were not different between primary and metastatic OC cells. Expression levels of Cystatins were higher in metastatic OC cells than primary cells, which may cause their greater resistance to TRAIL-induced apoptosis whereas CB (mediator of TRAIL-induced apoptosis in OC) levels remain unchanged.
	TNFRSF5	CD40, p50	20q13.12 Size-11592bp Introns -13 Exons-34	Rentoft M et al (2009)	Performed gene expression profiling of tongue squamous cell carcinomas based on DNA repair genes	Employed gene expression array methodology specifically developed for analysis of degraded RNA and investigated the expression of 502 cancer-related genes in archival paraffin-embedded SCCHN of the tongue from young ( $\leq 40$ ) and elderly patients ( $\geq 50$ ).	Genes detected was de-regulated in tumors compared to non-malignant controls No significantly differential expression was detected between young and old patients. TNFRSF5 was differentially expressed ( $< 2$ fold) in cancer cells.
				Sandra Gibson et al (2006)	Analysed apoptosis-associated genes and pathways in oral cancer cells	Comparison of gene expression patterns of normal human epidermal keratinocytes (NHEK cells) and the oral cancer cell line Tu183 when the cells were exposed to different apoptosis-inducing stimuli was done.	Microarray analysis, supported by immunoblotting, revealed that TNFRSF5 (encodes receptor CD40) was likely to be involved in the proapoptosis signal. When Tu183 cells were exposed to the CD40 ligand they showed apoptosis, while NHEK cells did not.
	TNFRSF1A	TNFR1, CD120a	12p13.31 Size-13358bp Introns -36 Exons-51	Gupta R et al (2008)	Assessed SNPs in TNF-alpha (-308, -238) and TNF receptor 1 (TNFR1; -609) promoters and at four sites of TNF receptor 2 gene (TNFR2; exon 9 site 1176; exon 10 sites 1663, 1668 and 1690)	PCR-RFLP and PCR-sequence-specific primers (PCR-SSP) techniques, respectively was performed in 94 patients and 130 healthy controls.	TNF-alpha-308 G allele was significantly lower, whereas A allele was significantly higher in patients compared with controls. No significant change was observed at 238 promoter site between the two groups. Both TNF receptors, TNFR1 -609 TT (Pc=0.006; OR=15.3) and



							TNFR2 1690 CT (Pc=0.018; OR=5.6) genotypes were significantly lower in patients compared with controls. Thus TNF-alpha-308 G/A may be related to susceptibility, whereas -609 TT TNFR1 and 1690 C/T TNFR2 SNPs may be protective to tobacco-related oral squamous cell carcinoma making them useful marker.
	TNFRSF25	DR3, Apo3, TRAMP, LARD	1p36.31 Size-5045bp Introns -46 Exons-50				
	TNFRSF10	TRAILR1, CD261, Apo2, TRICK 2	8p21.3 Size-34675bp Introns -11 Exons-19	Han B et al (2015)	Demonstrated that CFZ decreases the survival of several human cancer cell lines and induces apoptosis via extrinsic apoptotic pathway. FADD deficiency protected cancer cells from undergoing apoptosis	CFZ increased total and cell surface levels of DR5 in different cancer cell lines; accordingly it enhanced TRAIL-induced apoptosis. DR5 deficiency protected cancer cells from induction of apoptosis by CFZ either alone or in combination with TRAIL	DR5 upregulation causes CFZ-induced apoptosis and enhances TRAIL-induced apoptosis. CFZ inhibited degradation of DR5
				Huong LD et al (2012)	Effect of $\beta$ -Phenylethyl isothiocyanate (PEITC) on induction of apoptosis to inhibit cell growth and molecular mechanism in oral cancer was studied	The apoptotic effect was investigated using 4'-6-Diamidino-2-phenylindole (DAPI) staining or Western blotting. MTS assay was used to examine cell viability. Inhibitors were used to determine the molecular target and mechanism of PEITC-mediated apoptosis.	PEITC inhibited the growth of HN22 human oral cancer cells and induced caspase-dependent apoptosis in HN22 cells as evidenced by nuclear fragmentation and the activation of caspase 3. It increased cleaved caspase 8, truncated BID, and death receptor 5 (DR5) through the activation of p38 MAPK. This was confirmed by blockage of PEITC-induced cleavages of Poly(ADP-ribose) Polymerase, caspase-3, caspase-8, and DR5 by p38 MAPK inhibitor, SB203580. Also

							PEITC activated p38 and augmented DR5 to induce apoptosis in other human oral cancer cells. Thus DR5 is potential molecular target for PEITC-induced apoptosis in oral cancer via p38 MAPK.
BCL-2 family	HRK	Bcl-2 Interacting Protein, DP5	12q24.2 Size-25298bp Introns - 6 Exons-11				
	BIK	BIP1, BP4, NBK	22q13.2 Size-18965bp Introns - 4 Exons-5	Reis PP et al (2002)			
	BAX	BCL2L4, NKX3-1, BAPX2	19q13.3 Size-4235bp Introns - 13 Exons-5	Kim JS et al (2015)	Examined the anticancer properties of berberine in KB oral cancer cells with a specific focus on its cellular mechanism.		
				Jordan RC et al (1996)	Demonstrated differential expression of bcl-2 in squamous cell carcinomas of the oral cavity.		
	BCLAF1		6q23.3 Size-33225bp Introns - 24 Exons-73	Lee YY et al (2012)	Examined different H2AX-coordinated complexes in cells exposed to different IR doses. Also function of BCLAF1 in the $\gamma$ H2AX-coordinated		

					cell fate-determining pathways under different radiation conditions was determined.		
				Liu H et al (2007)	Demonstrated that, upon exposure to genotoxic stress, PKC delta activates and interacts with the death-promoting transcription factor Btf to co-occupy CPE-TP53. Inhibition of PKC delta activity decreases the affinity of Btf for CPE-TP53, thereby reducing TP53 expression at both the mRNA and the protein levels.		
Caspase Family	CASP1	IL1BC	11q22.3 Size-9808bp Introns - 18 Exons- 40	Ribeiro IP et al (2014)	Evaluated the clinical applicability of a multiplex ligation-dependent probe amplification (MLPA) probe panel directed to head and neck cancer.	Thirty primary oral squamous cell tumors were analyzed using the P428 MLPA probe panel.	Detected genetic imbalances in 26 patients and observed a consistent pattern of distribution of genetic alterations in terms of losses and gains for chromosomes 3, 8, and 11. Specific genes were highlighted due to frequent losses of genetic material--RARB, FHIT, CSMD1, GATA4, and MTUS1--and others due to gains--MCCC1, MYC, WISP1, PTK2, CCND1, FGF4, FADD, and CTTN. Also the gains of MYC and WISP1 genes suggested higher propensity of tumors localized in the floor of the mouth. This study proved the value of this MLPA probe panel for a first-tier analysis of oral tumors.

				Tsai WC et al (2004)	Studied the mRNA profile of genes in betel quid chewing oral cancer patients.	The cDNA microarray analysis was used to analyse the mRNA expression patterns of 1177 genes in ten oral cancer patients with betel quid chewing history.	84 genes (Chiefly caspase-1, STAT-1, COX-2 and pleiotrophin) involving cell adhesion, cell shape, growth, apoptosis, angiogenesis, metastasis, and metabolism were deregulated but was not associated with clinical staging.
	CASP3	CPP32B	4q35.1 Size- 21814bp Introns - 15 Exons- 18	Pan C et al (2008)	Demonstrated that Aurora kinase inhibitory small molecule VX-680 inhibited histone H3 phosphorylation at Ser10 (in vivo substrate residue of Aurora kinase) in oral squamous cell carcinoma (OSCC) KB cells.	MTT assay was done to demonstrate inhibition of Aurora kinase, leading to reduced KB cell growth. Western blot analysis revealed that VX-680 caused cleavage of two critical apoptotic associated proteins, PARP and caspase-3. In contrast, expression of cell survival factor Bcl-2 was reduced by VX-680 treatment in a dose-dependent manner.	Nuclear characteristic of DNA fragmentation, indicative of apoptotic cell death, was observed in OSCC cells with Aurora kinase inhibitory VX-680. Thus Aurora kinase inhibitory VX-680 is a novel therapeutic target in oral cancer.
				Andressakis D et al (2008)	Investigated the potential role of caspase-3 and caspase-8 protein expression in the biological behaviour of tongue squamous cell carcinoma.	Immunohistochemical analyses of 87 primary tongue squamous cell carcinomas, using monoclonal anti-caspase-3 and anti-caspase-8 antibodies. A digital image analysis assay was also performed in order to evaluate the results.	Reduced expression of caspase-8 and -3 proteins was observed in 30/87 and 79/87 cases, respectively. No significant association between overall expression of either marker and survival probability was seen. However the size of tumors strongly correlated with survival status. Down-regulation of caspase-3 activation is a crucial process for induction of apoptosis and response to therapeutic strategies.
				Lin CC et al (2007)	Studied the effects of Berberine on cell growth, apoptosis and cell cycle regulation in human oral squamous carcinoma HSC-3 cells	Phase contrast microscopy was done to confirm Berberine- induced dose- and time-dependent irreversible inhibition of cell growth and cellular DNA synthesis.	Berberine induced morphological changes in HSC-3 cells. Berberine activated caspase-3, induced G0/G1-phase arrest, production of reactive oxygen species (ROS)

						Propidium iodide/annexin V staining for flow cytometric analysis was done.	and Ca <sup>2+</sup> , dysfunction of mitochondrial membrane potential (MMP), to induce apoptosis.
				Kim KS et al (2005)	Investigated induction of apoptosis by Ginkgo biloba extract (EGb 761) of oral cavity cancer cells and attempted to characterize the apoptotic pathway activated by EGb 761	The inhibition of SCC 1483 oral cavity cancer cells proliferation was noted from 250 micro/ml of EGb 761. Apoptosis was observed after 24 h of incubation with 250 microg/ml EGb 761 and occurred in a time- and dose-dependent manner. Apoptosis was confirmed by DNA fragmentation and PARP cleavage. Co-treatment with the caspase inhibitor (z-VAD-fmk) inhibited apoptosis and PARP cleavage induced by EGb 761. Caspase-3 activity was upregulated by EGb 761 but reduced to the control level by co-treating with z-VAD-fmk	EGb 761 induces apoptosis of oral cavity cancer cells and caspase-3 is activated in this apoptosis. Therefore, EGb 761 may be considered as a possible chemopreventive agent against oral cavity cancer.
				Hague A et al (2003)	Studied Caspase-3 expression in terminally differentiated normal oral epithelium and in oral squamous cell carcinomas and correlated with tumor stage.	Caspase-3 expression was compared in 39 samples of normal oral epithelium and 54 oral squamous cell carcinomas to determine how caspase-3 expression alters during oral carcinogenesis,	Morphologically apoptotic cells stained positively for cleaved (active) caspase-3. In normal oral epithelium, cleaved caspase-3 positive-cells were only rarely detected. Squamous cell carcinomas had more intense nuclear and cytoplasmic caspase-3 staining, which increased with STNMP. Both caspase-3 staining intensity and the percentage of cells positive for caspase-3 were inversely associated with differentiation
	CASP7	ICE-LAP3	10q25.3 Size- 51746bp	Coutinho-Camillo CM et al (2011)	Studied Caspase expression in oral squamous cell	By tissue microarray of 229 cases of oral squamous cell carcinoma (OSCC), we	All proteins examined were expressed in primary OSCC samples. Caspase 8 and 9 were

			Introns - 21 Exons- 34		carcinoma	analyzed the immunoeexpression of caspases 3, 6, 7, 8, 9, and 10.	prominently expressed, and caspases 3, 6, 7, and 10 were occasionally expressed. Disease-free survival differed significantly between caspase 7 high-expressing and low-expressing patients, and multivariate analysis suggested that expression of caspase 7 is an independent prognostic factor for patients with OSCC and is a predictor of locoregional recurrence of OSCC.
	CASP9	PPP1R56	1p36.21 Size- 35703bp Introns - 19 Exons- 38	Ding H et al (2005)	Evaluated second generation celecoxib derivatives, lacking COX-2 inhibitory activity, in a premalignant and malignant human oral cell culture model to determine their potential anticancer effect and mechanisms responsible for the COX-independent apoptotic activity	Celecoxib and its derivatives delayed the progression of cells through the G(2)/M phase and induced apoptosis. The derivatives with apolar substituents at the terminal phenyl moiety of celecoxib greatly enhanced apoptosis and cell cycle delay. Derivatives induced apoptosis was mediated by the cleavage and activation of caspase-9 and caspase-3, but not caspase 8, implicating the mitochondrial pathway for apoptosis induction. Thus second generation celecoxib derivatives disrupts mitochondrial membrane potential activating caspase 9 and hence can be used as apoptotic agents in chemoprevention and therapy.	
				Lahusen T et al (2003)	Alsterpaullone(Alp) a novel cyclin-dependent kinase inhibitor, induces	Alp induced apoptosis and promoted loss in clonogenicity in the Jurkat cell line. It	

					apoptosis by activation of caspase-9	caused early activation of both caspase-8 and -9, leading to cleavage of caspase-3 and poly(ADP-ribose) polymerase (PARP). Caspase inhibitor ZVAD blocked the cleavage and activation of most caspases except caspase-9. Alp also disrupts mitochondrial potential in the presence of ZVAD, suggesting that the activation of caspase-9 by Alp follows mitochondrial perturbation.	
	CASP10	MCH4	2q33.1 Size- 46526bp Introns - 16 Exons- 43	Cengiz B et al (2007)	Analyzed the loss of heterozygosity (LOH) of long arm of chromosome 2 by using 16 polymorphic microsatellite markers and defined the deletion mapping of the region with putative tumor suppressor genes	Fine deletion mapping of chromosome 2q21-37 was done in 39 matched oral normal and cancer tissues,	LOH was detected at least one location in 85% tumor tissues. Frequent deletions were detected at the locations of microsatellite markers, D2S2304 (35%), D2S111 (40%), D2S155 (35%), D2S1327 (29%), D2S164 (29%), D2S125 (68%) and D2S140 (32%). Three preferentially deleted regions at 2q21-24, 2q33-35 and 2q37.3 were observed. Several candidate tumor suppressor genes in these regions such as LRP1B, CASP8, CASP10, BARD1, ILKAP, PPP1R7, and ING5, are located. These genes roles in oral carcinogenesis.
CARD Family	NOD1	CARD4, NLRC1	7p14.3 Size- 54258bp Introns - 29 Exons- 36	Wang X et al (2014)	Examined protein expression of NOD1 signaling in normal, premalignant and malignant specimens of oral cavity.	Immunohistochemical expression of NOD1, Receptor-interacting protein 2 (RIP2), Caspase12, human $\beta$ Defensin1, 2 and 3 (hBD1, 2, 3) was examined in 15 normal controls, 30 cases of oral leukoplakia (OLK) and 60	The expression of NOD1, RIP2, Caspase12, hBD1, 2, and 3 decreased along with the progression of OSCC. NOD1 expression was correlated significantly to tumor differentiation, lymph node metastasis, and tumor size. Thus

						cases of OSCC. The immunostaining score was assessed by 2 pathologists, respectively	NOD1, RIP2 and Caspase12 could be potential novel biomarkers for oral carcinogenesis.
	APAF1	CED4	12q23.1 Size-90293bp Introns - 36 Exons- 56	Lo Muzio L et al (2014)	Investigated the expression profile of genes involved in the apoptotic mechanism in 21 paired tissue samples (OSCC and adjacent normal oral mucosa)	cDNA macroarray was done in order to identify differentially expressed genes in oral cancer compared to normal tissue. Findings were validated the by quantitative real-time PCR, Western blot, and immunohistochemical analyses	Different expression levels of CRADD, FADD, ATM, APAF1, and TP63 genes in OSCC compared to normal mucosa was seen. Real-time PCR showed an overexpression of FADD and a downregulation of ATM. Western blot analysis confirmed that both CRADD and APAF-1 were decreased in OSCC compared to normal oral mucosa. In Immunohistochemistry, OSCC exhibited increased expression of p63 compared to normal tissue. Along with a statistically significant positive correlation between p63 expression and the histological grade.
	CARD8	NADPP1, TUCAN, DACAR	19q13.3 3 Size-75177bp Introns - 40 Exons- 90				
Death Family	DAPK1	ROCO3	9q21.3 Size-211406bp Introns - 35 Exons- 85	Melchers LJ et al (2015)	Identified methylation tumor markers that have a predictive value for presence of regional lymph node metastases in patients with oral and oropharyngeal squamous cell carcinoma (OOSCC).	Significantly differentially expressed genes were retrieved from four reported microarray expression profiles comparing pN0 and pN+ head-neck tumours, and one expression array identifying functionally hypermethylated genes. Additional metastasis-associated genes were	5 out of 28 methylation markers (OCLN, CDKN2A, MGMT, MLH1 and DAPK1) were frequently differentially methylated in OOSCC. Of these, MGMT methylation was associated with pN0 status and with lower immunoeexpression. DAPK1 methylation was associated with pN+ status but



						included from the literature. Methylation-specific PCR (MSP) primers were designed and tested on 8 head-neck squamous cell carcinoma cell lines and technically validated on 10 formalin-fixed paraffin-embedded (FFPE) OOSCC cases. Predictive value was assessed in a clinical series of 70 FFPE OOSCC with pathologically determined nodal status.	did not associate with protein expression. Thus Both DAPK1 and MGMT, have predictive value for nodal metastasis in a clinical group of OOSCC.
				Noorlag R et al (2014)	Investigated the role of promoter hypermethylation of 24 well-described genes	Using methylation specific multiplex ligation-dependent probe amplification (MS-MLPA), the role of promoter hypermethylation of 24 well-described genes (some of which are classic TSGs), in 166 HPV-negative early oral squamous cell carcinomas (OSCC), and 51 HPV-negative early oropharyngeal squamous cell carcinomas (OPSCC) in relation to clinicopathological features and survival was demonstrated	Early OSCC showed frequent promoter hypermethylation in RARB (31% of cases), CHFR (20%), CDH13 (13%), DAPK1 (12%), and APC (10%). More hypermethylation independently correlated with improved disease specific survival in early OSCC and could therefore be used as prognostic biomarker.
				Lim AM et al (2014)	Investigated previously reported hypermethylated genes with quantitative methodology in oral tongue squamous cell carcinomas (OTSCC).	The methylation status of 12 genes in 115 OTSCC samples was assessed by quantitative analyses: methylation sensitive high resolution melting (MS-HRM), sensitive-melting analysis after real time-methylation specific PCR (SMART-MSP), and bisulfite pyrosequencing	Either no or infrequent locus-specific methylation was identified by MS-HRM for DAPK1, RASSF1A, MGMT, MLH1, APC, CDH1, CDH13, BRCA1, ERCC1, and ATM. The most frequently methylated loci were RUNX3 (18/108 methylated) and ABO (22/107 methylated)
				Ovchinnikov DA et al. (2012)	Tumor-suppressor Gene Promoter hypermethylation in	Sensitive methylation-specific polymerase chain reaction (MSP) assay was used to	The specificity for this MSP panel was 87% and the sensitivity was 80%. In the early

					Saliva of Head and Neck Cancer Patients.	determine specific methylation events in the promoters of RASSF1A, DAPK1, and p16 genes from DNA isolated from saliva of patients with HNSCC (n = 143) and healthy nonsmoker controls (n = 31)	stages of HNSCCs, a sensitivity of 94% and a specificity of 87%, and a high $\kappa$ concordance value of 0.8, was observed. Thus suggesting that the promoter methylation of RASSF1A, DAPK1, and p16 MSP panel is useful in detecting hypermethylation events in a noninvasive manner in patients with HNSCC.
	FADD	MORT1	11q13.3 3 Size- 4228bp Introns - 1 Exons-2	Upadhyay P et al (2017)	Evaluated Genomic characterization of tobacco/nut chewing HPV-negative early stage tongue tumors. Since Nodal metastases status plays a decisive role in the choice of treatment, about 70% patients can be spared from surgery with an accurate prediction of negative pathological lymph node status.	Performed sequencing of 54 samples derived from HPV negative early stage tongue cancer patients habitual of chewing betel nuts, areca nuts, lime or tobacco using whole exome (n=47) and transcriptome (n=17) sequencing. Additionally, gene expression meta-analyses were carried out for 253 tongue cancer samples. The candidate genes were validated using qPCR and immuno-histochemical analysis in an extended set of 50 early primary tongue cancer samples.	Somatic analysis revealed a classical tobacco mutational signature C:G>A:T transversion in 53% patients that were mutated in TP53, NOTCH1, CDKN2A, HRAS, USP6, PIK3CA, CASP8, FAT1, APC, and JAK1. Similarly, significant gains at genomic locus 11q13.3 (CCND1, FGF19, ORAOV1, FADD), 5p15.33 (SHANK2, MMP16, TERT), and 8q24.3 (BOP1); and, losses at 5q22.2 (APC), 6q25.3 (GTF2H2) and 5q13.2 (SMN1) were observed. Also an up-regulation of metastases-related pathways and over-expression of MMP10 was observed in 48% tumors.
				Ribeiro IP et al (2014)	Evaluated the clinical applicability of a multiplex ligation-dependent probe amplification (MLPA) probe panel directed to head and neck cancer.	Thirty primary oral squamous cell tumors were analyzed using the P428 MLPA probe panel.	Detected genetic imbalances in 26 patients and observed a consistent pattern of distribution of genetic alterations in terms of losses and gains for some chromosomes, particularly for chromosomes 3, 8, and 11. Specific genes were highlighted due to frequent losses of genetic material--RARB, FHIT, CSMD1, GATA4, and MTUS1-

							-and others due to gains-- MCCC1, MYC, WISP1, PTK2, CCND1, FGF4, FADD, and CTTN. Also the gains of MYC and WISP1 genes suggested higher propensity of tumors localized in the floor of the mouth. This study proved the value of this MLPA probe panel for a first-tier analysis of oral tumors.
				Lo Muzio L et al (2014)	Investigated the expression profile of genes involved in the apoptotic mechanism in 21 paired tissue samples (OSCC and adjacent normal oral mucosa)	cDNA macroarray, in order to identify differentially expressed genes in oral cancer compared to normal tissue. Findings were validated the by cDNA macroarray, quantitative real-time PCR, Western blot, and immunohistochemical analyses	cDNA macroarray analysis showed different expression levels of CRADD, FADD, ATM, APAF1, and TP63 genes in OSCC compared to normal mucosa. Real-time PCR showed an overexpression of FADD and a downregulation of ATM. Moreover, Western blot analysis confirmed that both CRADD and APAF-1 were decreased in OSCC compared to normal oral mucosa. In Immunohistochemistry, OSCC exhibited increased expression of p63 compared to normal tissue. Along with a statistically significant positive correlation between p63 expression and the histological grade.
				Sugahara K et al (2011)	Studied Combination effects of distinct cores in 11q13 amplification region on cervical lymph node metastasis (LNM) of oral squamous cell carcinoma	Array-based comparative genomic hybridization (CGH) with individual gene-level resolution and real-time quantitative polymerase chain reaction (QPCR) were conducted using primary tumor materials resected from 54 OSCC patients with (n=22) or without (n=32) cervical	Frequent gain was observed at the 11q13 region exclusively in patients with cervical LNM, which was confirmed by real-time QPCR experiments using 11 genes (TPCN2, MYEOV, CCND1, ORAOV1, FGF4, TMEM16A, FADD, PPFIA1, CTTN, SHANK2 and DHCR7) in this region. It was revealed

						LNM.	that two distinct amplification cores existed, which were separated by a breakpoint between MYEOV and CCND1 in the 11q13 region. The combination of copy number amplification at CTTN (core 2) and/or TPCN2/MYEOV (core 1), selected from each core, was most significantly associated with cervical LNM (P=0.0035). Two amplification cores at the 11q13 region may have biological impacts on OSCC cells to metastasize to local lymph nodes.
				Prapinjumrune C et al (2010)	Elucidated the role of FADD in oral squamous cell carcinoma (SCC).	The DNA amplification of FADD from 30 samples of tongue SCC was analyzed using real-time PCR and the protein expression of FADD from 60 samples of tongue SCC was analyzed using immunohistochemistry.	FADD is an adaptor for relaying apoptotic signals, plays an important role in the growth and regulation of the cell cycle. The DNA amplifications of FADD were observed in 13 cases and were significantly correlated with the histopathological differentiation grade of SCCs. The positive expressions of FADD were significantly correlated with lymph node metastasis of SCCs and the 5-year disease-specific survival rates. A positive association between FADD expression level and the histopathological differentiation grade was found to be limited to T1 SCCs. DNA amplification was moderately correlated with expression of FADD in 30 samples of tongue SCC. Hence SCC cells with the expression of FADD are more likely to become metastatic and

							to worsen survival rates.
				Iwase M et al (2008)	Examined whether inhibition of EGFR signaling would affect the susceptibility of oral squamous cell carcinoma (OSCC) cells to Fas-mediated apoptosis.	Treatment of OSCC cells with an anti-EGFR monoclonal antibody, C225, and an EGFR tyrosine kinase inhibitor, AG1478, which target the extracellular and intracellular domains of the receptor, respectively, inhibited phosphorylation of EGFR and its downstream effector molecule Akt and amplified the induction of Fas-mediated apoptosis accompanied by caspase-8 activation but not Bid cleavage. Caspase-3 and -8 inhibitors reduced the effect of EGFR inhibitors on Fas-mediated apoptosis in OSCC cells.	The pro-apoptotic activity of EGFR inhibitors in OSCC cells depends on the extrinsic pathway of the caspase cascade. Although EGFR inhibitors did not affect the expression of Fas, the Fas-associated death domain protein, or procaspase-8 in OSCC cells, the inhibition downregulated cellular FLICE-inhibitory protein (c-FLIP). And knockdown of c-FLIP in HSC-2 cells with a small interfering RNA strongly enhanced Fas-mediated apoptosis.
				Järvinen AK et al (2008)	Characterized genome-wide copy number and gene expression changes on microarrays for 18 oral tongue SCC (OTSCC) cell lines.	Demonstration of number of altered regions including nine high-level amplifications such as 6q12-q14 (CD109, MYO6), 9p24 (JAK2, CD274, SLC1A1, RLN1), 11p12-p13 (TRAF6, COMMD9, TRIM44, FJX1, CD44, PDHX, APIP), 11q13 (FADD, PPFIA1, CTTN), and 14q24 (ABCD4, HBLD1, LTBP2, ZNF410, COQ6, ACYP1, JDP2) where 9% to 64% of genes showed overexpression. Across the whole genome, 26% of the amplified genes had associated overexpression in OTSCC	Genomic alterations with associated gene expression changes play an important role in the malignant behavior of head and neck SCC. The identified genes may lead to the identification of novel candidates for targeted therapies.
				Tomioka H et al (2006)	Performed comprehensive gene expression profiling for	cDNA microarray was used to analyze expression patterns of 16 617 genes in nine OSCC	47 genes with altered expression were extracted and classified into 10 categories. By

					understanding OSCC.	patients. To validate the microarray data, the expression of genes, including TGFBI, FADD and DUSP1 was analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR)..	hierarchical clustering analysis, the nine cases were divided into two clusters. 47 genes are suggested as having a functional significance in oral squamous cell carcinogenesis.
CIDE Domain Family	CIDEA	CIDE-A	18p11.2 1 Size- 23277bp Introns - 8 Exons- 16				
	CIDEB	CIDE-B	14q12 Size- 6335bp Introns - 11 Exons- 17				
P53 Family	Tp53BP2/A PCC2/53BP 2/Bbp	Bbp, ASPP2, PPP1R13A	1q41 Size- 66074bp Introns - 29 Exons- 61	Chia YC et al (2010)	Studied the effects of TS on various human oral squamous carcinoma cell lines (HOSCC) including UM1, UM2, SCC-4, and SCC-9.	Cell lines were treated with TS leaf extract and screened for viability, apoptosis, necrosis, and apoptotic gene expression. FAC Scan analysis and Microarray and semi-quantitative RT-PCR analysis were done. Normal human oral keratinocytes (NHOK) served as a control for cytotoxic assays.	Viability of TS-treated HOSCC was reduced, whereas that of NHOK was not affected. The leaf extract induced apoptosis and necrosis, depending on cell type. 3,4,5-trihydroxybenzoic acid (Gallic acid, one of the major bioactive compounds purified from TS extract) up-regulated pro-apoptotic genes such TNF- $\alpha$ , TP53BP2, and GADD45A, and down-regulated the anti-apoptotic genes survivin and cIAP1, resulting in cell death. Thus Gallic acid, is responsible for the anti-neoplastic effect of Toona sinensis leaf extract.



**Appendix Table B. Anti apoptotic genes.**

Gene family	Gene	Other names	Location, exon and introns	Studied by	Study design	Methodology	Inference
Bcl-2 Family	Bcl-2	B Cell Lymphoma 2, PPP1R50	18q21.33 Size- 196783bp Introns -3 Exons-8	Schoelch ML et al (1999)	Apoptosis-associated proteins and the development of oral squamous cell carcinoma.	90 archived paraffin-embedded specimens from 25 patients and 8 control specimens were evaluated in immunohistochemically stained sections for tumor suppressor protein p53, p53 binding protein mdm-2, and apoptosis regulatory proteins Bcl-2, Bcl-X, Bax, and Bak. The initial histologic diagnosis for 17/25 patients was either focal keratosis, mild dysplasia, or moderate dysplasia; the initial diagnosis for the remaining eight patients ranged from severe dysplasia to moderately differentiated squamous cell carcinoma.	30 specimens 9 of which were dysplasias were p53 positive. Similarly, in 57 specimens including 27 dysplasias of various grades were Bak positive with increase in staining intensity with disease progression, which was independent from p53 status. Bcl-X was expressed in 73 specimens. 10 specimens were positive for Bcl-2 (all were dysplasias or carcinomas), and only 2 specimens were positive for Bax. 11 specimens were positive for mdm-2; 6 of which were also positive for p53. Thus apoptosis-associated proteins are altered in variable patterns in both premalignant and malignant oral epithelial lesions. p53 and especially Bak and Bcl-X are expressed early; Bax is largely absent; and Bcl-2 and mdm-2 show sporadic expression in the development of oral premalignant and malignant disease.
				Yao L et al (1999)	Studied correlations of bcl-2 and p53 expression with the clinicopathological features in tongue squamous cell carcinomas.	The expression of both bcl-2 and p53 proteins in 52 primary tongue squamous cell carcinomas (SCCs) was immunohistochemically explored in correlations with clinicopathological features, patient's prognosis and apoptosis index (AI) of this tumor type.	Bcl-2 and p53 expression were identified in 50% and 60% cases, respectively. bcl-2 expression was associated with tumor histologic grade and marginally with mode of tumor invasion but not with lymph nodal involvement. The three combined staining patterns of bcl-2-/p53-, bcl-2+/p53- and bcl-2-/p53+, and bcl-2+/p53+ were significantly correlated with tumor



						histologic grade mode of tumor invasion and pN status. Also, bcl-2 protein expression was associated with decreased AI. Thus suggesting that combined investigation of the bcl-2 & p53 aid in assessment of tumor aggressiveness, and suppressing mechanism of bcl-2 oncogene in regulation of apoptosis in tongue SCC.	
				Ravi D et al (1998)	Analyzed the significance of angiogenesis in relation to apoptosis, expression of apoptosis regulatory p53, bax and bcl-2 proteins as well as tissue proliferation in lesions of the oral cavity, defined by cyclin D1 expression	Immunohistochemical analysis of oral cavity carcinomas for expression of apoptosis regulatory p53, bax and bcl-2 and cyclin D1 proteins.	The correlation between CD34 expression, cyclin D1 and TUNEL reactive cells suggests that increased angiogenesis, decreased apoptosis and deregulated proliferation occur simultaneously during tumor progression in the oral mucosa. Presence of a mutant p53, increased bcl-2 expression and altered bax expression are involved in this complex process.
				Costa A et al (1998)	Described Biological markers GST-pi, p53, bcl-2 and bax as indicators of pathological response to primary chemotherapy in oral-cavity cancers.	Immuno-histochemical expression of GST-pi, p53, bcl-2 and bax was detected, and autoradiographical determination of the S-phase cell fraction, as thymidine labeling index (TLI), were investigated within a prospective randomized phase III clinical trial on squamous-cell carcinoma of the oral cavity, including surgery or primary chemotherapy (PCT) on 100 previously untreated patients with resectable T2-4N0-2M0 carcinoma	In patients treated with surgery alone, 3-year disease-free survival (DFS) appeared to be weakly, but not significantly, related only to GST-pi and p53 expression. In patients treated with PCT, pathological response and DFS were independent of p53 expression and cell proliferation. Absence of bcl-2 was associated with high probability of 3-year DFS. All patients with bcl-2-positive tumors relapsed within 1 year of surgery, whereas a 60% probability of 3-year DFS was observed for patients with bcl-2-negative tumors.
				Singh BB et al	Performed Immunohistochemical	Immunohistochemical analysis with monoclonal antibodies to bcl-2	Severe epithelial dysplasias had a higher percentage of

				(1998)	evaluation of bcl-2 oncoprotein in oral dysplasia and carcinoma.	oncoprotein in formalin-fixed paraffin-embedded tissue sections	immunoreactivity than did mild and moderate dysplasias and squamous cell carcinomas. Expression of this oncoprotein was directly proportional to the degree of epithelial dysplasia, and nondysplastic basal cells contiguous to neoplastic lesions also expressed bcl-2. Also down-regulation of bcl-2 was seen in differentiating carcinomas, suggesting its role in relatively early stages of oral tumor progression. Also differentiating neoplastic cells with marginal or no bcl-2 reactivity showed heterogeneous cell labeling of varying intensity for differentiation-associated cytokeratin (CK13), indicating their inverse topographic relationship.
				Birchall MA et al (1997)	Apoptosis, mitosis, PCNA and bcl-2 in normal, leukoplakic and malignant epithelia of the human oral cavity	32 oral biopsies samples were taken, 10 normal (N), 10 leukoplakia (dysplasia, D = 5; hyperplasia, H = 5) and 12 squamous cell carcinoma (C: 11). Distant normal tissue was also collected (HN, DN, CN). Based on counts of 1000 cells/slide, mitotic (MI), apoptotic (AI) and proliferating cell nuclear antigen (PCNA: PI) indices were calculated and bcl-2 expression recorded.	AI correlated with MI, but not PI or bcl-2 expression. PCNA was higher in H and HN than other groups. Bcl-2 was reduced in C and CN (P < 0.001). Peak mitosis shifted basally in dysplasia, whilst peak apoptosis remained unaltered. Reduced bcl-2 in carcinoma and related 'normal' epithelium was unexpected, and may contribute to the high incidence of carcinomas in these patients.
				Jordan RC et al (1996)	Demonstrated differential expression of bcl-2 in squamous cell carcinomas of the oral cavity.	bcl-2 protein immunexpression in adjacent serial sections of 30 squamous cell carcinomas of the oral cavity and correlated this with tumour differentiation.	Examination of normal epithelium showed bcl-2 expression confined to basal keratinocytes and dendritic cells. Overall, moderate or marked immunostaining for bcl-2 was identified in 18/30 (60%)

							carcinomas Upregulation of bcl-2 protein was identified in dysplastic epithelium adjacent to invasive tumour
	MCL1	Myeloid Cell Leukaemia Sequence 1	1q21.2 Size-5188bp Introns -6 Exons-10	Maji S et al (2015)	Reported that knocking down Mcl-1 sensitized OSCC cells to ABT-737, which binds to Bcl-2/Bcl-xL but not Mcl-1.	Report of a BH3 mimetic, Sabutoclax, which functions as an inhibitor of all anti-apoptotic Bcl-2 proteins, induced cancer-specific cell death in Mcl-1-dependent manner through both apoptosis and toxic mitophagy.	In vivo studies demonstrated that Sabutoclax alone decreased tumor growth in a carcinogen-induced tongue OSCC mouse model. In a combination regimen, Sabutoclax and COX-2 inhibitor, Celecoxib, synergistically inhibited the growth of OSCC in vitro and also significantly reduced OSCC tumor growth in vivo. Hence Mcl-1 is an important therapeutic target for oral squamous cell carcinomas.
				Sachita K et al (2015)	YM155 is a small-molecule pro-apoptotic agent, which inhibit survivin expression and induce apoptosis in various cancer cells. The function and molecular mechanism of YM155 in human oral cancer cells were studied.	The apoptotic effects and related signaling pathways of YM155 were evaluated using trypan blue exclusion assay, 4'-6-diamidino-2-phenylindole staining, Western blotting, RT-PCR, and siRNA.	YM155 inhibited the growth, caused caspase-dependent apoptosis in MC3 and HN22 cells and suppressed the level of survivin protein expression through proteasome-dependent protein degradation. YM155 also reduced myeloid cell leukemia-1 (Mcl-1) protein, but it did not alter Mcl-1 mRNA. It was associated with the facilitation of lysosome-dependent protein degradation. The modifications of Mcl-1 and survivin by YM155 were caspase-independent manner. Treatment of MC-3 and HN22 cells with YM155 inhibited specificity protein 1 (Sp1) and the knockdown of Sp1 by siRNA demonstrated that Mcl-1 was regulated by Sp1 protein. Since YM155 causes apoptosis of human oral cancer cell lines through downregulation of Sp1 and Mcl-1. Therefore, it may be a potential

						anticancer drug candidate for the treatment of oral cancer.
				Palve VC et al (2012)	Studied the role of Mcl-1 isoforms in radiation response of oral squamous carcinoma cells (OSCC) and the association of Mcl-1 isoform expression with radiosensitivity of OSCC, using siRNA strategy.	<p>The time course expression of Mcl-1 splice variants (Mcl-1L, Mcl-1S &amp; Mcl-1ES) was studied by RT-PCR, western blotting &amp; immunofluorescence, post-irradiation in oral cell lines [immortalized FBM (radiosensitive) and tongue cancer AW8507 &amp; AW13516 (radioresistant)] of relatively differing radiosensitivities. The effect of Mcl-1L knockdown alone or in combination with ionizing radiation (IR) on cell proliferation, apoptosis &amp; clonogenic survival, was investigated in AW8507 &amp; AW13516 cells. Further the expression of Mcl-1L protein was assessed in radioresistant sublines generated by fractionated ionizing radiation (FIR).</p> <p>3-6 fold higher expression of anti-apoptotic Mcl-1L versus pro-apoptotic Mcl-1S was observed at mRNA &amp; protein levels in all cell lines, post-irradiation. Sustained high levels of Mcl-1L, downregulation of pro-apoptotic Bax &amp; Bak and a significant reduction in apoptosis was observed in the more radioresistant AW8507, AW13516 versus FBM cells, post-IR. The ratios of anti to pro-apoptotic proteins were high in AW8507 as compared to FBM. Treatment with Mcl-1L siRNA alone or in combination with IR significantly increased apoptosis viz. 17.3% (IR), 25.3% (siRNA) and 46.3% (IR plus siRNA) and upregulated pro-apoptotic Bax levels in AW8507 cells. Combination of siRNA &amp; IR treatment significantly reduced cell proliferation and clonogenic survival of radioresistant AW8507 &amp; AW13516 cells, suggesting a synergistic effect of the Mcl-1L siRNA with IR on radiosensitivity. However during development of radioresistant sublines using FIR, high expression of Mcl-1L was observed. Thus suggesting that Mcl-1L isoform has an important role in the survival and radioresistance of OSCC and may be a promising therapeutic target.</p>
				Shin JA et al (2012)	Demonstrated effect of Mithramycin A on myeloid cell leukemia-1 in oral squamous	<p>The Mcl-1 promoter activators (TPA) and epidermal growth factor (EGF) enhanced neoplastic</p> <p>Mith treatment decreased Mcl-1 expression and neoplastic cell transformation, accompanied by</p>

					cell carcinomas and tumor xenograft through activation of Bax and oligomerization.	transformation of JB6 cells. The response was accompanied by enhanced expression of Mcl-1. Mithramycin A (Mith) exhibited decreased TPA-induced JB6 cell transformation and Mcl-1 expression. Mcl-1 was overexpressed in human oral tumors compared with normal oral mucosa and also in several OSCC cell lines including HN22 and HSC-4 cells	induction of several markers of apoptosis. This increases pro-apoptotic protein Bax, resulting in the Bax translocation into mitochondria and its oligomerization. Hence Mcl-1 has a critical role in the growth and survival of OSCC and Mith may be a potential anticancer drug candidate for clinical treatment of OSCC.
				Mallick S et al (2010)	Evaluated cell cycle and apoptosis-related proteins Myeloid Cell Leukemia-1 (Mcl-1) and Proliferating Cell Nuclear Antigen (PCNA) for predicting response and outcome in oral cancer patients treated with definitive radiotherapy.	Pre-treatment oral cancer biopsy samples from 39 patients were examined for Mcl-1 and PCNA proteins using immunohistochemistry and correlated with clinico-pathological variables using disease-free survival (DFS) as the primary endpoint.	High expression of Mcl-1 was evaluated. On univariate analysis, high PCNA, Mcl-1, node positivity and co-expression of PCNA and Mcl-1, had a significant impact on DFS. On multivariate analysis, low PCNA/Mcl-1 co-expressing tumors were associated with improved DFS. Thus suggesting that PCNA has potential predictive value in patients undergoing primary radiotherapy, aiding in assessing risk of nodal metastases and in combination with Mcl-1 has potential prognostic value to differentiate patients with poor DFS.
				Park IS et al (2010)	The effects of NSAIDs (aspirin or indomethacin) and COX-2 inhibitor (NS-398) on growth of YD-8 human oral squamous carcinoma cells have been studied	Aspirin showed strongest inhibitory effects on viability and survival of YD-8 cells. Aspirin treatment resulted in severe cell shrinkage and nuclear DNA fragmentation in YD-8 cells, suggesting the aspirin-induced apoptosis in YD-8 cells. Western blot demonstrated that aspirin treatment caused activation of caspases, down-regulation of Mcl-1 protein, dephosphorylation of ERK-1/2 and AKT, and also IkappaB-	Aspirin induced apoptosis in YD-8 cells via activation of caspases, caspase-dependent Mcl-1 proteolysis, inactivation of ERK-1/2 and AKT, and activation of NF-kappaB. Thus suggesting that aspirin may be a potential anti-cancer drug against human oral squamous carcinoma.

						alpha proteolysis-dependent NF-kappaB activation in YD-8 cells.	
				Kuo CL et al (2005)	Explored the biochemical influence of berberine-induced COX-2 reduction and apoptosis	KB cells were treated with berberine, and the apoptosis was measured by morphology and caspase-3 activity. The effects of prostaglandin E2 (PGE2) on berberine-mediated cell growth were also determined. The expression of COX-2, Bcl-2, Mcl-1, Akt and phosphorylated Akt in berberine-treated KB cells, with or without PGE2, were assessed by Western blots.	Berberine induced apoptosis in KB cells, and was partially reversed by incorporation of PGE2. Berberine treatment inhibited COX-2 and Mcl-1 expression dose-dependently, but not Bcl-2. PGE2 induced COX-2 and Mcl-1 expression and reversed the repressive effect of berberine on Mcl-1. Thus berberine-induced apoptosis is COX-2-dependent and related to decreased Akt phosphorylation and Mcl-1 expression.
	BAG-1	Bcl-2 related Athano gene	9p13.3 Size-16901bp Introns - 14 Exons-34	Coutinho-Camillo CM et al (2010)	Studied the expression of proteins that inhibit (Bcl-2, Bcl-x, Bcl-xL, Bcl-2-related protein A1, BAG-1) or promote (Bak, Bax, Bim/Bod, Bim-Long, Bad, Bid, PUMA) apoptosis and determine possible correlations between the expression of these proteins and clinicopathological features of oral squamous cell carcinoma (OSCC).	229 cases of OSCC, arranged in a tissue microarray, were immunohistochemically analysed.	Absence of vascular invasion was associated with increased expression of Bak, Bax, Bcl-xL, Bcl-2-related protein and PUMA. Increased expression of Bim/Bod and BAG-1 was associated with the presence of perineural infiltration. An increase in Bid and Bim-Long expression was associated with moderately to well-differentiated tumors. Increased expression of the Bcl-2-related protein and PUMA was associated with tumors occurring in the floor of mouth and increased expression of PUMA was also associated with recurrence of the tumor. Thus suggesting the expression of apoptotic molecules might be used as a prognostic indicator for OSCC.
				Shindoh M et al (2000)	Demonstrated a possible correlation between BAG-1 expression levels and the probability of oral squamous cell	BAG-1 expression levels in 22 patients diagnosed with early lesions (T1 and T2) of oral SCCs using immunohistochemistry and western	High levels of BAG-1 were detected in 59% cases. Which was more frequent in cases with nodal metastasis (89%), suggesting that

					carcinoma (SCC) progression.	blotting was done.	BAG-1 expression levels may correlate with the pathological stage of oral SCCs.
	Bcl-2A1	Bcl-2 Related Protein A1, BFL1	15q25.1 Size-10558bp Introns -3 Exons-5	Saleh A et al (2010)	Demonstrated the use of formalin-fixed paraffin-embedded tissues in microarray experiments to identify genes differentially expressed between cancerous and normal oral tissues.	Gene expression analyses were conducted in 43 tissues using the Illumina DASL assay. RNA yield of 2.4 and 0.8 ug/mm <sup>3</sup> from tumor and normal tissues, were taken. Using unsupervised hierarchical clustering, distinct gene expression profiles for tumor and normal samples was generated, and differentially expressed genes was identified	Majority of genes were involved in regulation of apoptosis and cell cycle, metastasis and cell adhesion including BCL2A1, BIRC5, MMP1, MMP9 and ITGB4. Representative genes were further validated in independent samples suggesting that these genes may be directly associated with oral cancer development.
	Bcl-2L1	Bcl-2 like Protein 1, PPP1R52	2q13 Size-59538bp Introns -12 Exons-15	<u>Si-Rui Ma et al (2015)</u>	Investigated the diagnostic and prognostic role of AGR2 on head and neck squamous cell carcinoma (HNSCC) with an emphasis on its correlation of cancer stemloid cells (CSC) and epithelial mesenchymal transition (EMT).	In HNSCC cell lines, knock-down of AGR2 induced apoptosis, reduced sphere formation, and down-regulated Survivin, Cyclin D1, Bcl2, Bcl2l1, Slug, Snail, Nanog and Oct4	
IAP Family	NAIP	NLR family apoptosis Inhibitory Protein, BIRC1	5q13.2 Size-56632bp Introns -29 Exons-45				
	BIRC3	Baculoviral IAP Repeat Containing 3	11q22.2 Size-21954bp Introns -38 Exons-28	Nagata M et al (2011)	Correlated Overexpression of cIAP2 with 5-FU resistance and a poor prognosis in oral squamous cell carcinoma.	DNA microarray was performed using parental and 5-FU-resistant OSCC cell lines. The effects of cIAP2 downregulation on 5-FU sensitivity and apoptosis were evaluated. An immunohistochemical analysis of cIAP2 and related proteins, cIAP1 and X-linked IAP, was performed in 54 OSCC patients who were treated with 5-FU-based chemoradiotherapy and surgery.	The downregulation of cIAP2 significantly enhanced the sensitivity of the 5-FU-resistant cells to 5-FU, with a significant increase in apoptosis. The immunohistochemical analysis demonstrated a high cIAP2 tumor expression to significantly correlate with the pathological response to chemoradiotherapy. Furthermore, a Cox regression analysis revealed cIAP2 expression status and the pathological response to

							chemoradiotherapy to be significant prognostic factors for OSCC patients.
	BIRC4/ XIAP	Baculoviral IAP Repeat Containing 4	Xq24-25 Size-542 56bp Introns - 15 Exons-33				
	TRAF2	TNF Receptor Associated Factor 2, TRAF3	9q34.3 Size- 44696bp Introns - 42 Exons-32				
	TRAF3	TNF Receptor Associated Factor 3, RNF118	14q32.32 Size- 134025bp Introns - 20 Exons-31				
	TRAF4	TNF Receptor Associated Factor 4, CART1	17q11.2 Size- 6975bp Introns - 17 Exons-53	Jianbin Yang et al (2015)	Examined the expression of TRAF4 on OSCC cell lines and investigated its effect on cell growth, invasion and migration.	Human tongue squamous cell carcinoma cell lines Tca8113, SCC-4, SCC-25 and SCC-9 were cultured. The human normal oral keratinocyte cell lines (HOK) were used as "normal". Total RNA was extracted followed by the reverse transcription to cDNA. It was then subjected to real-time PCR followed by amplification to , human full-length TRAF4 cDNA . Cells were then western blotted analysed for proliferation, apoptotic, cell migration and invasion assays.	Compared with HOK, up-regulation in TRAF4 mRNA levels was demonstrated in OSCC cell lines, especially in SCC-25 and SCC-4 cells which was confirmed by western blot analysis. TRAF4 overexpression with pcDNA3.1-TRAF4 vector transfection promoted cell proliferation and inhibited cell apoptosis. TRAF4 elevation also increased cell invasion and migration. TRAF4 up-regulation induced the expression of $\beta$ -catenin and the downstream target molecules of cyclinD1, c-myc, Bcl-2, MMP-9 and MMP-2, indicating that TRAF4 could induce the activation of Wnt/ $\beta$ -catenin pathway



CARD Family	NOL3	Nucleolar Protein 3, MYP	16q22.1 Size-5582bp Introns - 16 Exons-35				
DED (Death Effector Domain) Family	CFLAR	CASP 8 and FADD like apoptosis regulator, I-FLICE	2q33.1 Size-60584bp Introns - 36 Exons-66	Yajima T et al (2009)	Demonstrated that resistance to cytotoxic chemotherapy induced apoptosis in side population cells of human oral squamous cell carcinoma cell line Ho-1-N-1.	Isolated SP cells from oral squamous cell carcinoma cell line, Ho-1-N-1. Ho-1-N-1 contained 3.0% SP cells. Ho-1-N-1 SP cells showed self-renewal capacity, generating both SP and non-SP cells. Differentially expressed genes between Ho-1-N-1 SP and non-SP cells using GeneChip microarray and quantitative real-time RT-PCR was assessed. Chemoresistance to anticancer agents, including 5-fluorouracil and carboplatin, were compared between Ho-1-N-1 SP and non-SP cells using flow cytometry and tetrazolium salt microtiter plate assay.	SP cells expressed high levels of ATP-binding cassette transporters with related multidrug resistance (MDR) genes. The expression of ABCB1 and ABCG2 were significantly up-regulated in Ho-1-N-1 SP cells. In addition, the expression of CFLAR, BCL2 and BCL2A1 which are associated with anti-apoptosis, were also significantly increased in the SP cells. Ho-1-N-1 SP cells survived significantly longer and SP ratio remarkably increased after anticancer agent treatment compared to non-SP cells. Immunocytochemical staining and apoptosis assay suggested an anti-apoptotic potential for Ho-1-N-1 SP cells. Ho-1-N-1 SP cells survived with various agents due to high level expression of ABC transporters, and anti-apoptotic proteins.
Other genes	AKT1	V-akt murine thymoma Viral oncogene homolog 1, RAC	14q32.33 Size-26403bp Introns - 28 Exons-63	Kuo YY et al (2013)	Examined the effect of caffeic acid phenethyl ester (CAPE) treatment on cell proliferation, cell cycle, and signaling protein expression in TW2.6 human oral cancer cells. And demonstrated suppression of proliferation and survival of TW2.6 human oral cancer cells by CAPE via inhibition of Akt signaling.	Hoechst dye-based proliferation assay, MTT assay, Soft agar colony formation assay, plasmid-based luciferase reporter assay was done followed by flow cytometric analysis to determine if cell cycle progression of TW2.6 oral cancer cells is affected by CAPE. TUNEL assay to determine if CAPE treatment at higher concentrations may induce apoptosis in TW2.6 oral	CAPE treatment suppressed cell proliferation and colony formation of TW2.6 human oral squamous cell carcinoma (OSCC) cells dose-dependently. CAPE treatment decreased G1 phase cell population, increased G2/M phase cell population, and induced apoptosis in TW2.6 cells. Treatment with CAPE decreased protein abundance of Akt, Akt1,

					<p>cancer cells. Also co-treatment of CAPE with commonly used chemotherapy drug 5-fluorouracil drug was done to assess whether it can suppress growth of TW2.6 cells more effectively than 5-fluorouracil treatment alone.</p>	<p>Akt2, Akt3, phospho-Akt Ser473, phospho-Akt Thr 308, GSK3<math>\beta</math>, FOXO1, FOXO3a, phospho-FOXO1 Thr24, phospho-FoxO3a Thr32, NF-<math>\kappa</math>B, phospho-NF-<math>\kappa</math>B Ser536, Rb, phospho-Rb Ser807/811, Skp2, and cyclin D1, but increased cell cycle inhibitor p27Kip. Overexpression of Akt1 or Akt2 in TW2.6 cells rescued growth inhibition caused by CAPE treatment. Co-treating TW2.6 cells with CAPE and 5-fluorouracil, a commonly used chemotherapeutic drug for oral cancers, exhibited additive cell proliferation inhibition.</p>	
				<p>Cohen Y et al (2011)</p>	<p>Described mutational analysis of PTEN/PIK3CA/AKT pathway in oral squamous cell carcinoma.</p>	<p>The frequency of mutations in AKT1, PTEN, PIK3CA, and RAS (K-RAS, N-RAS, H-RAS) genes in 37 cases of oral squamous cell carcinoma (OSCC) was studied. Mutational analysis of PTEN, RAS, PIK3CA and AKT genes was performed using chip-based matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometry and by direct sequencing.</p>	<p>The only gene mutated s was the PIK3CA. Missense mutations of the PIK3CA gene were found in 4 of our cases; no correlation has been found with oral location, stage and survival. Hence PIK3CA is important to OSCC tumorigenesis and can contribute to oncogene activation of the PIK3CA/AKT pathway in OSCC</p>
					<p>Evaluated the role of AKT1 in the tongue squamous cell carcinoma (TSCC) and the mechanisms of AKT1 in the migration and invasion of TSCC.</p>	<p>Immunohistochemistry (IHC) was conducted to detect the expression of AKT1 in TSCC. The role of AKT1 in the migration and invasion of TSCC was determined investigated whether AKT1 was the target gene of miR-138 using dual luciferase reporter assays and Western blot.</p>	<p>AKT1 dysregulation was frequent in TSCC, which was correlated with lymph node metastasis reduced overall survival. UM1 cells with higher migratory and invasive abilities had more robust AKT1 protein expression than UM2 cells with lower migratory and invasive abilities. The migration and invasion abilities were inhibited in UM1 cells upon AKT1 knockdown, meanwhile</p>

							resulted in a decline of metastasis-related proteins (vimentin, slug, and pERK1/2), and upregulation of E-cadherin. Luciferase assays revealed that AKT1 was directly targeted by miR-138, and ectopic transfection of miR-138 reduced the expression of AKT1 protein. Hence upregulation of AKT1, a miR-138 target gene, contributes to the aggressive behaviors and poor prognosis of TSCC.
				Lim J et al (2005)	Investigate the association between the expression of activated Akt, clinicopathological factors, and E-cadherin, PCNA, and VEGF expression to validate of Akt as prognostic factor in OSCC.	Phosphorylated Akt (p-Akt), E-cadherin, PCNA, and VEGF expression were assessed immunohistochemically in 84 OSCCs. The results were analysed in relation to clinicopathological factors.	p-Akt was expressed in 29 cases and was significantly correlated with lymph node metastasis, TNM stage, and E-cadherin expression. p-Akt, E-cadherin, and PCNA expression, differentiation, tumor size, lymph node metastasis, TNM stage, and recurrence correlated with prognosis. p-Akt expression is an independent prognostic factor in patients with OSCC.
	BRAF	V-raf murine sarcoma viral oncogene homolog B1	7q34 Size-205602bp Introns - 21 Exons-88	Tan DS et al (2014)	Demonstrated common actionable genetic alterations infrequently harbored in Tongue carcinoma.	A high-sensitivity mass spectrometry-based mutation profiling platform was used to determine the EGFR mutation status, and other actionable alterations in 66 TSCC patients. Somatic mutation profiling was performed using Sequenom LungCarta v1.0, and correlated with clinical parameters.	Mutations were identified in 20/66 of samples and involved TP53, STK11, MET, PIK3CA, BRAF and NRF2. The most common mutations were in p53 and MET followed by STK11 and PIK3CA. BRAF and NRF2 mutations, which are novel in TSCC, were demonstrated in one sample each.
				Bruckman KC et al (2010)	Performed mutational analyses of the BRAF, KRAS, and PIK3CA genes (involved in cell cycling and apoptosis), in oral squamous cell carcinoma.	Mutations of BRAF, KRAS, and PIK3CA were evaluated by direct genomic sequencing of exons 1 of KRAS, 11 and 15 of BRAF, and 9 and 20 of PIK3CA in OSCC specimens.	Both BRAF and KRAS mutations were detected with a mutation frequency of 2%. PIK3CA mutations were detected at 3% implicating that mutations in the BRAF, KRAS, and PIK3CA genes make a minor contribution to OSCC tumorigenesis, and

							pathway-specific therapies targeting these 2 pathways should be considered for OSCC in a subset of patients with these mutations.
		Bifunctional Apoptosis Regulator, RNF47	16p13.12 Size-36422bp Introns - 18 Exons-36	BFAR			

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