RESEARCH

Different potential mitochondrial drug targets in oral carcinogenesis

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ABSTRACT

Aim: To identify the genes associated with mitochondria in oral carcinogenesis by using bioinformatics approach.
Methods: This study involves identification of the mitochondria associated genes in oral carcinogenesis by using bioinformatics approach, including gene ontology, pathway analysis, network construction and centrality and module analyses.
Results: We have identified 30 differentially expressed genes (DEGs), of which 11 were up-regulated and 19 were down-regulated in oral squamous cell carcinoma (OSCC). With the help of bio-computational approach, we have identified CASP3, BID, DIABLO, BCL2L11 (BAM, BID, BOD), BCL-XL, TP53, CASP7, CASP8, BCL2, MCL1, APAF1, CASP9, CASP2, BAX, AKT1 and CASP10 genes which are associated with mitochondria in OSCC.
Conclusions: CASP3, BID, DIABLO, BCL2L11 (BAM, BID, BOD), BCL-XL, TP53, CASP7, CASP8, BCL2, MCL1, APAF1, CASP9, CASP2, BAX, AKT1 and CASP10 genes are potential mitochondrial associated drug targets. Their mutual interaction might yield a model that can be used in diagnosis and treatment of oral cancer at an early stage.

KEYWORDS:*OSCC; Micro-array; BCL-2; Apoptosis*

Citation: Anthony et al. Different potential mitochondrial drug targets in oral carcinogenesis. Polymorphism 2021; 6: 1-25.

INTRODUCTION

Cancer is a genetic disease that chiefly consists of unregulated cell growth and division caused by the changes to genes or damage to DNA. In cancer, a cell divides and grow uncontrollably, forming malignant tumors, and invade nearby parts of the body. Cancer may also spread to more distant parts of the body through the lymphatic system or bloodstream. Oral cancer is currently a major global health issue [Lai CH et al, 2013] and most commonly found in many South Asian underdeveloped countries, especially among men in comparison to women. In India, considering the mortality rate among various cancers the primary reason for death in men is due to oral cancer [Jain A et al, 2014]. Oral Squamous cell carcinoma (OSCC) occurs at lips, hard palate, upper and lower alveolar ridges, anterior two-thirds of the tongue, sublingual area, buccal mucosa, retromolar trigons, and the floor of the mouth [Vogel DWT et al, 2010] and is the most common subtype of Head and Neck Squamous Cell Carcinoma. Globally, the incidence of OSCC is increasing and accounted for 8.8 million deaths in the year 2015 [https://www.who.int/en/news-room/fact-sheets/detail/cancer]. A high prevalence of tobacco and alcohol consumption and the Human Papilloma Virus (HPV) are some of the causative agents of the OSCC [Jadhav KB et al, 2013]. The late diagnosis and lack of clinical interventions are some of the salient reasons for the high mortality rate due to OSCC. Diagnosis of oral cancer at later stages implies that the neoplastic cells become aggressive and become resistant to standard therapeutics [Jain A et al, 2014]. Despite the vast amount of research and several conventional therapeutics advancements for oral cancer patients, many drawbacks have to be get addressed like surgical resection leads to constant defacement, altered individuality, and devitalizing physiological consequences. Similarly, chemotherapies and radiotherapies result in toxic effects thus affecting the welfare and quality of patient life [Ketabat F et al, 2019]. Thus, the prognosis for the OSCC patient remains poor and burdensome task with a five-year survival rate that encourages the further research on the factors which modify the disease outcome [Jadhav KB et al, 2013] [Brinkman et al, 2006].

Available research reports reveal that cancer involves aggressive modification in both the mitochondrial and nuclear genome; and that via a succession of cellular DNA alterations the tumor development proceeds, each grants an uncontrolled growth and ultimately lead to the intensifying transformation of normal cells into cancerous cells [Dai H et al, 2016]. Cancer consists of characteristic features such as apoptosis, progression, invasion, metastasis, and angiogenesis, each of these features can be detected and is amenable to treatment at the molecular level. The most common molecular event related to the establishment of cancer is the dysfunction of the apoptotic pathway in mitochondria [Jain A et al, 2014]. The circular human mitochondrial DNA is double-stranded 16.6 kb DNA comprising of 13 genes coding respiratory chain protein subunits, 22 tRNAs, and 2 rRNAs. There are several thousands of mitochondria in each cell and each mitochondrion contains an average of five copies of mitochondrial DNA (mtDNA). The vital function of mitochondria is to produce energy that supports cellular activities through the oxidative phosphorylation pathway that generates reactive oxidative species (ROS), aging, and initiation of apoptosis. By the insufficiency of protective histone proteins, mtDNA becomes an easy target for oxidative DNA damage. Therefore, mtDNA mutation is accumulated by the limited DNA repair. The mutation rate of mitochondrial DNA is 10 times higher than the nuclear DNA mutation rate [Tan DJ et al, 2004]. Besides, a mutation that occurs in mitochondrial DNA leads to mitochondrial dysfunction which results in oncogenesis. Recent studies have reported the association of somatic

mtDNA mutation with tumorigenesis, throughout mitochondria [Tan DJ et al, 2004].
In the development of cancer, immune response, tissue homeostasis, and cellular death play an essential role. "Programmed Cell Death" is another term of Apoptosis. It has multiple characteristics of both unique morphological and biochemical features. Apoptosis is often down-regulated during cancer development since the anti-apoptotic proteins are over-expressed. Conversely, a large number of anti-cancerous drugs induce apoptosis in susceptible cells. Thus, apoptosis plays a pivotal role in cancer development and treatment [Gerl R et al, 2005].

Previous studies account for the inter-relation of mtDNA mutation and apoptosis in various types of cancer. There are many a pathway through which apoptosis is dysregulated in mitochondria of cancerous cells. One mechanism is free radical-induced cell death where defective mitochondria overproduce free radicals. There are many genes involved in the apoptotic pathway. Mechanisms for few genes like the B cell Lymphoma-2 (BCL-2) and B cell Lymphoma-2 Associated X, Apoptosis Regulator (BAX) are understood but still, there are many whose roles and exact function is still poorly understood. In the mechanism of apoptosis, the caspases play the central role as they are both the initiators and executioners. The apoptotic pathway has three important pathways Extrinsic Apoptotic Pathway (Death Receptor Pathway), Intrinsic Apoptotic Pathway (Mitochondrion Pathway), and Initiation Pathway (Intrinsic Endoplasmic Reticulum Pathway). The dysregulation of the apoptotic pathway leads to cancer. In cancer, during oncogenesis, there is over-expression of anti-apoptotic proteins while down-expression of pro-apoptotic proteins. There is a proliferative expression of Inhibitors of Apoptotic Proteins (IAPs). Caspases expression decreases while TP53 expression increases. In an impaired receptor signaling pathway, there is a reduced expression of the death receptor and signals [Wong RSY, 2011, Hanahan D et al, 2000]. Thus, we see there is a varied expression of different genes that are either drivers or followers during oncogenesis. Because there is difficulty in identifying which genes are drivers and which are followers, the search for an ideal drug target in oral cancer up till now has been unfruitful. In the present study, we focus on the potential therapeutic drug target to trigger mitochondrial-dependent cell death in oral carcinogenesis. The novelty of the present study has been the identification of potential therapeutic drug targets to trigger mitochondrial-dependent cell death involved in cellular apoptosis in oral carcinogenesis by using a bioinformatics approach. The main objective of the present study is to identify suitable therapeutic drug targets that trigger mitochondrial regulated cell death which is the first study of the type.

MATERIALS AND METHODS

Since, no patient samples are used in this study so there is no ethical issue.

Dataset

The GSE38823 dataset was extracted using keywords Oral Squamous Cell Carcinoma (OSCC) and Microarray, from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo), a repository at the National Centre for Biotechnology Information (NCBI) [Barret T et al, 2006, Zhang H, 2017, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE38823]. The GSE38823 dataset was depend on the GPL6883 platform Illumina HumanRef-8 v3.0 expression beadchip, submitted by Lee CH et al [Zhang H et al, 2017]. The GSE38823 dataset had 16 samples, which included 8 samples of OSCC and 8 samples of normal tissue [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE38823].

Data Processing

The data from the dataset was further used for the analysis of Differentially Expressed Genes (DEGs)

between OSCC and normal samples by using the GEO2R [https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE38823] tool [Zhang H et al, 2017, https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE38823]. This tool helps in contrasting two or more groups of samples in a target to identify the differentially expressed genes across the experimental conditions. In our study, we took only 30 differentially expressed genes as these genes were associated with mitochondria in apoptotic pathway. To correct the false positive results, the adjusted p values (adj. p) were applied by the default Benjamini-Hochberg false discovery rate method. The |log2FC| value is 0 to 10 then the genes from differentially expressed genes come under up-regulated (uDEGs) genes and when |log2FC| value is 0 to -10 then the genes from differentially expressed genes come under down-regulated genes (dDEGs) [Zhang H et al, 2017].

Gene Ontology (GO) OF DEGS

The database of gene ontology (http://www.geneontology.org) contributes an ontology of defined terms constituting several gene product properties which includes biological process (BP), cellular component (CC) and molecular function (MF) [https://en.wikipedia.org/wiki/Gene_ontology]. In this study, we have used DAVID (database for annotation, visualization and integrated discovery), [http://david.abcc.ncifcrf.gov/], an online tool for gene functional classification which will help to understand the biological importance of genes used in this study. In the present study, in order to analyze the functions of DEGs, gene ontology analysis was conducted using DAVID Tool by setting $p<0.05$ as the cutoff point [Hanahan D et al, 2000]. In our study, we took two groups of genes up-regulated and down-regulated.

Construction of protein- protein interaction and module analysis

In the present study, we have to construct and visualize the PPI (Protein-Protein Interaction) network on the CYTOSCAPE software. The STRING database plugin was used for PPI Network construction by setting H confidence cut off 0.90 and maximum additional interaction cut off 0. Further centrality analysis was done by CytoNCA plugin by using four centrality measures like Closeness, Degree, Subgraph, and Betweenness. The centrality analysis was used to identify potential mitochondrial drug target in oral carcinogenesis. In addition, module analysis was also done of the PPI Network. MCODE plugin was used for the module analysis by setting degree cut off =2, Node Score cut off =0.2, K core cut off =2 and Maximum Depth =100 [https://cytoscape.org/download.html]. The highest score genes were selected as a hub gene of the centrality analysis. Gene ontology in every table is categorized into Biological Process, Cellular Component, and Molecular Function and KEGG pathway.

RESULTS

Identification of differentially expressed genes

30 differentially expressed genes as the main data for analysis because only these 30 genes were associated with mitochondria in apoptotic pathway. The following are the 30 differentially expressed genes which were taken BCL2, FADD, FASLG, CASP8, CASP6, BCL2L2, HRK, BOK, TP53, MCL1, BIRC6, BIRC5, BCL2L1, BAX, FAS, CASP9, CASP7, PARP1, PRKD1, DIABLO, CASP3, APAF1, MCL1, AKT1, BAD, BID, CASP2, CASP10, BCL2L11, BCL2L12 and BCL2L10.

Gene ontology enrichment analysis

For the further understanding the function of identified DEGs, all DEGs were uploaded to DAVID Tool to identify significant Gene Ontology Categories. Table 1 shows the information of gene ontology analysis of up regulated genes and down regulated genes in OSCC. The Gene Ontology of up-regulated genes resulted into four main classification like Biological Process, Cellular Component, Molecular Function and KEGG Pathway. Same is with the case of Down-regulated genes.

Table 1: Gene Ontology analysis of differentially expressed genes associated with OSCC

Go-term Category	Term/ Gene Function	Gene Count	%	P Value	Benjamini Value
Up regulated genes					
Biological process	GO:0006915-apoptotic process	9	0.53	6.83E-11	1.86E-08
Biological process	GO:0042981-regulation of apoptotic process	7	0.41	7.82E-10	1.07E-07
Biological process	GO:0097192-extrinsic apoptotic signaling pathway in absence of ligand	5	0.29	2.92E-09	2.65E-07
Biological process	GO:0043065-positive regulation of apoptotic process	5	0.29	1.93E-05	8.76E-04
Biological process	GO:0043066-negative regulation of apoptotic process	5	0.29	9.81E-01	0.002432
Cellular component	GO:0005737-cytoplasm	7	0.41	0.038128	0.238233
Cellular component	GO:0005739-mitochondrion	6	0.35	3.81E-04	0.010608
Cellular component	GO:0005741-mitochondrial outer membrane	5	0.29	8.68E-07	4.86E-05
Cellular component	GO:0005783-endoplasmic reticulum	3	0.17	0.072837	0.34525
Cellular component	GO:0031265-CD95 death-inducing signaling complex	2	0.11	0.003288	0.059631
Molecular Function	GO:0005515-protein binding	11	0.65	0.001453	0.016157
Molecular Function	GO:0046982-protein heterodimerization activity	5	0.29	1.05E-04	0.002925
Molecular Function	GO:0005123-death receptor binding	3	0.17	3.77E-05	0.002111
Molecular Function	GO:0051434-BH3 domain binding	2	0.11	0.002368	0.018785
Molecular Function	GO:0035877-death effector domain binding	2	0.11	0.002368	0.018785
KEGG Pathway	hsa04210:Apoptosis	6	0.35	2.77 E-09	1.74E-07
	hsa05200:Pathways in cancer	5	0.29	6.11 E-04	0.012753

	hsa05206:MicroRNAs in cancer	4	0.23	0.003408	0.042105
	hsa04151:PI3K-Akt signaling pathway	4	0.23	0.005799	0.059238
	hsa04722:Neurotrophin signaling pathway	3	0.17	0.007889	0.060467

Protein-protein interaction network construction

The top centrality hub genes are CASP3, BID, DIABLO, BCL2L11 (BAM, BID, BOD), BCL2L1 (BCL-XL, BCL2L, BCLX), TP53, CASP7, CASP8, BCL2, MCL1, APAF1, CASP9, CASP2, BAX, AKT1, and CASP10. By the module analysis, we got 3 (i.e. module a, b, c) modules from PPI Network. Table 2 represents the top 15 DEGs identified by four centrality method with the highest PPI scores were. Table 3, 4 and 5 represents the gene ontology of the module a, b and c respectively. The Figure 1 represents the Protein-Protein Interaction Network and Figure 2 represents Module a, Module b and Module c respectively.

Table 2: Top 10 DEGs with the highest score of four centrality analysis respectively

Closeness		Degree		Subgraph		Betweenness	
CASP3	0.23	CASP3	16	CASP3	3417.68	CASP3	88.56
BCL2L1	0.228	BCL2L1	15	BCL2L1	3342.01	BID	70.70
BID	0.223	BID	14	CASP8	3140.66	DIABLO	69.76
CASP8	0.223	CASP8	14	APAF1	2862.16	BCL2L11	63.32
TP53	0.221	TP53	13	TP53	2519.77	BCL2L1	62.43
BCL2	0.221	APAF1	13	BID	2425.96	TP53	52.45
APAF1	0.221	BCL2	12	BCL2	2345.96	CASP7	31.88
MCL1	0.216	CASP7	11	CASP2	1944.42	CASP8	27.95
BCL2L11	0.216	CASP2	10	CASP9	1596.73	BCL2	22.42
CASP7	0.216	BCL2L11	10	CASP7	1584.61	MCL1	21.69
CASP9	0.216	CASP9	10	CASP10	1379.07	APAF1	21.07
BAX	0.214	MCL1	9	AKT1	1243.07	CASP9	16.31
CASP2	0.214	CASP10	9	BCL2L11	1189.59	CASP2	9.87
DIABLO	0.214	DIABLO	9	BAX	1183.27	BAX	9.11
AKT1	0.213	BAX	8	MCL1	1154.80	AKT1	6.28

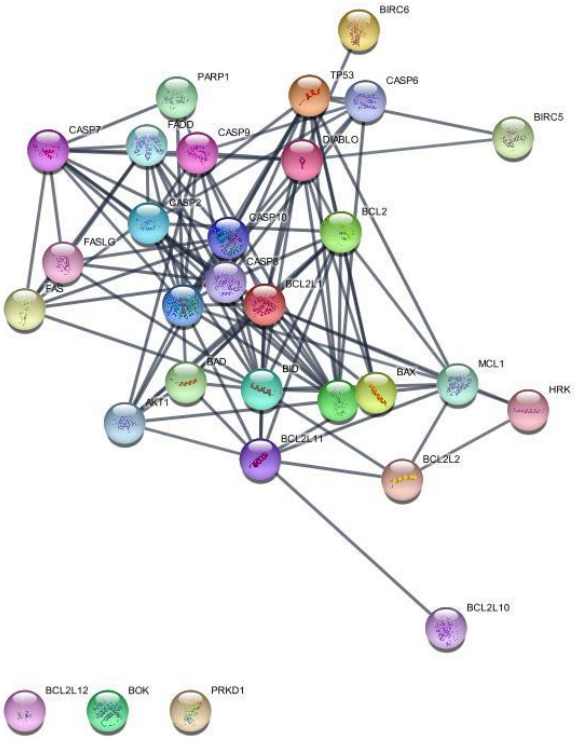

Figure 1. PPI Network

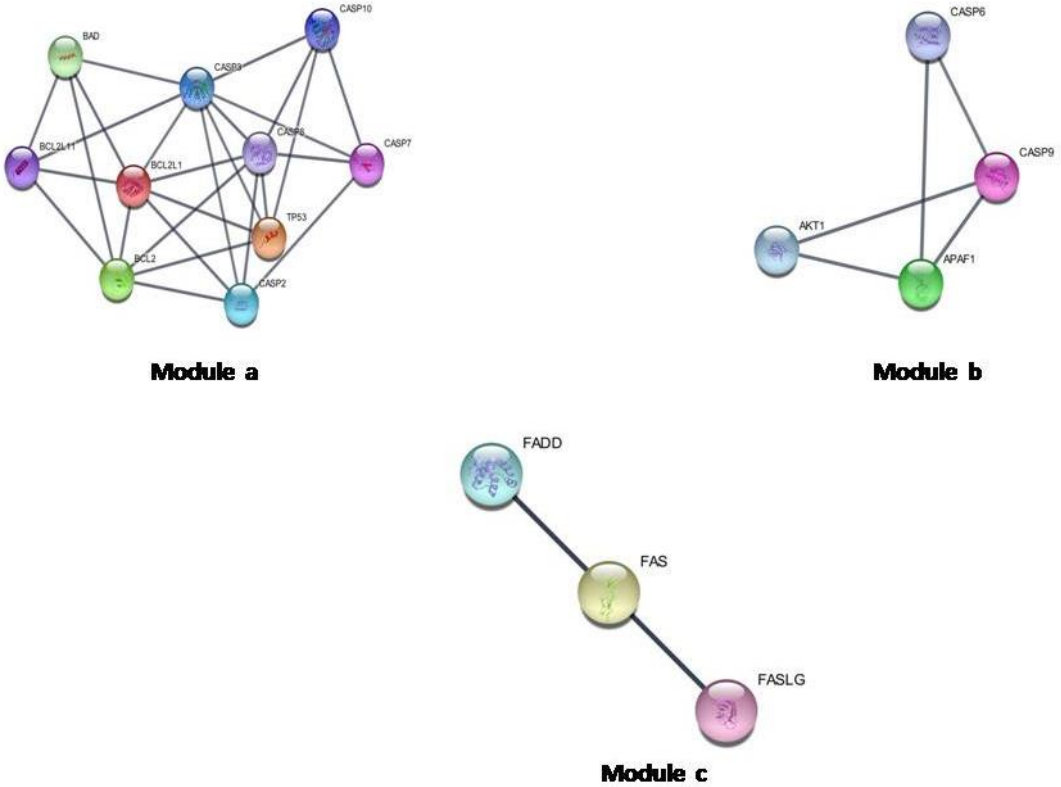

Figure 2. Modules

Table 3: The below table gives information of Gene Ontology and KEGG Pathway analysis of selected module (a)

Category	Term / Gene Function	Count	%	P value	Benjamini
BIOLOGICAL PROCESS	GO:0006915-apoptotic process	9	0.56	1.41 E-11	2.17 E-09
	GO:0097192-extrinsic apoptotic signaling pathway in absence of ligand	6	0.37	3.14 E-12	9.69 E-10
	GO:0008630-intrinsic apoptotic signaling pathway in response to DNA damage	5	0.31	6.72 E-09	4.15 E-07
MOLECULAR FUNCTION	GO:0005515-protein binding	10	0.62	0.002794	0.016037
	GO:0004197-cysteine-type endopeptidase activity	5	0.31	1.92 E-08	1.11 E-06
	GO:0042802-identical protein binding	5	0.31	4.05 E-04	0.005858
CELLULAR COMPONENT	GO:0005829-cytosol	10	0.62	2.16 E-07	9.29 E-06
	GO:0005739-mitochondrion	7	0.43	1.04 E-05	1.49E-04
	GO:0005737-cytoplasm	7	0.43	0.020072	0.135246
KEGG PATHWAY	hsa04210:Apoptosis	8	0.50	2.71 E-14	1.81 E-12
	hsa05200:Pathways in cancer	6	0.37	2.88 E-05	3.22 E-04
	hsa04151:PI3K-Akt signaling pathway	5	0.31	3.71 E-04	0.002756

Table 4: The below table gives information of Gene Ontology and KEGG Pathway analysis of selected module (b)

Category	Term / Gene Function	Count	%	P value	Benjamini
BIOLOGICAL PROCESS	GO:0042981-regulation of apoptotic process	3	0.40	4.76 E-04	0.03602
	GO:0006915-apoptotic process	3	0.40	0.003338	0.097857
	GO:0043065-positive regulation of apoptotic process	2	0.26	0.052648	0.695738

MOLECULAR FUNCTION	GO:0042802-identical protein binding	3	0.40	0.005724	0.071914
	GO:0097153-cysteine-type endopeptidase activity involved in apoptotic process	2	0.26	0.002309	0.058324
	GO:0004197-cysteine-type endopeptidase activity	2	0.26	0.010802	0.089833
CELLULAR COMPONENT	GO:0005829-cytosol	4	0.53	0.006014	0.047115
	GO:0043293-apoptosome	3	0.26	3.29 E-04	0.005255
KEGG PATHWAY	hsa04210:Apoptosis	4	0.53	6.97 E-07	5.02 E-05
	hsa05222:Small cell lung cancer	3	0.40	4.49 E-04	0.01604
	hsa04115:p53 signaling pathway	2	0.26	0.02894	0.161551

Table 5: The below table gives information of Gene Ontology and KEGG Pathway analysis of selected module (c)

Category	Term / Gene Function	Count	%	P value	Benjamini	Reference
BIOLOGICAL PROCESS	GO:0097296-activation of cysteine-type endopeptidase activity involved in apoptotic signaling pathway	3	0.53	5.53 E-07	2.43 E-05	http://david.abcc.ncifcrf.gov/
	GO:0006915-apoptotic process	3	0.53	0.001138	0.009069	
	GO:0097192-extrinsic apoptotic signaling pathway in absence of ligand	2	0.35	0.004046	0.02929	
MOLECULAR FUNCTION	GO:0005123-death receptor binding	2	0.35	0.001895	0.029889	http://david.abcc.ncifcrf.gov/
	GO:0005164-tumor	2	0.35	0.003433	0.027136	

	necrosis factor receptor binding					
	GO:0042802-identical protein binding	2	0.35	0.086773	0.383754	
CELLULAR COMPONENT	GO:0005886-plasma membrane	3	0.53	0.051125	0.182225	http://david.abcc.ncifcrf.gov/
	GO:0031264-death-inducing signaling complex	2	0.35	7.68 E-04	0.008798	
	GO:0009897-external side of plasma membrane	2	0.35	0.02324	0.126464	
KEGG PATHWAY	hsa04210:Apoptosis	3	0.53	7.99 E-05	0.002156	http://david.abcc.ncifcrf.gov/
	hsa05200:Pathways in cancer	3	0.53	0.003256	0.017457	
	hsa04668:TNF signaling pathway	2	0.35	0.030869	0.081176	

Table 6: The Role of identified genes in oral carcinogenesis.

GENES	FULL FORM	MECHANISM of ACTION	ROLE in ORAL CANCER	REFERENCE	REFERENCE
CASP3	CASPASE 3	In the apoptotic cell, CASP3 is activated by both Extrinsic and Intrinsic Apoptotic Pathway. It is participates in the cascade activation of caspases which is responsible for the execution of apoptosis.	In Ho et al study, they examine the berberine anti-cancer activity in SCC-4 human tongue cancer cells. The result reported the berberine induced apoptosis by the generation of reactive oxidative species (ROS), increase in Cytosolic Ca2+. Therefore, the apoptosis is associated with reduction in mitochondrial membrane potential which changes the bcl-2/bax	[Ho YT et al, 2009]	[https://string-db.org/cgi/network.pl?taskId=YyY6cH1nONlv]

			ratio, release of cytochrome c, and thus activates the Caspase 3. The Real-time PCR study reported,berberine stimulates the gene expression of Caspase 8, 9, 3 and endonuclease G. The study demonstrated the berberine induced apoptosis in SCC-4 cells via ROS, Mitochondria, Caspase-3 dependent and Mitochondrial apoptotic pathway. Thus berberine can be used as a potential candidate in the future study of human tongue cancer.		
BID	BH3 interacting domain death agonist	BID is a pro-apoptotic member of Bcl-2 protein family which contains BH3 domain. In the apoptosis, it directly activates the Bax and Bak which permits the release of Cytochrome c.	Gillenwater et al reported in a study, they have evaluated the activity of Suberoylanilidehydroxamic acid (SAHA) in regulating cell growth and apoptosis in Head and Neck Squamous cell carcinoma (HNSCC) cells compared with premalignant leucoplakia and normal cells. The result suggests SAHA triggered the mitochondrial apoptotic pathway for apoptosis, including the release of cytochrome c, caspase 3 and caspase 9 activation, and poly(ADP-ribose) polymerase cleavage in	[Gillenwater AN et al, 2007]	[https://string-db.org/cgi/network.pl?taskId=YyY6cH1nONlv]

			HNSCC. In addition, SAHA also activates the extrinsic apoptotic pathway which included an increased expression of Fas and Fas Ligand (FasL), activating caspase 8 and cleavage of Bid. Thus SAHA activated extrinsic and intrinsic apoptotic pathway with Fas and FasL expression.		
DIABLO	DIABLO Homolog	DIABLO gene codes for DIABLO protein. This protein has as pro-apoptotic function in apoptotic cell. In the mitochondrial pathway through cytochrome c release and activation of Apaf-1, it promotes apoptosis by activating caspases. It also inhibits the activity of Inhibition of Apoptosis protein family.	Coutinho-Camillo et al in their study analyzed the expression of apoptosis-regulating miRNAs in 20 OSCC and 5 normal oral mucosa tissue samples by using Real time RT-PCR. They also used bioinformatics algorithm to recognize target genes of miRNAs like BCL2, CASP2, CASP7, CASP8, DIABLO. The results suggests the BCL2 expression was low and CASP2, CASP7, CASP8, DIABLO expression was high. The study concludes the apoptosis regulation plays a hallmark for tongue squamous cell carcinoma pathogenesis.	[Coutinho-Camillo CM et al, 2015]	[https://string-db.org/cgi/network.pl?taskId=YyY6cH1nONlv]
BCL-XL	B-cell lymphoma -extra large	BCL-XL gene encodes anti-apoptotic protein Bcl-xl. It potentially inactivates apoptosis by inhibiting the caspases. It inhibits cell death by	In Kok et al study they have demonstrated the Norcantharidin (NCTD) also promote apoptosis in Human oral cancer cell lines like SAS and Ca9-22.	[Kok SH et al, 2005]	[https://string-db.org/cgi/network.pl?taskId=YyY6cH1n

		obstructing the voltage-dependent anion channel by binding to it and prevents the release of Cytochrome c from Mitochondria.	The results showed the induction of apoptosis via mitochondrial apoptotic pathway. Moreover, NCTD enhanced the expression of Bax in SAS cells while it down regulated the expression of Bcl-2 in Ca9-22 and Bcl-xl in SAS cells. Thus, the study concluded, NCTD up regulates the expression of pro-apoptotic proteins and down regulates the expression of anti-apoptotic proteins. Oral cancer cells with mutant p53 or over-expression of Bcl-XL showed the resistant to chemotherapy but NCTD overcomes the chemoresistance of these cells. Thus, NCTD could be used in treating the human oral cancers.		ONlv]
CASP7	Caspase 7	Caspase 7 helps in activation of Cascade of caspase that executes the apoptosis. It cleaves and operates the sterol regulatory element binding proteins (SREBPs) and also cleaves the poly (ADP-ribose) polymerase (PARP).	Camillo et al study reported that they have used tissue microarray method to analyze the immunoexpression of caspases 3, 6, 7, 8, 9 and 10 in 229 OSCC cases. In the results they explored the over-expression of Caspase7 which was associated with advanced stage of OSCC. In addition, Free-survival rate of disease is higher in low	[Coutinho-Camillo CM et al, 2011]	[https://string-db.org/cgi/network.pl?taskId=YyY6cH1nONlv]

			expressed patients and vice-versa.		
CASP8	Caspase 8	Caspase 8 is the initiator and most upstream protease. It is responsible for Death-Receptor Apoptotic Pathway. The ligand, receptor, and adaptor protein form an aggregate complex called Death-inducing signaling complex (DISC). DISC further activates the pro-caspase 8.	In Min et al study they examined the ability of Shikonin to operates apoptosis in cultured Tca-8113 oral cancer cells. They treated the Tca-8113 oral cancer cells with variety of concentration of Shikonin (10-40 micron). The study explored the shikonin induced apoptosis in Tca-8113 oral cancer cells by the activation of Caspase 8, 9, 3 and Bcl-2 protein low expression. In addition, shikonin also inactivated the NF-kappaB pathway in Tca-8113 oral cancer cells.	[Min R et al, 2008]	[https://string-db.org/cgi/network.pl?taskId=YyY6cH1nONlv]
BCL2	B-cell lymphoma 2	BCL2 gene encodes both pro-apoptotic and anti-apoptotic proteins. Anti-apoptotic protein inhibits the apoptosis process while pro-apoptotic proteins regulate the apoptosis. Both of them regulate the apoptosis process by governing the mitochondrial membrane permeability. Anti-apoptotic proteins inhibit the caspase activity either by obstructing the release of cytochrome c from the mitochondria or by binding to APAF1.	Ravi et al studied the immunocytochemical evaluation of bcl-2 and p53 proteins in hyperplastic oral mucosa, dysplastic oral mucosa and invasive oral cancer. The result suggests the p53 expression was not significant while bcl-2 expression was absent in hyperplastic leukoplakia lesions. While both p53 and bcl-2 proteins were expressed in apparent leukoplakia dysplasia. Moreover, p53 and bcl-2 were highly expressed in	[Ravi D et al, 1996]	[https://string-db.org/cgi/network.pl?taskId=YyY6cH1nONlv]

			all invasive cancer lesions. Thus, it concludes in early oral carcinogenesis there is a possibility of alterations in p53 and over-expression of bcl-2 protein.		
MCL1	Myeloid leukemia cell differentiation 1	MCL1 belongs to Bcl-2 protein family. MCL1 inhibits the apoptosis process.	In Kuo et al they have examined the biochemical impact of berberine induced COX-2 reduction and apoptosis. In this study they have treated KB cells with berberine and measured the apoptosis morphologically and by caspase activity. In the same study they also determined the effects of prostaglandin E2 (PGE2) on berberine mediated cell growth. Wertern Blotting was also been used to explore the expression of COX-2, Bcl-2, Mcl-1, Akt and phophorylatedAkt with or without berberine treated KB cells. The result concludes that the berberine –induced apoptosis might be COX-2 dependent and is related to decreased Akt phosphorylation and Mcl-1 expression.	[Kuo CL et al, 2005]	[https://string-db.org/cgi/network.pl?taskId=YyY6cH1nONlv]
APAF1	Apoptotic protease-activating	APAF1 activity is mediates through cytochrome c activation	In the study, Chang et al reported that they have used water soluble PLGA	[Chang PY et al, 2013]	[https://string-db.org/cg

	factor 1	which leads further activation of pro-caspase 9. Activated pro-caspase 9 leads to activation of caspase 3 and apoptosis process.	curcumin nanoparticles (Cur-NPs) to investigate its effects on CAL27-cisplatin-resistant human oral cancer cells (CAR cells). The study concluded the Cur-NPs triggered intrinsic apoptotic pathway by ROS production and regulating the production of Multiple Drug Resistant Protein 1 in CAR cells. In addition, Cur-NPs up regulated the expression of cleaved Caspase 3 and 9, Cyt c, Apaf-1, AIF, BAX and also down regulated the expression of Bcl-2 proteins.		i/network.pl?taskId=YyY6cH1nONlv]
CASP9	Caspase 9	Caspase 9 is the initiator caspase and is involved in Mitochondrial Apoptotic Pathway. It binds to APAF1 that leads to protease activation whichfurther cleaves and activates CASP3. Activated CASP3 further leads to apoptosis process.	Lai et al study observed the rhein induced S-phase arrest through the inhibition of p53, cyclin A and E. It also induced the apoptosis through Endoplasmic Reticulum stress whichincreased the production of reactive oxidative species (ROS) and Ca2+ release and the activation of Caspase 3, 8, and 9 in human tongue cancer cell lines (SCC-4). The results showed the rhein decreased the Bcl-2 expression, release of cytochrome c from mitochondria and activation of Caspase 9	[Lai WW et al, 2009]	[https://string-db.org/cgi/network.pl?taskId=YyY6cH1nONlv]

			and 3. It can be concluded that rhein induced apoptosis in SCC-4 cells via caspase activation, ROS production and Mitochondrial Apoptotic Pathway.		
CASP2	Caspase 2	Caspase 2 executes the apoptosis process by activating cascades of caspase. It functions either by activating some proteins which were required for apoptosis of cell or inactivating the cell survival proteins.	The study by Kingsley et al demonstrated and compared the anti-proliferative effect of whole soy protein extract (SPE) on CAL27 and SCC25 oral cancer cell lines *in vitro*. The results explored the SPE induced apoptosis by down-regulation of mRNA expression in the oral cancer cell lines as well as up-regulation of Caspase 2 and 8. Thus the study concluded that the diet rich in fruits, vegetables and soy protein helps in protection against development and progression of oral cancer.	[Kingsley K et al, 2011]	[https://string-db.org/cgi/network.pl?taskId=YyY6cH1nONlv]
BAX	BCL2 associated X	BAX belongs to pro-apoptotic protein family. It regulates and accelerates the apoptotic process of the cell. It has conformation changes under stress condition that causes the translocation of mitochondrial membrane which leads to release of	Loro et al studied the expression of bcl-2 and bax in apoptosis in fresh frozen samples of normal oral epithelium (OE) and OSCC by the immunohistochemistry and TUNEL method. In oral epithelium (OE), bcl-2 was expressed in both basal and suprabasal	[Loro LL et al, 1999]	[https://string-db.org/cgi/network.pl?taskId=YyY6cH1nONlv]

		Cytochrome c. It further activates CASP3 and thereby apoptosis process.	compartment. In OSCC compared with OE, bax was highly expressed in suprabasal layer of OE and central parts of OSCC than in basal layers and parts of OSCC. They concluded, in OSCC compared with OE there were decreased in bcl-2 expression as well as lowered bcl-2/baxratio which induced apoptosis. The expression of bax is helpful in histological grading of tumor in OSCC.		
AKT1	RAC-alpha serine/ threonine-protein kinase	AKT1 regulates many processes like metabolism, proliferation, cell survival, growth and angiogenesis. AKT1 has major role in cell survival through phosphorylation of MAP3K5. Thus prevents apoptosis of the cell.	In Hara et al study, they demonstrated the Hepatocyte Growth Factor (HGF) and c-Met expression in human salivary gland cancer tissues. In this study they have used two human salivary gland cancer cell lines: green fluorescent protein-adenoid cystic carcinoma 2 (GFP-ACC2) and GFP-ACCM. Western blot analysis was used to detect the all AKT isoforms (AKT1, AKT2, AKT3) on two cell lines. The result suggests, HGF-stimulated invasive growth of human salivary gland cancer cells required all AKT isoform. Thus by targeting AKT isoform could be effective in treating salivary gland	[Hara S et al, 2008]	[https://string-db.org/cgi/network.pl?taskId=YyY6cH1nONlv]

			cancers.		
CASP10	Caspase 10	CASP10 executes the activation of cascade of caspases which are responsible for apoptosis. It recruits both Fas and TNFR-1 receptor in FADD dependent manner. It also cleaves and activates the Caspase 3, 4, 6, 7, 8 and 9.	Yasumoto et al have used two cell lines derived from the human squamous cell carcinoma (SAS). They have investigated the expression of apoptosis-related genes after irradiation by using cDNA array analysis method. The results suggested that after irradiation in SAS cell lines with tp53, have an increased expression of DFF40, Caspase 3, Caspase 8, Caspase 9, Caspase 10 and CRADD.	[Yasumoto J et al, 2003]	[https://string-db.org/cgi/network.pl?taskId=YyY6cH1nONlv]
TP53	Tumor Protein 53	TP53 gene encodes the p 53 protein which regulates the cell cycle and suppress tumor. The main function of TP53 is growth arrest, DNA repair and apoptosis regulation.	In this Ogden et al estimated the p53 expression in oral mucosal diseases like they took biopsies of normal, benign, pre-malignant and malignant oral tissues. They used polyclonal antibody CM1 and immunoperoxidase technique for the expression study. The result suggests total 37 oral cancer in which 20 were found with the expression of the p53 protein. The p53 were not expressed in normal, benign and pre-malignant oral mucosa but it was expressed in malignant oral lesions.	[Ogden GR et al, 1992]	[http://www.bioinformatics.org/p53/introduction.html] [https://string-db.org/cgi/network.pl?taskId=YyY6cH1nONlv]

DISCUSSION

OSCC is a solid neoplasm and it is the most prevalent subtype of Head and Neck Squamous cell carcinoma. Globally it accounts for exceeding 200,000 new cancer cases every year. OSCC may appear at Lips, Tongue, Cheeks, Floor of mouth, and Roof of the mouth. The most commonly known risk factors of OSCC are tobacco chewing, alcohol consumption, and betel quid usage. Another risk factor is the Human Papillomavirus (HPV) infection. Around 35-50% OSCC patient has 5-year survival rate [https://www.cancer.net/cancer-types/oral-and-oropharyngeal-cancer/statistics]. Globally cancer has the second most mortality rate after cardiovascular diseases, therefore, a lot more attention should be given to the molecular mechanism of cancer. For the execution of oncogenes and inexecution of tumor suppressor genes accompanied in OSCC, a multi-stage process is involved. This induces an imbalance between cell death and growth because of the loss of the apoptotic mechanism leading to the transformation of the normal epithelium to the neoplastic epithelium. The most conventional molecular events related to cancer development are the "Dysfunction of Apoptosis in Mitochondria" [Pandey R et al, 2019].

In our study, with the help of gene ontology we identified the up-regulated genes which were associated with the Apoptotic pathway, Pathways in cancer, PI3K-Akt signaling pathway, p53 signaling pathway, and RIG-I-like receptor signaling pathway. In addition to the Apoptotic pathway, Pathways in cancer, p53 signaling pathway the down-regulated genes were predominantly identified in, TNF signaling pathway, P13K-Akt signaling pathway, and VEGF signaling pathway. In the previous studies, it has been reported that by the dysregulation of the apoptotic pathway, RIG-I-like receptor signaling pathway, TP53 gene, VEGF proteins, and PI3K-Akt contributes to the oral carcinogenesis [Martins F et al, 2016, Lindemann A et al, 2018, Liang Y et al, 2018, Kowshik J et al, 2014].

The study has been focused, to identify the potential drug targets that trigger the mitochondrial cell-dependent death in oral carcinogenesis. Apoptosis is referred to as "Programmed Cell Death". It occurs to maintain the cell populations in tissues, as a defense mechanism such as immune response or when cells are damaged by disease. The apoptotic process is regulated through both the Intrinsic and Extrinsic Apoptotic Pathway. Both the apoptotic pathway helps in originating cell death by the activation of initiator Caspases. After which the executioner caspases also get activated which induces Apoptosis. The dysregulated apoptotic pathway leads to diseases like cancer. The defective apoptotic pathway has an imbalance in pro-apoptotic and anti-apoptotic proteins and affecting the down expression of caspases causing carcinogenesis [Elmore S, 2007]. In a eukaryotic cell, Mitochondria are the cell organelles that produce Adenosine Triphosphate (ATP), the chief energy molecule used by the cell. It is sometimes referred to as "the powerhouse of the cell" [Annesley SJ et al, 2019]. Apart from ATP production, mitochondria have other activities that affect the cellular physiology of the cell. ROS are produced inside the mitochondria; these are involved in many other physiological activities of the cell. However, if ROS is produced irrationally then it can damage the enzyme, lipids, and does a mutation in mtDNA. Previous studies report the major role of oxidative stress in oral carcinogenesis [Carew JS et al, 2002][Lightowlers RN et al, 1997] [Prior SL et al, 2006] [Fliss MS et al, 2000] [Saranath D et al, 1993]. The excessive ROS production leads to mtDNA mutations resulting in Mitochondrial Dysfunction [Staniek K et al, 2002].

Also, mitochondrial dysfunction often changes the gene expression inhibiting the apoptotic process. Previous studies account for the inter-relation of mtDNA mutation and increased oxidative stress in various types of cancer. In free radical-induced cell death, mitochondria are involved [Pandey R et al, 2019]. Thus, there is a strong relation between mitochondria and apoptosis. Furthermore, it is known that the mitochondria assist in initiating the early apoptosis of the cell. In our study, we are trying to identifying the potential drug targets that trigger the mitochondria cell-dependent in oral carcinogenesis.

In this study, we have identified CASP3, BID, DIABLO, BCL2L11 (BAM, BID, BOD), BCL2L1 (BCL-XL, BCL2L, BCLX), TP53, CASP7, CASP8, BCL2, MCL1, APAF1, CASP9, CASP2, BAX, AKT1, and CASP10 genes as the potential mitochondrial drug targets of oral carcinogenesis. Caspase 3 is an initiator and executioner of the apoptotic pathway. The activation of Caspase 3 induces the apoptotic process. By the caspase 3 up-regulation, the apoptosis process gets hindered leading to oral carcinogenesis [Huang JS et al, 2017]. BID is a pro-apoptotic protein of the BCL-2 gene family. It consists of the BH-3 domain which helps in the activation of BAK and BAX, results in Cytochrome C release. The inactivated or non-transformed BID contributes to oral carcinogenesis [Gillenwater AN et al, 2007]. DIABLO (Direct IAP binding protein with low pl) gene encodes the DIABLO protein. It binds to the Inhibitor of Apoptosis proteins (IAPs) and activates the caspases. Thus, DIABLO gene gets over-expressed in many cancers [Coutinho-Camillo CM et al, 2015]. The Bcl-2 protein family has an anti-apoptotic protein called as Bcl-XL. It potentially hinders the apoptotic process by inhibiting cytochrome c release from the mitochondria. In oral carcinogenesis, the BCL-XL gene is over-expressed. [Kok SH et al, 2005]. Caspase 7 helps in activating the cascade of caspases and induces the apoptotic process. It helps in cleaving and activating the sterol regulatory element-binding protein (SREBPs) and poly (ADP-ribose) polymerase (PARP). In oral cancer, caspase 7 was reported to be over-expressed and associated with the advanced stage of OSCC [Coutinho-Camillo CM et al, 2011]. Caspase 8 is the most upstream protease and is the initiator of the apoptotic process. It participates in the Extrinsic Apoptotic Pathway or Death-Receptor Apoptotic Pathway. The DISC complex helps in activating the pro-caspase 8 into caspase 8. The pro-caspase 8, an inactivated form of Caspase 8 in carcinogenesis contributes to the inhibition of apoptosis. [Min R et al, 2008]. BCL-2 gene encodes the Bcl-2 protein. It encodes two types of protein that is pro-apoptotic and anti-apoptotic proteins. Bcl-2, anti-apoptotic protein expression is high in oral carcinogenesis. [Ravi D et al, 1996]. MCL-1 gene encodes the Mcl-1 protein. In carcinogenesis, Mcl-1 anti-apoptotic protein is over-expressed leading to hindrance in the apoptotic process [Kuo CL et al, 2005]. APAF1 gene forms a complex called Apoptosome mediated by the cytochrome c release. Further, Apoptosome activates the pro-caspase 9 into caspase 9 and caspase 9 activates the caspase 3. The activation of caspase 3 contributes to the apoptotic process. The APAF1 gene is down-expressed in many cancers [Chang PY et al, 2013]. Caspase 9 is involved in the Intrinsic Apoptotic Pathway or Mitochondrial Apoptotic Pathway and it is the initiator caspase of this pathway. The Apoptosome activates the pro-caspase 9 into caspase 9. Further, the caspase 9 activates the caspase 3. The inactivated form of caspase 9 inhibits the apoptotic process [Lai WW et al, 2009]. Caspase 2 helps in the execution of the apoptotic process by caspase cascades execution. It functions either by activating some proteins which are mandatory for apoptosis of a cell or by inactivating the cell survival proteins. The study by Kingsley et al reported that caspase 2 is down-

regulated in oral carcinogenesis [Kingsley K et al, 2011]. BAX is the pro-apoptotic protein and accelerates the apoptotic process. The activated BAX helps in making a condition called mitochondrial outer membrane permeability (MOMP). The MOMP condition contributes to the cytochrome c release. It further operates the Caspase 3 and conducts apoptosis. Loro et al study reported that the BAX expression is lower in oral carcinogenesis [Loro LL et al, 1999]. AKT1 regulates many processes like metabolism, proliferation, cell survival, growth, and angiogenesis. AKT1 has a major role in cell survival through phosphorylation of MAP3K5. Thus, prevents the cell from apoptosis. Hara et al study reported the AKT1 has a major role in oral carcinogenesis by preventing the cell to undergo apoptosis [Hara S et al, 2008]. CASP10 executes the caspases cascade activation that is accountable for apoptosis. It recruits both Fas and TNFR-1 receptors in FADD dependent manner. It also split and executes the Caspase 3, 4, 6, 7, 8, and 9. Yasumoto et al study suggested that inactivated caspase 10 participates in oral carcinogenesis [Yasumoto J et al, 2003].

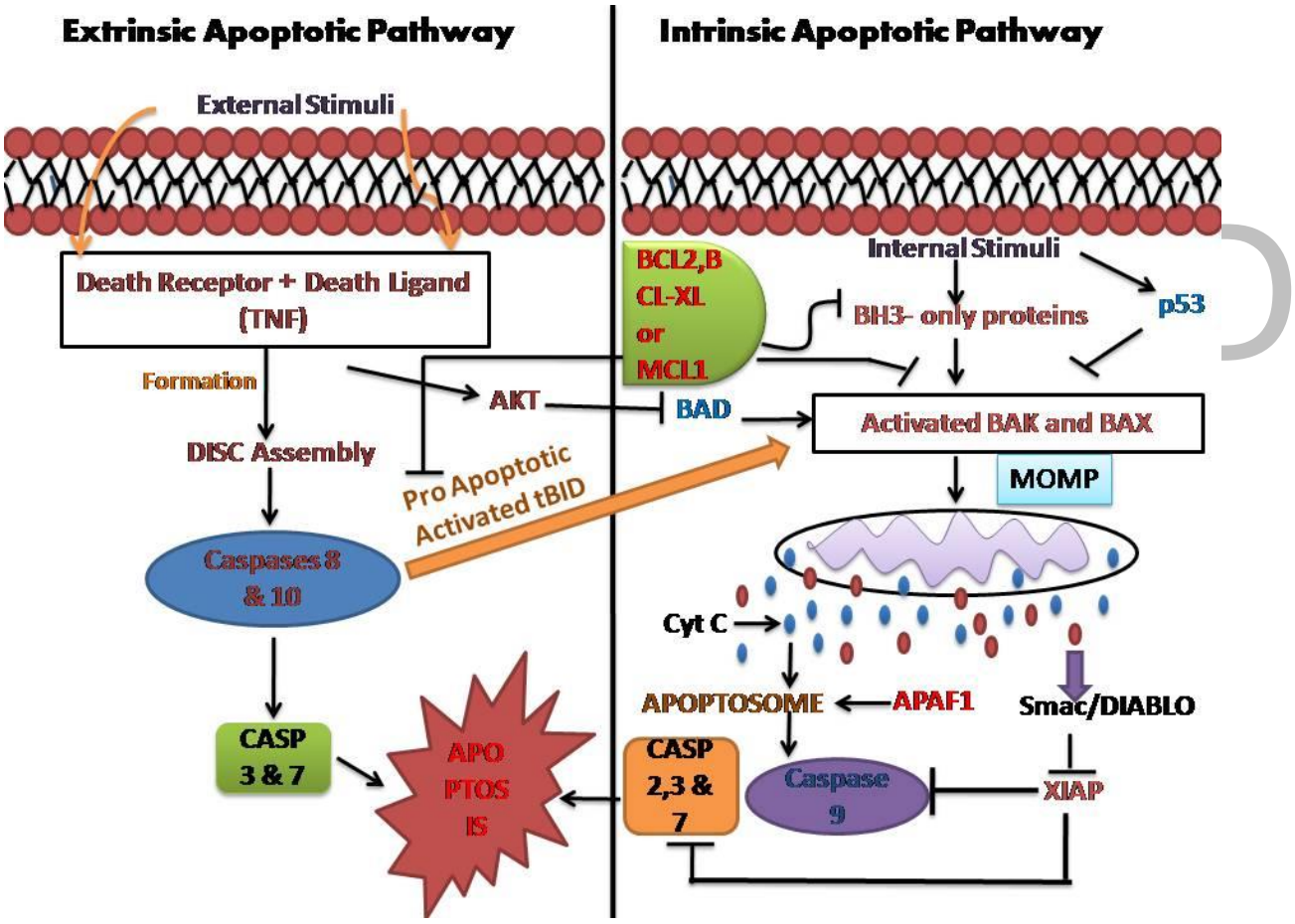

Figure 3. Overview of Mitochondrial apoptotic pathways

TP53 gene encodes the p53 protein, which regulates the cell cycle and suppresses tumor. The main function of TP53 is growth arrest, DNA repair, and apoptosis regulation. Ogden et al in a study reported that the TP53 gene gets mutated which leads to several cancers like oral cancer [Ogden GR et al, 1992]. Figure 3 represents the extrinsic and intrinsic apoptotic pathway of the CASP3, BID, DIABLO, BCL2L11 (BAM, BID, BOD), BCL2L1 (BCL-XL, BCL2L, BCLX), TP53, CASP7, CASP8, BCL2, MCL1, APAF1, CASP9, CASP2, BAX, AKT1, and CASP10 genes in the Apoptotic Pathway. Table 6 represents the role of identified mitochondrial drug targets in oral carcinogenesis.

Conclusion

In the present study, we identified the potential therapeutic drug target to trigger mitochondrial-dependent cell death in oral carcinogenesis. The identified potential mitochondrial drug targets are mostly of Mitochondrial Apoptotic Pathway. In a cancerous cell, there is an imbalance in anti-apoptotic and pro-apoptotic genes, caspase down-regulation, IAP up-regulation, and TP53 gene gets mutated. Targeting the apoptotic and associated mitochondrial genes will serve as potential drug targets in oral carcinogenesis. However how all together behave in a cancerous cell is a topic to be investigated. All these genes' interaction amongst each other can yield startling results and may be possible that statistical correlation might be produced which would be helpful in diagnosing and treating oral carcinogenesis.

Acknowledgements

This work was made possible by the support of DHR-MRU, KGMU, Dr Chandra Shekhar Mukhopadhyay, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India and Mrs Jaya Singhal, Research Scholar, KGMU.

Conflict of interest statement

The authors have declared to have no conflict of interest.

Authors' contributions

Pandey R conceived the idea. Anthony E conducted the experiments. Anthony E wrote the manuscript. Pandey R and Mehrotra D supervised the work and manuscript.

Source of Funding

This study received no specific funding.

Declaration of originality

The author declares that the work/review presented in this manuscript is original and has not been copied from elsewhere without appropriate citations.

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