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Review Article

Role of ATF-4 In Oral Lesions: A systematic scoping review

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ABSTRACT:
Background-Established role of ATF4 has been well documented in other human cancers, however it has not been attempted to explore the putative role of it in oral carcinogenesis and oral lesions. Therefore, this systematic scoping review aims to investigate the potential role of ATF4 in oral lesions. **Methods**- The search is conducted with the help of search engines like PubMed, Google Scholar by using databases of PubMed , Scopus, and Web of Science using mesh terms. **Results** –Total articles retrieved from electronic search was 4, amongst articles meeting inclusion criteria was 3, with 2 elimination of duplicates.Based on this data, total articles evaluated for this systematic scoping review was 3, where they used techniques like western blot analysis, double-labeling immune ofluorescence and quantitative PCR technique in oral lesions like oscc and okc to rule out the role of ATF4. **Conclusion** -Several molecular pathways of tumorigenesis depict ATF4 as important signaling molecule and transcription factor in carcinogenesis. Though a little knowledge is available in case of role of ATF4 in oral lesions/malignancy, potential role of the same cannot be neglected in terms of its active involvement in various signaling pathways in development of oral lesions and oral cancer.
Keywords: ATF4, oral squamous cell carcinoma, oral cancer, oral lesions, odontogenic keratocysts, PERK, ER stress.

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INTRODUCTION

Activating transcription factor 4, also known as ATF4, is a protein in humans that is encoded by the ATF4 gene. It belongs to the ATF/CREB (activating transcription factor/cyclic AMP response element binding protein) family of basic region-leucine zipper (bZip) transcription factors, which have the consensus binding site cAMP responsive element. ATF4 is a stress responsive gene, which is upregulated by several factors/stressors,including oxygen deprivation (hypoxia/anoxia), aminoacid deprivation, endoplasmic reticulum stress (ER stress), oxidative stress, and by the growth factor heregulin.[1]

ATF4 can function as a transcriptional activator, as well as a repressor. It is also a protective gene regulating the adaptation of cells to stress factors such as ER and oxidative stress, and a developmental gene, required for skeletal and eye development and haematopoiesis.[1]Some of the genes that are induced by ATF4 include receptor activator of nuclear factor-kappa B (RANK) ligand (RANKL), osteocalcin, E-selectin, VEGF, Gadd153, gadd34, asparagine synthetase, TRB3, and several genes involved in mitochondrial function, amino acid metabolism and redoxchemistry. ATF4 can induce osteoblast-specific gene expression in non-osteoblastic cells too, and therefore, ATF4 might have a role in bone metastasis. The accumulation of ATF4 in cancer modulating the RANK/RANKL system is a possibility since it has recently been reported that ATF4 is a regulator of RANKL. ATF4 regulates the synthesis of type I collagen, and could contribute to the establishment of benign tumours and cancer.[1]It is a stress-inducible subunit of several different bZIP transcription factors.

ATF4 may promote the growth and invasion of cancer cells partly by promoting the activation of various pathways. Activation of the endoplasmic reticulum (ER) stress and ER stress response, also known as the unfolded protein response (UPR), is common to various degenerative disorders. Therefore, signaling components of the UPR are currently emerging as potential targets for intervention and treatment of

human diseases. One UPR signaling member, activating transcription factor 4 (ATF4), has been found up-regulated in many pathological conditions, pointing to therapeutic potential in targeting its expression. In cells, ATF4 governs multiple signaling pathways, including autophagy, oxidative stress, inflammation, and translation, suggesting a multifaceted role of ATF4 in the progression of various pathologies. Therefore, it is determined that ATF4 expression is frequently up-regulated in various cancers like gastric cancer, esophageal squamous cell carcinoma, colorectal cancer, lung cancer, pancreatic cancer, breast cancer and hepatocellular cancer tissues compared with adjacent non-cancerous epithelial samples. Moreover, it is found that ATF4 overexpression correlated with the TNM stage and lymph node metastasis. In addition, positive ATF4 expression indicated poorer prognoses than negative ATF4 expression. These findings highlight the importance of ATF4 dysfunction in promoting tumor progression and metastasis and implicate it as a potential therapeutic target for treatment therapy.

The established role of ATF4 has been well documented in other human cancers, however it has not been attempted to explore the putative role of it in oral carcinogenesis and oral lesions. So, this systematic scoping review aims to give overview of the literature on the role of ATF4 through IHC studies and various methods like western blot analysis,double-labeling immunofluorescence, real-time quantitative PCR and to conclude its role in pathogenesis and prognosis of oral lesions.

METHODS

The literature search was carried out on the basis of role of ATF4 through IHC studies and other techniques like western blot analysis, double-labeling immunofluorescence and quantitative PCR technique in oral lesions from 2014 to 2022. The search is conducted by using search engines like PubMed, Google Scholar by using databases of PubMed , Scopus, and Web of Science using following terms :

INCLUSION CRITERIA

- Studies done on oral lesions.
- Studies published from 2014 to 2022.
- Human studies.
- Articles published in English language.

EXCLUSION CRITERIA

- Studies done on lesions other than oral.
- Surveys and questionnaire based studies.
- Review letters, personal opinions, book chapters and conference abstracts.
- Articles published in languages other than english.

Total articles retrieved from electronic search was 4, out of which articles meeting inclusion criteria was 3, with 2 elimination of duplicates. Based on this data, total articles evaluated for this systematic scoping review was 3. Two authors independently assessed each remaining full-text article to determine eligibility for inclusion in the study. Disagreement was resolved by consensus, and Institutional Board Review approval was not required.

RESULTS

Fig.1:

(((Head and neck squamous cell carcinoma*[MeSH Terms]) OR ("Head and neck squamous cell carcinoma"[Text Word])) OR ("Head and neck squamous cell carcinoma"[Title/Abstract])) OR (Squamous cell carcinoma of the head and neck[MeSH Terms])) OR ("Squamous cell carcinoma of the head and neck"[Text Word])) OR ("Squamous cell carcinoma of the head and neck"[Title/Abstract])) OR (Oral squamous cell carcinoma*[MeSH Terms])) OR ("Oral squamous cell carcinoma"[Text Word])) OR ("Oral squamous cell carcinoma"[Title/Abstract])) OR (Oral cavity squamous cell carcinoma*[MeSH Terms])) OR ("Oral cavity squamous cell carcinoma"[Text Word])) OR ("Oral cavity squamous cell carcinoma"[Title/Abstract])) OR (Squamous cell carcinoma of the mouth[MeSH Terms])) OR ("Squamous cell carcinoma of the mouth"[Text Word])) OR ("Squamous cell carcinoma of the mouth"[Title/Abstract])) OR (Oropharyngeal squamous cell carcinoma*[MeSH Terms])) OR ("Oropharyngeal squamous cell carcinoma"[Text Word])) OR ("Oropharyngeal squamous cell carcinoma"[Title/Abstract])) OR (Mouth neoplasm*[MeSH Terms])) OR ("Mouth neoplasm"[Text Word])) OR ("Mouth neoplasm"[Title/Abstract])) OR (Oral neoplasm*[MeSH Terms])) OR ("Oral neoplasm"[Text Word])) OR ("Oral neoplasm"[Title/Abstract])) OR (Cancer of mouth[MeSH Terms])) OR ("Cancer of mouth"[Text Word])) OR ("Cancer of mouth"[Title/Abstract])) OR (Mouth cancer*[MeSH Terms])) OR ("Mouth cancer"[Text Word])) OR ("Mouth cancer"[Title/Abstract])) OR (Oral cancer*[MeSH Terms])) OR ("Oral cancer"[Text Word])) OR ("Oral cancer"[Title/Abstract])) OR (Oral epithelial dysplasia*)) OR ("Oral epithelial dysplasia"[Text Word])) OR ("Oral epithelial dysplasia"[Title/Abstract])) OR (Oral lichen planus*[MeSH Terms])) OR ("Oral lichen planus"[Text Word])) OR ("Oral lichen planus"[Title/Abstract])) OR (Oral submucous fibrosis*[MeSH Terms])) OR ("Oral submucous fibrosis"[Text Word])) OR ("Oral submucous fibrosis"[Title/Abstract])) OR (Odontogenic cyst*[MeSH Terms])) OR ("Odontogenic cyst"[Text Word])) OR ("Odontogenic cyst"[Title/Abstract])) OR (Odontogenic keratocyst[MeSH Terms])) OR ("Odontogenic keratocyst"[Text Word])) OR ("Odontogenic keratocyst"[Title/Abstract])) OR (Oral leukoplakia*[MeSH Terms])) OR ("Oral leukoplakia"[Text Word])) OR (("Oral leukoplakia"[Text Word]) AND ("Oral leukoplakia"[Title/Abstract]))) OR (Ameloblastoma*[MeSH Terms])) OR ("Ameloblastoma"[Text Word])) OR ("Ameloblastoma"[Title/Abstract])) AND (((((((((("ATF4"[Text Word]) OR ("ATF4"[Title/Abstract])) OR ("Activating transcription factor - 4"[MeSH Terms])) OR ("Activating transcription factor- 4"[Text Word])) OR ("Activating transcription factor-4"[Title/Abstract])) OR (ATF4 gene*[MeSH Terms])) OR ("ATF4 gene"[Text Word])) OR ("ATF4 gene"[Title/Abstract])) OR (ATF4 Protein*[MeSH Terms])) OR ("ATF4 Protein"[Text Word])) OR ("ATF4 Protein"[Title/Abstract]))) AND (((((((((((Diagnosis*[MeSH Terms]) OR ("Diagnosis"[Text Word])) OR ("Diagnosis"[Title/Abstract])) OR (Prognosis*[MeSH Terms])) OR ("Prognosis"[Text Word])) OR ("Prognosis"[Title/Abstract])) OR ("Diagnostic marker"[Text Word])) OR ("Diagnostic marker"[Title/Abstract])) OR ("Prognostic marker"[Text Word])) OR ("Prognostic marker"[Title/Abstract])) OR ("Pathogenesis"[Text Word])) OR ("Pathogenesis"[Title/Abstract]))

Fig.2:

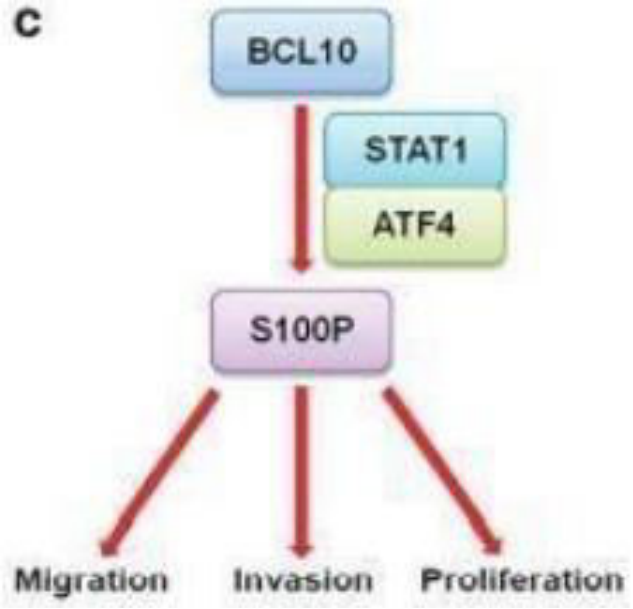
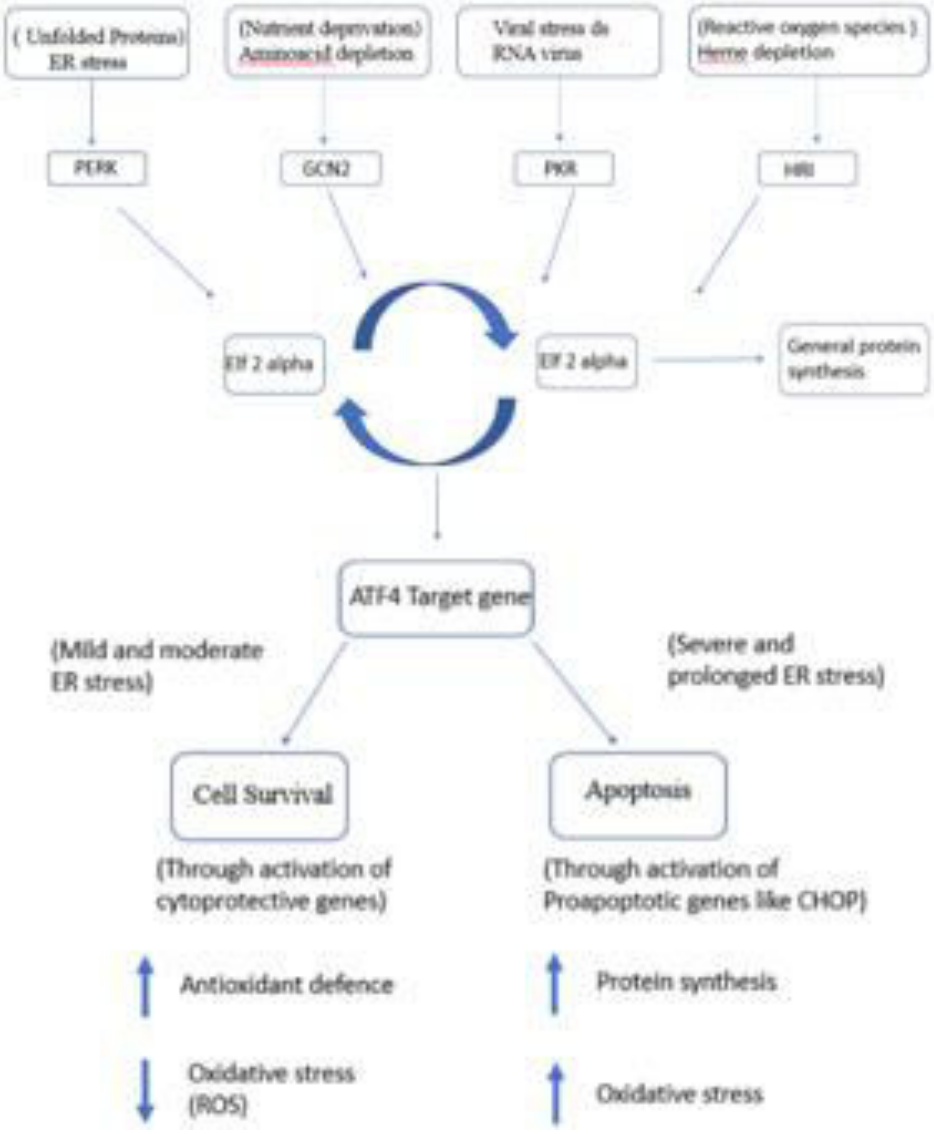

C
BCL10
STAT1
ATF4
S100P
Migration
Invasion
Proliferation

Fig.3:


(Unfolded Proteins)
ER stress
(Nutrient deprivation)
Amino acid depletion
Viral stress ds
RNA virus
(Reactive oxygen species)
Heme depletion
PERK
GCN2
PKR
HRI
EIF 2 alpha
EIF 2 alpha
General protein
synthesis
ATF4 Target gene
(Mild and moderate
ER stress)
(Severe and
prolonged ER stress)
Cell Survival
Apoptosis
(Through activation of
cytoprotective genes)
(Through activation of
Proapoptotic genes like CHOP)
Antioxidant defence
Protein synthesis
Oxidative stress
(ROS)
Oxidative stress

Sr. No.	Year	Author	Title	Oral Lesions/ Participants	Intervention	Immunoreactive areas of expression	Other study methods	Mode of action	Study results	Outcome/ Conclusion
1.	2014	H-H Chang[6] et al	B-cell lymphoma/leukemia 10 promotes oral cancer progression through STAT1/ATF4/S100P signaling pathway	Oral Squamous Cell Carcinoma (OSCC-93, Nomal oral mucosa-)	Immunohistochemical analysis with BCL10, S100P and NF-kB		1. Western blot analysis 2. Immunofluorescence analysis 3. MTT assay 4. Enzyme linked immunosorbent assay	Knockdown BCL10 significantly downregulated STAT1 and ATF4 levels on the S100P promoter region . In addition, S100P promoter activity significantly reduced in SAS/shBCL10 cells, and deletion of the STAT1/ATF4 binding site abolished S100P promoter activity. Together, these results demonstrated the importance of STAT1/ATF4 in BCL10-induced S100P induction.	Knocking down BCL10 expression significantly reduced cell migration, invasion and proliferation abilities.[3]	BCL10 upregulated its downstream effector S100P via direct binding to the STAT1/ATF4 sites, which led to attenuates OSCC migration, invasion and proliferation.[3]

Sr. No.	Year	Author	Title	Oral Lesions/ Participants	Intervention	Immunoreactive areas of expression	Other study methods	Mode of action	Study results	Outcome/ Conclusion
2.	2019	Wen-Qun Zhong et al	Elevated ATF4 Expression in Odontogenic Keratocysts Epithelia: Potential Involvement in Tissue Hypoxia and Stromal M2 Macrophage Infiltration	Odontogenic Keratocyst (OKC – 35 Normal Oral mucosa- 17)	Immunohistochemical analysis with ATF4, HIF alpha, and Mcsf	Positive staining of ATF4 as dense distribution of brown granules in cytoplasm throughout the epithelium, including the basal, superficial layer cells, and a small amount brown nuclear staining in the epithelial cells. The epithelium of OM samples barely had positive ATF4 staining.	1. Western blot analysis 2. Double-labeling Immunofluorescence 3. Real-time quantitative PCR 4. Hierarchical analysis	ATF4 is one of the stress responsive genes from ATF/CREB family. Under hypoxia, as the major transcriptional regulator in response to the Unfolded Protein Response (UPR), ATF4 can activate some genes to promote the restoration of ER function and survival of cells.	The expression of ATF4 was elevated and closely correlated with tissue hypoxia, M2-polarized macrophages infiltration, and angiogenesis in OKC.[4]	ATF4 could be activated by oxygen deprivation and playa critical role in the cell adaptation to hypoxia. ATF4 might further regulate transcriptions of relative genes in OKC and then promote cell survival and lesion growth.[4]

Sr. No.	Year	Author	Title	Oral Lesions/ Participants	Intervention	Immunoreactive areas of expression	Other study methods	Mode of action	Study results	Outcome/ Conclusion
3.	2022	Xin Wang[5] et al	Glaucocalyxin A impairs tumor growth via amplification of the ATF4/CHOP/CHAC1 cascade in human oral squamous cell carcinoma	Oral squamous cell carcinoma (OSCC-19, Normal Oral mucosa – 12)	Immunohistochemical analysis		1. Western blot analysis 2. Quantitative real time PCR 3. Bioinformatic analysis 4. Cell viability assay and colony formation assay	GLA promoted cell apoptosis by activating oxidative stress-related mitochondrial-mediated and ER stress-induced apoptotic pathways through the ATF4/CHOP/CHAC1 cascade mediated by ROS.	GLA could potently inhibit the OSCC growth *in vitro* and *in vivo* by inducing cell apoptosis through the ER stress-mediated ATF4/ CHOP/ CHAC1 cascade.[5]	GLA is a promising therapeutic agent which acts by activating the ATF4/ CHOP/ CHAC1 axis in OSCC patients.[5]

DISCUSSION

It has been shown that ATF4 is constitutively expressed in a wide variety of tissues including brain, heart, liver, spleen, thymus, lung and kidney, as well as in cell lines derived from T cells, B cells, monocytes and fibroblasts. It is suggested that ATF4

plays an important role in regulation of the high-level proliferation required during fetal-liver hematopoiesis, long-term memory, osteoblast differentiation, ER stress, amino acid deprivation and glucose metabolism etc. Consistent with the diversity of cellular processes reported to be regulatedby ATFs, the functions of ATFs are also diverse. ATFs play an important role in cell proliferation, apoptosis, differentiation and inflammation related pathological processes. The expression and phosphorylation status of ATFs are also related to neurodegenerative diseases and polycystic kidney disease. Various miRNAs target ATFs to regulate cancer proliferation, apoptosis, autophagy, sensitivity and resistance to radiotherapy and chemotherapy. Moreover, ATFs are necessary to maintain cell redox homeostasis.Therefore, deepening our understanding of the regulation and function of ATF4 will provide insights into the basic regulatory mechanisms that influence how cells integrate extracellular and intracellular signals into genomic responses through transcription factors.[2]

With a total of 3 articles included, this systematic scoping review attempted to rule out the role of ATF4 in oral lesions.

H-H Chang6 et al in the year 2014[3] performed a study in which they demonstrated BCL10 induced S100P expression through transcription factors signaling transducers and activators of transcription 1 and activating the transcription factor4 binding site in OSCC cells. The objective of this study was to elucidate the underlying mechanism of BCL10 in OSCC progression. The data showed that knockdown BCL10 significantly downregulated STAT1 and ATF4 levels on the S100P promoter region. In addition, they found that S100P promoter activity significantly reduced in SAS/shBCL10 cells, and deletion of the STAT1/ATF4 binding site abolished S100P promoter activity. Together, these results demonstrated the importance of STAT1/ATF4 in BCL10-induced S100P induction. They have previously demonstrated that BCL10 was overexpressed in advanced OSCC patients, which was correlated with TNM stage and survival rate. Herein, they reported that BCL10 promoted OSCC progression through the STAT1/ATF4/S100P/P65 signaling pathway. In conclusion, this study proposed that BCL10 upregulated its downstream effector S100P via direct binding to the STAT1/ATF4 sites, which led to attenuation of OSCC migration, invasion and proliferation abilities. Therefore, BCL10 could be regarded as a potential diagnostic or prognostic marker in OSCC patients in the future.

Wen-Qun Zhong et al in the year 2019[4] conducted a study in which , the expression levels of ATF4, HIF-1α, M-CSF, andRANKL in normal oral mucosa (OM) and OKC samples were detected by immunohistochemistry and the correlation between their expression levels was evaluated. The possible relationship between the expression levels of ATF4 and the infiltration of M2 macrophages and angiogenesis was also studied. to explore the relationships between HIF- 1α, ATF4, M- CSF, and M2-polarized macrophages, Spearman's rank correlation tests and linear regression analyses were performed. The results showed that there were positive correlations between the appearance of M2-polarized macrophages and the expression of HIF-1α, ATF4, and M-CSF respectively. Also, to verify the potential significance of ATF4 in angiogenesis in OKC, they investigated the correlations between MVD and the expression of ATF4 in OKC by using Spearman's rank correlation tests. Interestingly, the results showed that the expression level of ATF4 was significantly correlative with MVD in OKC samples. Collectively, these result implied that expression of ATF4 was closely associated with the presence of M2-polarizedmacrophages and angiogenesis in OKC. Similarly, the results from Western blot confirmed that hypoxia obviously upregulated the expression of ATF4 in a dose-dependent way. These results confirmed that hypoxia could enhance the expressions of ATF4 and M-CSF in epithelial cells.

In this study, they initially found the significant overexpression of ATF4 and its close correlation with hypoxia in the epithelial cells of OKC. Their data implicated that ATF4 could be activated by oxygen deprivation and play a critical role in the cell adaptation to hypoxia. ATF4 might further regulate transcriptions of relative genes in OKC and then promote cell survival and lesion growth. Based on these findings, they concluded the accumulation of ATF4 in the epithelium of OKC, and its potential association with M2-polarized macrophages infiltration and angiogenesis. Besides this; regulatory network, which involves ATF4 activation and ATF4-associated M-CSF upregulation, could be one of the pathophysiological mechanisms how OKC epithelium cells make response to hypoxia stress. In addition, their research also suggested the interaction between OKC epithelial cells and stromal cells (M2-polarized macrophages) occurred in the pathogenesis of OKC under the regulation of ATF4 in response to hypoxia.

Xin Wang et al in the year 2022[5] performed a study to elucidate the therapeutic potential and regulatory mechanisms of GLA in OSCC. The anti-tumor effect of GLA was investigated in vitro and in vivo to explore a new agent for improving therapeutic outcomes in OSCC. The results indicated that GLA notably induced cell apoptosis in OSCC. GLA could accumulate excessive ROS to activate mitochondria-mediated apoptosis in SCC25 and CAL27 cell lines. In addition, they discovered a new mechanism of GLA-induced ER-mediated apoptosis through the ATF4/CHOP/CHAC1 cascade using multiple methods.

Based on the findings, they hypothesize that GLA may promote cell apoptosis via amplification of the ER stress-excited ATF4/CHOP/CHAC1 cascade in OSCC. Therefore, it has been concluded that GLA promoted cell apoptosis by activating oxidative stress-

related, mitochondrial-mediated and ER stress-induced apoptotic pathways in OSCC and findings suggested that CHAC1 has critical roles in both GLA cytotoxicity and OSCC development, indicating that GLA is a promising therapeutic agent by activating the ATF4/CHOP/CHAC1 axis in OSCC patients.Under pathological conditions, especially in cancer biology and response to treatment, the characterization of ATF dysfunction is important for understanding how to therapeutically utilize ATF4 or other pathways controlled by transcription factors. In this systematic scoping review, we have demonstrated how ATF4 functions in promoting or suppressing cancer development and identify their role in tumour immunotherapy. There are several approaches to therapeutically target ATF4 for cancer therapy. First, inhibitors that interfere with ATF4 transcriptional function by blocking protein--protein or protein--DNA interactions or by regulating post-translational activation and degradation events. An alternative approach is to target the upstream eIF2a kinases and reduce overall ATF4 translation. Lastly, inhibition of pathways that are transcriptionally regulated by ATF4 may also be considered a valid approach for targeting ATF4-expressing cells.

CONCLUSION

ATF4 is an oxidative stress inducible transcription factor mainly regulated by HIF's in additionto oxidative stress, cellular stress like viral infection, ER stress and translation inhibition whichinduces a protection phenomenon by inhibiting protein synthesis, thus, helps in increase inATF4 expression. ATF4 acts as master regulator controlling the transcription of key genesessential for adaptive functions and especially modulates cellular gate in response to stress.Under chronic stress of sufficient magnitude, ATF4 induces apoptosis. Dysregulation in ATF4expression has been noticed in various carcinomas of human body. Several molecular pathwaysof tumorigenesis depict ATF4 as important signaling molecule and transcription factor incarcinogenesis. Though a little knowledge is available in case of role of ATF4 in oral lesions/malignancy, potential role of the same cannot be neglected in terms of its active involvementin various signaling pathways in development of odontogenic cysts and oral cancer. Anexclusive study conducted on ATF4 expression in OKCs indicated its potential inetiopathogenesis by facilitating M2 macrophage infiltration through hypoxia induced ATF4activation. Few in vitro studies on oral squamous cell carcinoma suggested the promising roleof ATF4 in oral carcinogenesis through its direct and indirect role in apoptosis regulatorymechanism. Lack of experimental and clinical studies put limitation to explore ATF4-centeredregulation network in pathogenesis of oral and maxillofacial pathologies.

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