INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

Bioinformatic Analysis of CPEB3 in Head and Neck Squamous Cell Carcinoma via mTOR Pathway and its microRNA Targets

Y Dinesh[1]*, Karthikeyan Ramalingam[2], Pratibha Ramani[2], Selvaraj Jayaraman[3]

1 Postgraduate student, Department of Oral and Maxillofacial Pathology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, India
2 Professor and Head, Department of Oral and maxillofacial Pathology, Saveetha Dental College and Hospital , Saveetha Institute of Medical and Technical Sciences, Chennai, India
3 Professor, Department of Biochemistry, Saveetha Dental College and Hospital , Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

OPEN ACCESS

Received: 11-01-2023
Accepted: 06-05-2023
Published: 24-06-2023

Citation: Dinesh Y, Ramalingam K, Ramani P, Jayaraman S (2023) Bioinformatic Analysis of CPEB3 in Head and Neck Squamous Cell Carcinoma via mTOR Pathway and its microRNA Targets. Indian Journal of Science and Technology 16(24): 1795-1801. https://doi.org/10.17485/IJST/v16i24.76

* **Corresponding author**.

drdineshdentist@gmail.com

Funding: None

Competing Interests: None

Published By Indian Society for Education and Environment (iSee)

ISSN
Print: 0974-6846
Electronic: 0974-5645

Abstract

Objectives: To analyse CPEB3 using bioinformatic tools in several types of cancer, various HNSCC cell lines based on cancer stages, tumour grades and the gene interaction, protein interaction, regulating pathway, microRNA and survival analysis. **Methods:** Bioinformatics tools used were UALCAN, TCGA, NCBI, GEPIA, Oncomine, cBioPortal, Human Protein Atlas, STRING, Metascape, miRNA Target Prediction Database. **Findings:** CPEB3 gene expression was considerably reduced in 520 HNSCC samples. Given that CPEB3 gene expression was downregulated with pathological grading grade 1 (n=62), grade 2 (n=303), grade 3 (n=125) and all 4 stages of HNSCC, this suggests that CPEB3 may be useful for determining the prognosis of HNSCC. CPEB3 gene and protein interaction revealed the high interaction with related proteins. CPEB3 expression in HNSCC causes negative regulation of cytoplasmic translation, cellular response to amino acid stimulus and downregulation of cell population proliferation. Significant low expression of CPEB3 on HNSCC patient survival p=0.013($p<0.05$). CPEB3 expression was lower in HNSCC patients with an altered mTOR pathway relative to healthy controls. miRDB results showed miR-184-5p and miR-21-5p regulate the expression of CPEB3 with corresponding target scores of 98 and 92. **Novelty:** This study is the first of its kind to utilise bioinformatic tools for analysis of the role of CPEB3 in several types of cancer and its related biological processes. Although there are several in silico studies on cancer, this study has involved several molecular biological processes and its impact on HNSCC. Further molecular studies based on gene knockout studies are warranted to consider CPEB3 as a potential target or a biomarker in HNSCC

Keywords: CPEB; HNSCC; mTOR; microRNA; insilico; Bioinformatics

1 Introduction

Head and neck squamous cell carcinoma (HNSCC) contributes a high number of cancer and mortality to the patients every year. GLOBOCAN data showed the prevalence of HNSCC is expected to rise by 30% by 2030. Surprisingly, despite the advancements in technology, the 5-year overall survival rate for HNSCC is poor and especially for patients with advanced HNSCC[1]. Recently several studies are done for better understanding of carcinogenesis of HNSCC in molecular level and utilise potential biomarkers for early diagnosis, therapeutic and post treatment monitoring in HNSCC[2,3]. There are several hypotheses put forth based on hallmarks of cancer, however several researchers are still working on a single or a panel of molecular biomarkers in HNSCC. CPEB is one such protein which is not widely studied in respect to HNSCC but has shown promising involvement in other sites involving cancer.

CPEB is a sequence-specific, highly conserved RNA-binding protein that regulates the translational activation and cytoplasmic polyadenylation of target messenger RNAs[4]. The CPEB family of proteins all share a similar structure, consisting of two RNA recognition motifs and a zinc finger domain necessary for RNA binding, with widely variable N-termini and somewhat conservative C-termini[5]. The CPEB family, which is highly expressed in vertebrates, consists of four members (CPEB1-CPEB4), with CPEB1 differing from CPEB2, CPEB4 in terms of binding specificity and regulatory domains[6,7]. CPEBs are related with several biological processes, including cell cycle progression, development, cellular senescence, and the evolution of malignant tumours. CPEB family binds with a particular region in the 3′ UTR of mRNA and regulates protein translation. Multiple CPEB-regulated mRNAs control the course of the apoptosis, cell cycle, mitosis and senescence[8].

In glioblastomas and pancreatic ductal adenocarcinomas, the direct relationship between abnormal expression of CPEBs and carcinogenesis has been shown[9]. CPEB3 modulates cytoplasmic polyadenylation in cancer cells and its expression was reduced in colorectal cancer hence it is regarded as a tumour suppressor[10,11]. CPEBs are found to be closely associated in carcinogenesis pancreatic, colorectal and neural benign and malignant tumours. The lacunae in the existing literature on whether CPEB3 is involved in the carcinogenesis of HNSCC. The aim of the study is to analyse the insilico association of CPEB3 in several types of cancer, various HNSCC cell lines based on cancer stages, tumor grades and the gene interaction, protein interaction, regulating pathway, microRNA and survival analysis.

2 Methodology

This proposed protocol points out the expression levels of CPEB3 in various cancers from the TCGA dataset, and the expression level of CPEB3 in different Head and neck cancer cell lines. Following the specificity in the presence of CPEB3 in HNSCC, a comparative with normal group and inter group analysis between different grading and stages of HNSCC. The gene and protein interaction of CPEB3 for potential target in HNSCC and gene ontology study to reveal the mechanism of CPEB3. Role of CPEB3 expression in HNSCC patient survival and potential role via mTOR pathway was further assessed.

Several bioinformatic tools have been used to analyse the role and function of CPEB3 in HNSCC. Bioinformatics tools used were TCGA, NCBI, Gene Expression Profiling Interactive Analysis (GEPIA), Oncomine, UALCAN, cBioPortal, Human Protein Atlas, STRING, Metascape, miRNA Target Prediction Database.

TCGA data: GEPIA tool makes it easier to extract data from the repository of expression data obtained via RNA sequencing. There are 33 different types of cancer included in this collection, which was compiled with the use of samples from The Cancer Genome Atlas (TCGA). We used GEPIA to evaluate the amounts of CPEB3 expression in a variety of malignant tissues as well as the importance of CPEB expression in terms of the patient's prognosis

The Oncomine database, which can be accessed at www.oncomine.org, provides cancer genetic data by integrating RNA and DNA-seq data GEO and the TCGA as well as data from published literature sources. Oncomine is often utilised in the process of detecting new biomarkers. Oncomine analysis was done to identify differentially expressed target genes and direct the direction of future research by identifying genes whose expression levels varied in various cancers.

UALCAN analyses the relative expression of a CPEB3 between HNSCC and normal samples using TCGA data from 31 different forms of cancer. In addition, the software is able to analyse various tumour sub-groups based on cancer stages, tumour grades, etc. (Based on the results of the student's t-test) a significance level of 0.05 was determined.

The genetic alterations CPEB3 in HNSCC patients were assessed by cBioPortal. Protein–protein interaction (PPI) network involving CPEB3 using the STRING database (https://string-bd.org/). In addition to that, we made use of Metascape (http://metascape.org) to do an analysis on the CPEB3 pathway and process enrichment. In the current investigation, the CPEB3 protein network by successfully combining these several techniques such as String and Metascape. Cytoscape was used for visualisation, while Metascape was utilised for annotation and integration of data from the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. GO enrichment analysis allows for the prediction of the functional functions that are related with CPEB3 mutations as well as genes that are strongly connected with CPEB3.

The Human Protein Atlas https://www.proteinatlas.org uses a variety of omics technologies, using this publicly available database, tumour-specific proteins were identified and compared CPEB3 protein expression between normal and HNSCC tissues in individuals.

The GEO database developed by the National Center for Biotechnology Information (NCBI) gives data on differential expression. In the current study several dysregulated microRNAs in HNSCC using this tool. The microRNA expression profiling data of more than 1000 cell lines were available in miRNA Target Prediction Database (miRDB). Prediction of HNSCC target miRNAs: To investigate the target miRNAs of CPEB3 in HNSCC, we utilised the online miRNA prediction tool miRDB (htt p://www.mirdb.org/miRDB/). The target microRNAs associated with the overall survival of HNSCC were discovered by the aforementioned rigorous research procedure.

3 Results and Discussion

The transcription and translation of CPEB3 in HNSCC data were analysed using the TCGA dataset, researchers analysed the expression levels of CPEB3 as well as the patterns of its mRNA expression in 17 different types of cancer (Figure 1). CPEB3 expression in 33 Head and Neck Cancer cell lines showed BIR11 found highest in BIR11 cell lines (Figure 2). When compared to normal samples, primary HNSCC tissues showed a substantial decrease in CPEB3 expression levels (p 0.05) (Figure 3). This included determining the cancer stage and tumour grade. We discovered that the mRNA down expression of CPEB3 had a strong correlation with the HNSCC cancer grades and stages (Figures 4 and 5). According to the findings of this study, patients whose cancer has progressed significantly are less likely to express CPEB3 at a higher level.

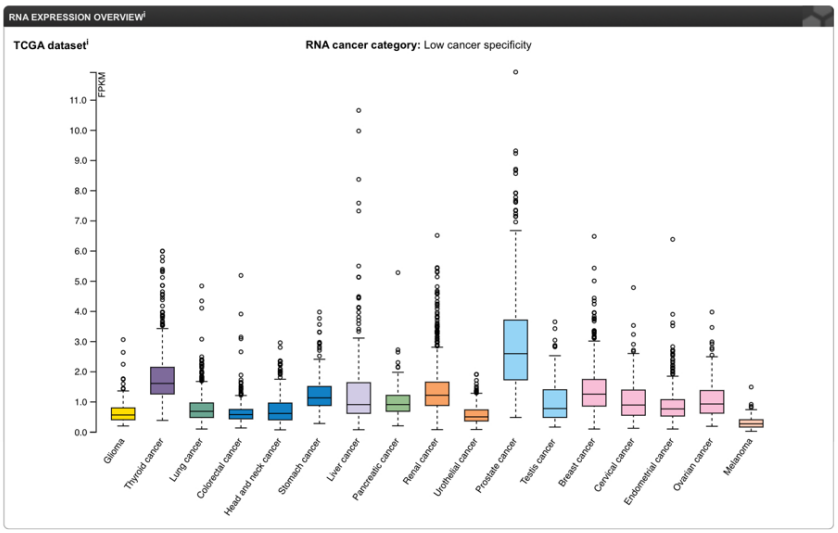

Fig 1. TCGA dataset of CPEB3 expression in various cancers

NEURL1 and IPO5 genes had high interaction with CPEB3 (Figure 6 a). CNOT series and OR4K15 protein interaction were found with CPEB3 protein (Figure 6 b). CPEB3 expression in HNSCC causes negative regulation of cytoplasmic translation, cellular response to amino acid stimulus and downregulation of cell population proliferation (Figure 7). CPEB3 was also significantly associated with p=0.013 (p<0.05) with a Overall survival in the UALCAN database to estimate the prognostic significance of CPEB3 in HNSCC patients (Figure 8).

CPEB3 via mTOR pathway: CPEB3 role in HNSCC, we investigated whether mTOR pathway is involved in CPEB3 using UALCAN. CPEB3 expression was lower in HNSCC patients with an altered mTOR pathway relative to healthy controls (Figure 9). miRDB results showed that CPEB3 is controlled by several miRNAs: microRNA-184-5p and microRNA-21-5p regulate the expression of CPEB3 with corresponding target scores of 98 and 92.

In recent years, standard clinicopathological signs have been shown to be insufficient for the early diagnosis of HNSCC as well as the prediction of its prognosis. Therefore, several studies are being done to identify potential biomarkers for early detection and post treatment monitoring of the HNSCC patients[12]. To this day, several studies have been carried out in individuals diagnosed with HNSCC in an effort to uncover prognostic biomarkers. Both types of biomarkers have their advantages and disadvantages[13].

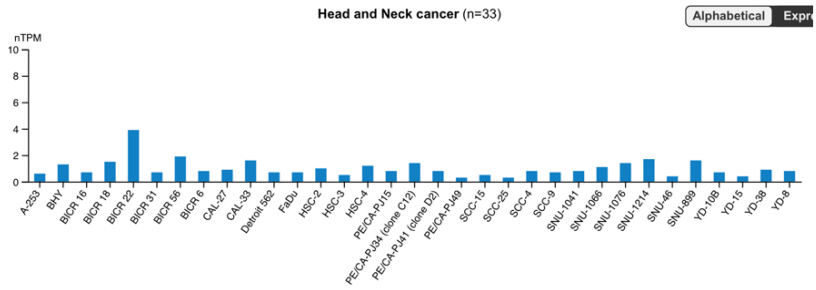

Fig 2. CPEB3 expression in various Head and Neck Cancer cell lines

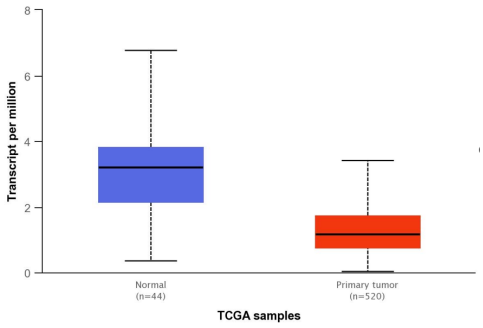

Fig 3. Expression of CPEB3 in HNSCC and healthy samples

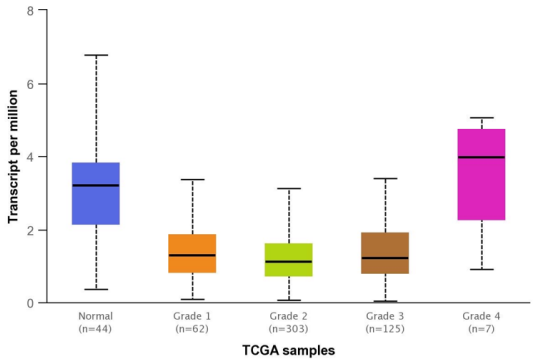

Fig 4. Expression of CPEB3 in grades of HNSCC and healthy samples

Downregulation of CPEB3 is found predominantly in several cancer types because of its cytoplasmic polyadenylation. The development and malignancy of glioma were shown to have a favourable correlation with the expression of CPEB3, as was discovered by Skubal et al[14]. Glioma patients showed negative correlation of CPEB3 with survival of patients but positive correlation with cancer proliferation. Tang et al[14,15] suggested that epigenetic alteration of miR-452-3p downregulated CPEB3 via EGFR pathway which led to anti apoptosis and invasion of cancer cells in the liver. When compared to normal tissues, the expression of CPEB3 was shown to be considerably reduced in melanoma. Indeed, higher levels of CPEB3 expression were linked to improved overall survival rates among melanoma patients.

In addition to this, CPEB3 prevents the proliferation of colorectal cancer cells, but inhibiting the protein leads to an increase in the rate of cell proliferation both *in vitro* and in animals lacking athymic nuclei[16]. Additionally, colorectal cancer cells showed an increase in their ability to migrate and invade when their CPEB3 levels were lowered, but raising their CPEB3 levels

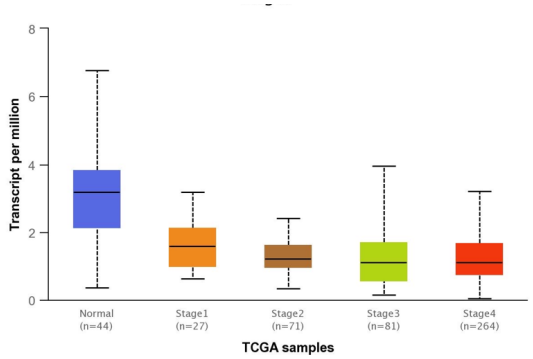

Fig 5. Expression of CPEB3 in different stages of HNSCC and healthy samples

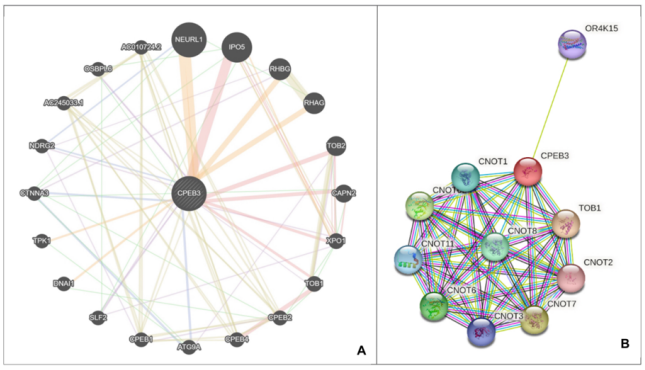

Fig 6. 6A:image represents interaction of CPEB3 with genes; 6B: image represents the protein interaction of CPEB3

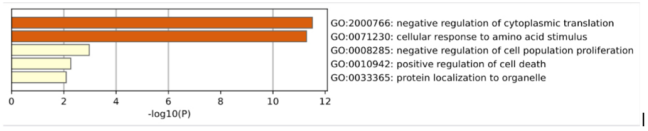

Fig 7. CPEB3 expression in HNSCC causes negative regulation of cytoplasmic translation, cellular response to amino acid stimulus and downregulation of cell population proliferation

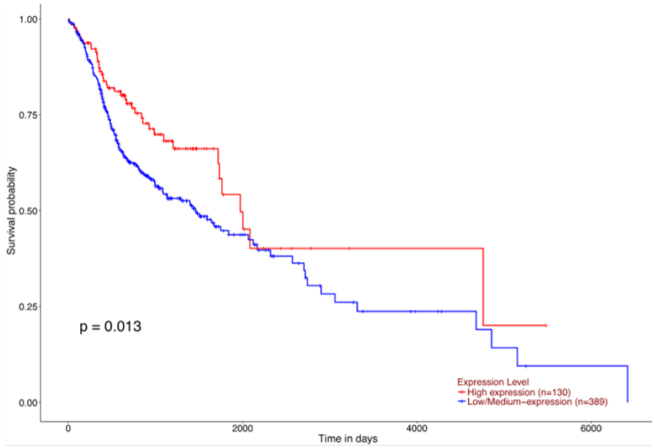

Fig 8. Effect of CPEB3 expression level on survival of patients with HNSCC p=0.013 (p<0.05)

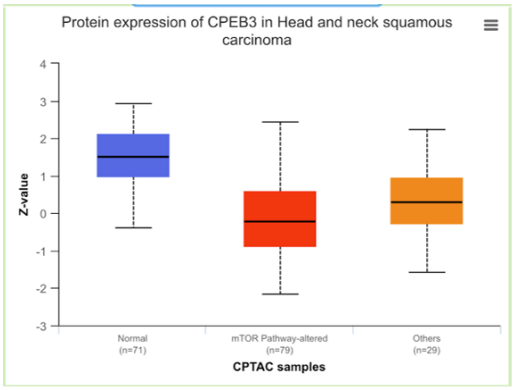

Fig 9. The graph represents protein expression of CPEB3 in Normal and mTOR pathway altered HNSCC samples

caused the reverse effect[17]. The conclusion that CPEB3 acts as a tumour suppressor in the advancement of colorectal cancer is supported by these findings, which are based on clinical data. Previous study has revealed that CPEB3 prevents the growth of hepatocellular carcinoma cells as well as their ability to metastasize, which is in agreement with our findings[18,19].

Recent developments in large-scale quantitative approaches, in particular next-generation sequencing and contemporary protein mass spectrometry, have made it easier to identify RNA binding proteins, their protein cofactors, and the RNA targets that they bind to throughout the whole genome. Numerous RNA binding proteins are shown to bind to thousands of RNA targets within cells at specific binding locations, as was revealed by research that utilised these approaches. In this paper, we present the profile of RNA that directly binds to CPEB3 by using the RIP-sequence technique. CPEB3, like other RNA-binding proteins, binds to over a thousand different RNAs. CPEB3 is also connected with signalling pathways like mTOR that are essential to the cancer progression. These pathways include the cellular stress response, cellular protein metabolism, and the cell cycle[20].

This study is first of its kind to analyse the CPEB3 in progression of HNSCC tumours. The results suggest that CPEB3 is vital in the initiation and progression of HNSCC tumours; therefore, additional research is urgently required. Thus, it is hypothesised that CPEB3, by downregulating CPEB3 gene expression and influencing the mTOR pathway, may be involved in the tumorigenesis of HNSCC. This result requires additional research in a subsequent study to confirm the underlying mechanisms involved.

The limitations of the study is that only in silico has been done in this study, only limited genes and protein were considered for interaction. Molecular docking and potential targets can be further assessed. This study has been limited to in silico analysis only. The future scope of research includes computational biology based research using multi omics data on cancer for prognosis and prediction of overall survival and disease free survival. Similar studies can be done on OPMDs for predicting malignant transformation. Invitro and clinical studies using patient samples need to be done in large population for accessing sensitivity and specificity of the marker.

4 Conclusion

In conclusion, this novel study provides insights on how CPEB3 gene expression was considerably reduced in 520 HNSCC samples. Given that CPEB3 gene expression was downregulated with pathological grading grade 1 (n=62), grade 2 (n=303), grade 3 (n=125) and all 4 stages of HNSCC, this suggests that CPEB3 may be useful for determining the prognosis of HNSCC. CPEB3 gene and protein interaction revealed the high interaction with related proteins. CPEB3 expression in HNSCC causes negative regulation of cytoplasmic translation, cellular response to amino acid stimulus and downregulation of cell population proliferation. Significant low expression of CPEB3 on HNSCC patient survival p=0.013($p<0.05$). Through the mTOR signalling pathway, CPEB3 is associated with many key pathways in HNSCC, resulting in cancer carcinogenesis. miRDB results showed miR-184-5p and miR-21-5p regulate the expression of CPEB3 with corresponding target scores of 98 and 92. Further molecular studies based on gene knockout studies are warranted to consider CPEB3 as a potential target or a biomarker in HNSCC.

References

1) Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *A Cancer Journal for Clinicians*. 2022;72(1):7–33. Available from: https://doi.org/10.3322/caac.21708.
2) Sarode SC, Gondivkar S, Sarode GS, Gadbail A, Yuwanati M. Hybrid oral potentially malignant disorder: A neglected fact in oral submucous fibrosis. *Oral Oncology*. 2021;121:105390. Available from: https://doi.org/10.1016/j.oraloncology.2021.105390.
3) Sridharan G, Ramani P, Patankar S, Vijayaraghavan R. Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma. *Journal of Oral Pathology & Medicine*. 2019;48(4):299–306. Available from: https://doi.org/10.1111/jop.12835.
4) Duran-Arqué B, Cañete M, Castellazzi CL, Bartomeu A, Ferrer-Caelles A, Reina O, et al. Comparative analyses of vertebrate CPEB proteins define two subfamilies with coordinated yet distinct functions in post-transcriptional gene regulation. *Genome Biology*. 2022;23(1). Available from: https://doi.org/10.1186/s13059-022-02759-y.
5) De Mingo DR, Pantoja-Uceda D, Hervás R, Carrión-Vázquez M, Laurents DV. Conformational dynamics in the disordered region of human CPEB3 linked to memory consolidation. *BMC Biology*. 2022;20(1):129. Available from: https://doi.org/10.1186/s12915-022-01310-6.
6) Ivshina MP, Van 't Spijker HM, Jung S, Ponny SR, Schafer DP, Richter JD. CPEB1 regulates the inflammatory immune response, phagocytosis, and alternative polyadenylation in microglia. *Glia*. 2022;70(10):1850–1863. Available from: https://doi.org/10.1002/glia.24222.
7) Wang G, Ji X, Li PX, Wang W. Human bone marrow mesenchymal stem cell-derived exosomes containing microRNA-425 promote migration, invasion and lung metastasis by down-regulating CPEB1. *Regenerative Therapy*. 2022;20:107–116. Available from: https://doi.org/10.1016/j.reth.2022.03.007.
8) Zeng W, Yue L, Lam KSW, Zhang W, So WKK, Tse EHY, et al. CPEB1 directs muscle stem cell activation by reprogramming the translational landscape. *Nature Communications*. 2022;13(1):947. Available from: https://doi.org/10.1038/s41467-022-28612-1.
9) Kozlov E, Shidlovskii YV, Gilmutdinov R, Schedl P, Zhukova M. The role of CPEB family proteins in the nervous system function in the norm and pathology. *Cell & Bioscience*. 2021;11(1). Available from: https://doi.org/10.1186/s13578-021-00577-6.
10) Chen J, Li L, Liu TYY, Fu HFF, Lai YHH, Lei X, et al. CPEB3 suppresses gastric cancer progression by inhibiting ADAR1-mediated RNA editing via localizing ADAR1 mRNA to P bodies. *Oncogene*. 2022;41(41):4591–4605. Available from: https://doi.org/10.1038/s41388-022-02454-z.
11) Wang H, Peng D, Gan M, He Z, Kuang Y. CPEB3 overexpression caused by miR-106b-5p inhibition inhibits esophageal carcinoma in-vitro progression and metastasis. *Anti-Cancer Drugs*. 2022;33(4):335–351. Available from: https://doi.org/10.1097/CAD.0000000000001265.
12) Jeevanandan G, Thomas E. Volumetric analysis of hand, reciprocating and rotary instrumentation techniques in primary molars using spiral computed tomography: An in vitro comparative study. *European Journal of Dentistry*. 2018;12(01):021–026. Available from: https://doi.org/10.4103/ejd.ejd_247_17.
13) Ponnulakshmi R, Shyamaladevi B, Vijayalakshmi P, Selvaraj J. In silico and in vivo analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. *Toxicology Mechanisms and Methods*. 2019;29(4):276–290. Available from: https://doi.org/10.1080/15376516.2018.1545815.
14) Skubal M, Gielen GH, Waha A, Gessi M, Kaczmarczyk L, Seifert G, et al. Altered splicing leads to reduced activation of CPEB3 in high-grade gliomas. *Oncotarget*. 2106;7(27):41898–41912. Available from: https://doi.org/10.18632/oncotarget.9735.
15) Tang H, Han X, Feng Y, Hao Y. linc00968 inhibits the tumorigenesis and metastasis of lung adenocarcinoma via serving as a ceRNA against miR-9-5p and increasing CPEB3. *Aging*. 2020;12(22):22582–22598. Available from: https://doi.org/10.18632/aging.103833.
16) Fang Y, Zhong Q, Wang Y, Gu C, Liu S, Li A, et al. CPEB3 functions as a tumor suppressor in colorectal cancer via JAK/STAT signaling. *Aging*. 2020;12(21):21404–21422. Available from: https://doi.org/10.18632/aging.103893.
17) Zhong Q, Fang Y, Lai Q, Wang S, He C, Li A, et al. CPEB3 inhibits epithelial-mesenchymal transition by disrupting the crosstalk between colorectal cancer cells and tumor-associated macrophages via IL-6R/STAT3 signaling. *Journal of Experimental & Clinical Cancer Research*. 2020;39(1):132. Available from: https://doi.org/10.1186/s13046-020-01637-4.
18) Alsawalha M, Rao CV, Al-Subaie AM, Haque SM, Veeraraghavan VP, Surapaneni KM. Novel mathematical modelling of Saudi Arabian natural diatomite clay. *Materials Research Express*. 2019;6(10):105531. Available from: http://dx.doi.org/10.1088/2053-1591/ab2f9b.
19) Shree KH, Shree H, Ramani K, P, Sherlin H, Sukumaran G, et al. Saliva as a Diagnostic Tool in Oral Squamous Cell Carcinoma - a Systematic Review with Meta Analysis. *Pathology & Oncology Research*. 2019;25:447–453. Available from: http://dx.doi.org/10.1007/s12253-019-00588-2.
20) Hannah RH, Ramani R, Ramanathan P, Jancy A, Gheena MR, S. CYP2 C9 polymorphism among patients with oral squamous cell carcinoma and its role in altering the metabolism of benzo[a]pyrene. 2020. Available from: http://dx.doi.org/10.1016/j.oooo.2020.06.021.