Expression Dynamics of Metastasis-Associated Markers in Oral Squamous Cell Carcinoma Cell Lines CAL27 and HSC-3

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Abstract

Metastasis remains the major cause of mortality in head and neck squamous cell carcinoma (HNSCC), driven by complex molecular alterations regulating proliferation and invasion. Building on prior bioinformatic predictions, this study experimentally validated nine hub genes CDC45, MCM5, ASF1B, RFC4, E2F1, TK1, CHTF18, CENPM and CDCA3 implicated in metastatic progression. Using quantitative real-time PCR, expression profiles were compared between metastatic HSC-3 and non-metastatic CAL-27 HNSCC cell lines. Five genes (MCM5, CDC45, E2F1, RFC4 and CENPH) were significantly upregulated in HSC-3, exhibiting 2.3–6.6-fold increases ($p < 0.01$), while ASF1B, CHTF18, TK1 and CDCA3 were downregulated.

The elevated expression of replication-associated genes underscores the role of aberrant DNA synthesis and cell-cycle progression in promoting metastatic competence. These results offer experimental validation of metastasis-related genes identified through bioinformatic analysis, underscoring MCM5, CDC45, E2F1, RFC4 and CENPH as promising prognostic indicators and potential therapeutic targets in HNSCC.

Keywords: Metastasis, Cell Carcinoma, Genes.

Introduction

Metastatic dissemination in cancers like head and neck Squamous Cell Carcinoma (HNSCC) continues to be a major factor influencing patient prognosis and treatment outcomes. Despite advances in surgical intervention and targeted therapy, metastasis is frequently associated with treatment refractoriness and poor survival, highlighting an urgent need to dissect the molecular hallmarks that distinguish metastatic from non-metastatic tumors. Such hallmarks include changes in transcriptional programs, alterations in cell signaling pathways and shifts in tumor cell phenotypes that together orchestrate the dissemination and colonization of cancer cells at distant sites[4].

A growing body of work has implicated cancer stem cells (CSCs) and epithelial–mesenchymal transition (EMT) programs in initiating metastatic colonization. These programs confer tumor cells with traits such as self-renewal capacity and enhanced adaptability to microenvironmental stresses, enabling them to seed new tumor foci at remote anatomical locations. There is compelling experimental evidence that disseminated tumor cells (DTCs) persisting in a CSC-like state are most competent for establishing secondary lesions[7]. The interplay between these cellular states and molecular drivers, including gene regulatory networks, appears to define the aggressiveness of the disease.

Our previous bioinformatics study, utilizing mRNA-based stemness index (mRNAsi) and Weighted Gene Co-expression Network Analysis on transcriptomic data from the TCGA[13]. HNSCC cohort, successfully identified a panel of nine key genes[20]. These hub genes CDC45, MCM5, ASF1B, RFC4, E2F1, TK1, CHTF18, CENPM and CDCA3 were found to be significantly associated with cancer stem cells and metastatic behavior in HNSCC[20]. All nine genes were observed to be up-regulated in HNSCC samples compared to normal tissues. These genes are implicated in various oncogenic pathways known to facilitate metastatic progression. For instance, CDC45, a gene crucial for DNA replication, is linked to cancer metastasis and CSC-like behavior, with increased expression associated with larger tumor size and advanced stage in other cancers[10,20].

MCM5, another component of the replicative helicase, also shows moderate to high expression in HNSCC[3,5,20]. ASF1B has been reported to promote cell proliferation, migration and invasion by modulating the P53-regulated epithelial–mesenchymal transition (EMT) signaling pathway across different cancer types[18,20,21,26]. RFC4, a key component of the DNA replication machinery, contributes to enhanced metastatic potential and stemness characteristics[9,20,24,25] whereas E2F1 functions as a pivotal regulator of genes driving metastasis in human breast cancer[6,8,20]. CDCA3 is involved in promoting tumorigenicity and metastasis[20,22]. TK1 contributes to metastasis in melanoma and breast cancer by facilitating tumor cell spread[2,20,29]. Furthermore, CHTF18, a DNA replication-involved protein, has shown prognostic significance in HNSCC patients[16,20] and CENPM facilitates metastatic progression by activating the mTOR/p70S6K signaling pathway[20,26,28].

To experimentally validate the differential expression patterns of these nine candidate genes and further elucidate their relevance in HNSCC metastasis, we conducted real-

time Polymerase Chain Reaction analysis. This study utilized two distinct HNSCC cell lines: CAL-27, representing a non-metastatic phenotype[1] and HSC-3, characterized by its metastatic capabilities[11,17]. This comparative study seeks to validate the differential expression of these genes *in vitro*, thereby reinforcing their potential as biomarkers or therapeutic targets for HNSCC metastasis.

Material and Methods

Cell lines and culture conditions: HNSCC cell lines, Cal-27 (originating from tongue carcinoma with low metastatic potential) and HSC-3 (originating from tongue carcinoma with high metastatic potential), were kindly provided by Dr. Amritha Suresh, Mazumdar Shaw Medical Foundation (MSMF), Bengaluru, India. Both cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM; Cat. No. AL007A, HiMedia, USA) supplemented with 10% fetal bovine serum (FBS; Cat. No. RM10434, HiMedia, India) and 1% penicillin–streptomycin (Cat. No. 15070063, Gibco, UK), under standard conditions at 37 °C in a humidified atmosphere containing 5% CO_2.

RNA extraction and cDNA conversion: Total RNA from the cell lines was extracted using NucleoSpin RNA, Mini kit for RNA purification (MN, 740955.50). RNA integrity was checked electrophoretically and quantified spectrophotometrically. For cDNA conversion, PrimeScript cDNA Synthesis Kit (TaKaRa Bio Inc. Cat: 6110A) was used. RNA was mixed with Oligo dT primers (50 µM) and RNase free dH_20 and incubated for 5 min at 65 ^{0}C. A reaction mixture was prepared using 5X PrimerScript buffer, RNase Inhibitor (20 U), PrimeScript RTase (200 U) along with RNA primer mixture. This reaction mixture was incubated for 30 min at 42 ^{0}C followed by 5 min at 95 ^{0}C and the final product stored at -20 ^{0}C.

Primer designing: Primers for the selected HNSCC marker genes were designed *in silico* using NCBI Primer-BLAST. Full-length mRNA reference sequences (RefSeq) for each gene were retrieved from the NCBI database and exon–exon junctions were preferentially targeted to avoid genomic DNA amplification. Primers were designed with the following parameters: length of 20–24 base pairs, GC content between 40–60%, melting temperature (Tm) ranging from 57–64 °C and an expected amplicon size of 80–200 bp to ensure optimal amplification efficiency in real-time PCR. Each primer pair was evaluated for self- and cross-dimer formation, 3′ complementarity, GC clamp and secondary structure stability using IDT OligoAnalyzer and NEB Tm Calculator.

Primers exhibiting strong secondary structures or high dimerization potential were excluded. Sequence specificity was confirmed by BLASTn analysis against the human genome (GRCh38), ensuring a single amplification product for each gene. The final selected primer pairs for markers such as CDC45, MCM5, ASF1B, RFC4, E2F1, TK1, CHTF18, CENPM and CDCA3[20] demonstrated ideal thermodynamic characteristics (Tm ~57–63 °C, GC ≈ 45–50%) with no evidence of primer-dimer formation. Primers were synthesized by a commercial oligonucleotide manufacturer (Barcode Biosciences, India).

Real-Time PCR and statistical analysis: qPCR was performed using DTI Green HiFid Taq HS Premix (DT0602, DSS TaKaRa Bio, India) SYBR-style master mix. Each reaction was carried out in a 20 µL volume containing 10 µL of DTI Green HiFid 2× Master Mix, 0.4 µL of forward primer (10 µM stock; final concentration 200 nM), 0.4 µL of reverse primer (10 µM stock; final concentration 200 nM), 2.0 µL of diluted cDNA template and nuclease-free water to make up the final volume. All reactions were performed in triplicate for each gene. Negative template controls (NTC) and negative reverse-transcriptase controls (No-RT) were included on each plate to monitor contamination and genomic DNA amplification.

Reactions were run on a BioRad CFX-96, real-time PCR instrument (user's lab instrument) with the following program: initial denaturation at 95 °C for 2 min, followed by 40 cycles of 95 °C for 5 s (denaturation) and 55.3 °C for 30 s (combined annealing/extension). After amplification, a dissociation (melt) curve was recorded from 65 °C to 95 °C with 0.5 °C increments to confirm single-product amplification.

Table 1
Relative mRNA expression of hub genes in HSC-3 versus Cal-27 cell lines

Gene	Mean ΔCt (HSC-3)	Mean ΔCt (Cal-27)	ΔΔCt (HSC vs Cal)	Fold Change (2^{-}ΔΔCt)	*p*-value
CHTF18	8.21	7.45	0.76	0.59	**0.0076**
CENPH	9.15	10.34	-1.19	2.28	**0.0058**
RFC4	4.46	5.94	-1.48	2.79	**0.0002**
TK1	10.08	9.75	0.33	0.80	*0.0461*
ASF1B	17.40	15.05	2.35	0.20	**0.0002**
CDCA3	6.68	6.26	0.42	0.75	0.127
CDC45	5.51	7.45	-1.94	3.84	**0.0017**
E2F1	6.37	8.29	-1.93	3.80	**0.0002**
MCM5	13.31	16.04	-2.73	6.64	**3.7×10^{-5}**

Primer specificity was confirmed by a single sharp peak in the melt curve. Primer efficiency for each assay was determined using a 5-point 10-fold serial dilution of pooled cDNA; acceptable efficiency range was 90–110% (slope ≈ −3.1 to −3.6) and $R^2 \geq 0.99$.

Reactions with atypical amplification (multiple peaks, late single replicate amplification only, or Cq variance >0.5 between technical replicates) were excluded and repeated. Quantification cycle (Cq) values were exported and mean Cq calculated for technical triplicates. Expression levels were normalized to the geometric mean of two endogenous controls (GAPDH and β-actin). Relative expression (fold change) between HSC-3 and Cal-27 was calculated using the ΔΔCq method and reported as 2^(−ΔΔCq). Statistical comparisons were performed on ΔCq values using appropriate unpaired t-test (Welch's t-test) and significance thresholds were reported.

Results and Discussion

Differential expression of hub genes between metastatic and non-metastatic HNSCC cell lines: Quantitative real-time PCR was performed to compare the expression of nine hub genes between HSC-3 (highly metastatic) and Cal-27 (low metastatic) oral squamous carcinoma cell lines. The geometric mean of β-ACTIN and GAPDH served as the normalization factor for ΔCt calculations. The relative expression levels were quantified using the $2^{-}\Delta\Delta Ct$ method and statistical significance was evaluated using a two-tailed *t*-test (Table 1 and figure 1).

Replication and DNA synthesis-associated genes significantly upregulated in metastatic HSC-3 cells: Among the nine hub genes, MCM5**,** CDC45, E2F1, RFC4 and CENPH were significantly upregulated in metastatic HSC-3 cells compared to non-metastatic Cal-27 (fold change ranging from 2.28- to 6.64-fold; $p < 0.01$). These genes are functionally involved in DNA replication licensing, helicase activity and S-phase progression, processes known to promote tumor proliferation and metastasis. MCM5 showed the highest upregulation (6.64-fold; $p = 3.7 \times 10^{-5}$), followed by CDC45 (3.84-fold; $p = 0.0017$) and E2F1 (3.80-fold; $p = 0.00017$). RFC4 and CENPH also exhibited significant overexpression (2.79- and 2.28-fold respectively), reinforcing their association with enhanced DNA replication and chromosomal segregation fidelity in metastatic cells.

Downregulation of ASF1B, TK1, CDCA3 and CHTF18 in metastatic cells: Conversely, ASF1B, CHTF18, TK1 and CDCA3 showed decreased expression in HSC-3 cells, with fold changes ranging from 0.20 to 0.80. Among these, ASF1B was markedly downregulated (0.20-fold; $p = 0.00018$), indicating a possible cell-type or stage-specific modulation. Although CDCA3 reduction did not reach statistical significance ($p = 0.127$), a general trend toward lower expression was evident for genes associated with chromatin remodeling and mitotic transition.

Statistical overview and expression trends: Five out of nine genes demonstrated statistically significant ($p < 0.01$) differential expression between metastatic and non-metastatic cell lines. The direction and magnitude of expression changes correlated well with the bioinformatic predictions made in the prior *in silico* analysis, validating the prognostic relevance of replication-associated genes in HNSCC metastasis.

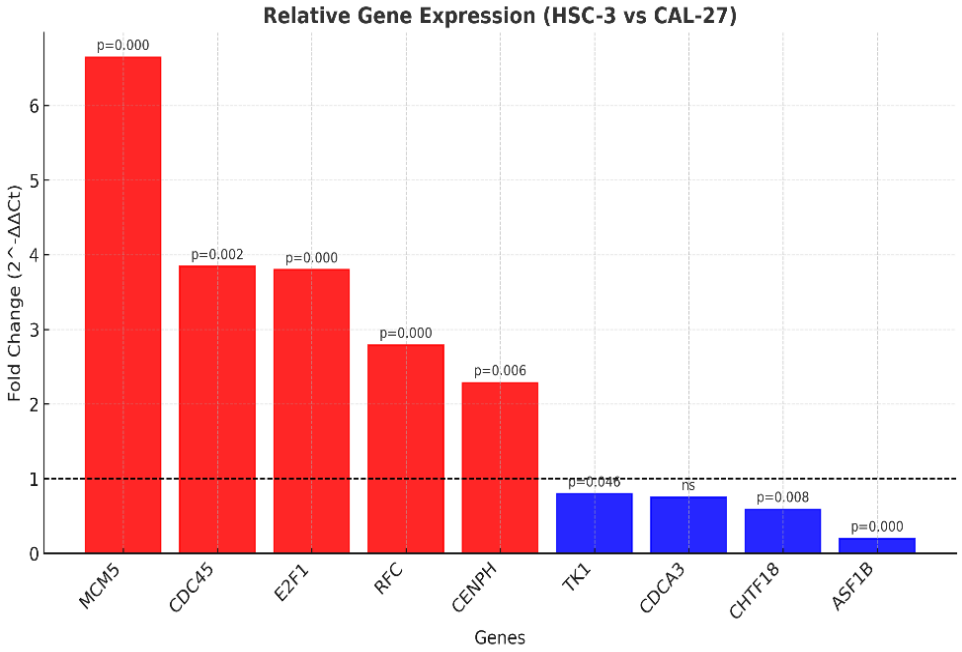

Figure 1: Relative gene expression in HSC-3 vs CAL-27 cell lines. Bar chart showing fold change ($2^{-}\Delta\Delta Ct$) for selected genes normalized to GAPDH and β-ACTIN. Red bars indicate upregulated and blue bars indicate downregulated genes in HSC-3 relative to CAL-27, highlighting differential expression of cell-cycle–related markers

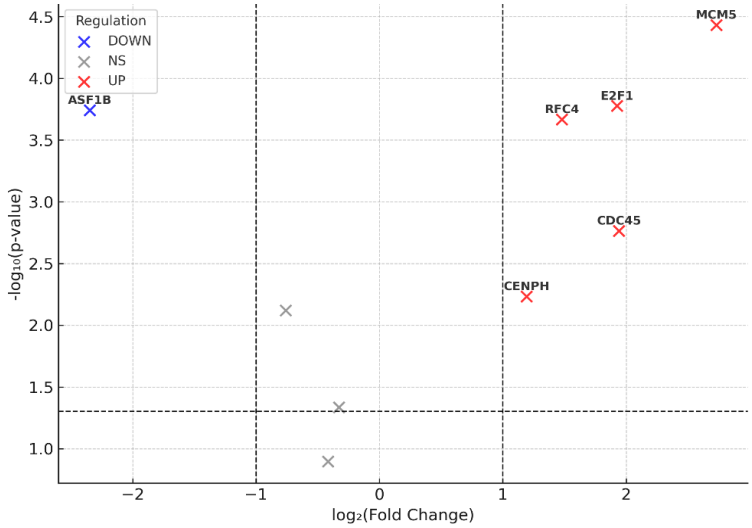

Figure 2: Volcano plot showing differential expression of hub genes between HSC-3 and Cal-27 cell lines. The volcano plot illustrates the $\log_2$ fold change (x-axis) against the $-\log_{10}$(p-value) (y-axis) obtained from qRT-PCR analysis comparing metastatic HSC-3 cells with non-metastatic CAL27 cells. Red points denote genes with significant upregulation (fold change ≥ 2, $p < 0.05$), blue points indicate significantly downregulated genes and gray points represent non-significant variations. Among the differentially expressed genes, MCM5, CDC45, E2F1, RFC4 and CENPH were notably elevated in HSC-3, while ASF1B and CHTF18 were reduced.

A volcano plot (Figure 2) visualization further emphasized the sharp contrast between upregulated and downregulated gene sets, highlighting MCM5**,** CDC45 and E2F1 as the most significantly elevated markers in HSC-3. This study provides experimental confirmation of bioinformatically identified metastasis-associated hub genes in head and neck squamous cell carcinoma (HNSCC). By validating nine candidate genes in metastatic (HSC-3) and non-metastatic (Cal-27) oral carcinoma cell lines, the findings establish a direct molecular link between replication-associated gene expression and metastatic potential.

Among the nine genes analyzed, MCM5, CDC45, E2F1, RFC4 and CENPH were significantly upregulated in HSC-3 cells, suggesting their contribution to enhanced proliferative and metastatic behavior. These genes are known to participate in DNA replication licensing, replication fork stability and chromosomal segregation, all of which are critical for maintaining genomic integrity during rapid tumor cell division. The marked upregulation of MCM5 (6.6-fold), CDC45 (3.8-fold) and E2F1 (3.8-fold) highlights their pivotal role in the replication initiation complex and cell-cycle regulation. Previous studies have reported similar associations of these genes with tumor aggressiveness and poor prognosis in various cancers including lung, liver and oral squamous carcinomas[14,23].

RFC4 and CENPH, which were moderately upregulated, are implicated in DNA repair and kinetochore assembly respectively. Mechanisms support chromosomal stability in highly proliferative metastatic cells[12,19]. The overexpression of these genes may provide a survival advantage to migrating cancer cells by facilitating rapid DNA synthesis and cell-cycle re-entry following stress or detachment. Conversely, ASF1B, CHTF18, TK1 and CDCA3 were found to be downregulated in the metastatic HSC-3 cell line. ASF1B, a histone chaperone regulating chromatin assembly and replication, exhibited the most significant downregulation, suggesting possible post-transcriptional control or cell-type-specific suppression during metastasis[15]. Although these results differ from bulk RNA-seq data that reported their upregulation in tumor tissues, such variation may reflect differences between *in vitro* and *in vivo* tumor microenvironments. It is also possible that certain replication-associated genes become transcriptionally repressed, once metastatic potential is established as observed in dormancy-associated or drug-resistant cancer subpopulations.

Collectively, these findings reinforce the earlier *in silico* prediction that DNA replication and cell-cycle–related pathways drive metastatic progression in HNSCC. The combined upregulation of MCM5**,** CDC45 and E2F1 in metastatic cells indicates a potential transcriptional axis promoting uncontrolled replication, enhanced DNA repair capacity and resistance to genotoxic stress which are key features of cancer stem cell (CSC) like behavior. Hence, these genes represent promising biomarkers for metastatic risk assessment and potential therapeutic intervention targets in HNSCC.

Conclusion

This study bridges bioinformatic prediction with biological validation, confirming that replication-associated hub genes, particularly MCM5**,** CDC45**,** E2F1**,** RFC4 and CENPH, are significantly upregulated in metastatic HNSCC cells. Their collective overexpression suggests a crucial role in promoting DNA synthesis, cell-cycle progression and metastatic competence.

The results not only validate the predictive accuracy of the earlier WGCNA and mRNAsi-based analysis but also emphasize the translational potential of these genes as biomarkers for metastasis and targets for anti-proliferative therapy in head and neck cancer. Future work will focus on protein-level validation**,** functional knockdown assays and clinical sample correlation to substantiate their diagnostic and therapeutic relevance.

References

1. Bais M.V., Kukuruzinska M. and Trackman P.C., Orthotopic non-metastatic and metastatic oral cancer mouse models, *Oral Oncol*, **51(5)**, 476-482 **(2015)**

2. Bitter E.E., Skidmore J., Allen C.I., Erickson R.I., Morris R.M., Mortimer T., Meade A., Brog R., Phares T., Townsend M., Pickett B.E. and O'Neill K.L., TK1 expression influences pathogenicity by cell cycle progression, cellular migration and cellular survival in HCC 1806 breast cancer cells, *PLoS One*, **18(11)**, e0293128 **(2023)**

3. Cao T., Yi S.J., Wang L.X., Zhao J.X., Xiao J., Xie N., Zeng Z., Han Q., Tang H.O., Li Y.K., Zou J. and Wu Q., Identification of the DNA Replication Regulator MCM Complex Expression and Prognostic Significance in Hepatic Carcinoma, *Biomed Res Int*, **2020**, 3574261 **(2020)**

4. Fares J., Fares M.Y., Khachfe H.H., Salhab H.A. and Fares Y., Molecular principles of metastasis: a hallmark of cancer revisited, *Signal Transduct Target Ther*, **5(1)**, 28 **(2020)**

5. Hao M., Wang H., Zhang C., Li C. and Wang X., Minichromosome maintenance protein 5 is an important pathogenic factor of oral squamous cell carcinoma, *Oncol Lett*, **20(4)**, 109 **(2020)**

6. Hollern D.P., Swiatnicki M.R., Rennhack J.P., Misek S.A., Matson B.C., McAuliff A., Gallo K.A., Caron K.M. and Andrechek E.R., E2F1 Drives Breast Cancer Metastasis by Regulating the Target Gene FGF13 and Altering Cell Migration, *Sci Rep*, **9(1)**, 10718 **(2019)**

7. Lambert A.W., Pattabiraman D.R. and Weinberg R.A., Emerging Biological Principles of Metastasis, *Cell*, **168(4)**, 670-691 **(2017)**

8. Li J., Bi W., Lu F., Pan B., Xiong M., Nasifu L., Nie Z. and He B., Prognostic role of E2F1 gene expression in human cancer: a meta-analysis, *BMC Cancer*, **23(1)**, 509 **(2023)**

9. Liu L., Tao T., Liu S., Yang X., Chen X., Liang J., Hong R., Wang W., Yang Y., Li X., Zhang Y., Li Q., Liang S., Yu H., Wu Y ., Guo X., Lai Y., Ding X., Guan H., Wu J., Zhu X., Yuan J., Li J., Su S., Li M., Cai X., Cai J. and Tian H., An RFC4/Notch1 signaling feedback loop promotes NSCLC metastasis and stemness, *Nat Commun*, **12(1)**, 2693 **(2021)**

10. Lu Y., Chen X., Liu F., Yu H., Zhang Y., Du K., Nan Y. and Huang Q., Systematic pan-cancer analysis identifies CDC45 as having an oncogenic role in human cancers, *Oncol Rep*, **48(4)**, 185 **(2022)**

11. Matsui T., Ota T., Ueda Y., Tanino M. and Odashima S., Isolation of a highly metastatic cell line to lymph node in human oral squamous cell carcinoma by orthotopic implantation in nude mice, *Oral Oncol*, **34(4)**, 253-256 **(1998)**

12. Morimoto M. et al, Expanding the genetic and phenotypic landscape of replication factor C complex-related disorders: RFC4 deficiency is linked to a multisystemic disorder, *Am J Hum Genet*, **111(9)**, 1970-1993 **(2024)**

13. Nguyen P. and Zeng E., A Protocol for Weighted Gene Co-expression Network Analysis With Module Preservation and Functional Enrichment Analysis for Tumor and Normal Transcriptomic Data, *Bio Protoc*, **15(18)**, e5447 **(2025)**

14. Nowinska K., Ciesielska U., Piotrowska A., Jablonska K., Partynska A., Paprocka M., Zatonski T., Podhorska-Okolow M. and Dziegiel P., MCM5 Expression Is Associated With the Grade of Malignancy and Ki-67 Antigen in LSCC, *Anticancer Research*, **39(5)**, 2325-2335 **(2019)**

15. Ouyang X., Lv L., Zhao Y., Zhang F., Hu Q., Li Z., Zhu D. and Li L., ASF1B Serves as a Potential Therapeutic Target by Influencing Cell Cycle and Proliferation in Hepatocellular Carcinoma, *Front Oncol*, **11**, 801506 **(2021)**

16. Rajabi-Moghaddam M. and Abbaszadeh H., Gene polymorphisms and prognosis of head and neck squamous cell carcinoma: a systematic review, *Rep Pract Oncol Radiother*, **27(6)**, 1045-1057 **(2022)**

17. Sakinah-Syed G., Liew J.S., Abdul Majid N. and Inche Zainal Abidin S.A., Alteration of primary cilia and intraflagellar transport 20 (IFT20) expression in oral squamous cell carcinoma (OSCC) cell lines, *PeerJ*, **13**, e18931 **(2025)**

18. Semenov O., Daks A., Fedorova O., Shuvalov O. and Barlev N.A., Opposing Roles of Wild-type and Mutant p53 in the Process of Epithelial to Mesenchymal Transition, *Front Mol Biosci*, **9**, 928399 **(2022)**

19. Thiru P., Kern D.M., McKinley K.L., Monda J.K., Rago F., Su K.C., Tsinman T., Yarar D., Bell G.W. and Cheeseman I.M., Kinetochore genes are coordinately up-regulated in human tumors as part of a FoxM1-related cell division program, *Mol Biol Cell*, **25(13)**, 1983-1994 **(2014)**

20. Vardhan N.R.H., Ladani R.K. and Reddy A.R., Integrated Bioinformatics Approach Unveils Genes Linked to Metastasis in Head and Neck Squamous Cell Carcinoma, *Journal of Biochemical Technology*, **14(4)**, 31-44 **(2023)**

21. Wang W., Xiao L., Pan D. and Hu L., ASF1B enhances migration and invasion of lung cancers cell via regulating the P53-mediated epithelial-mesenchymal transformation (EMT) signaling pathway, *Neoplasma*, **69(2)**, 361-369 **(2022)**

22. Wu J., Cui M., Wang J., Fan J., Liu S. and Lou W., CDCA3 promotes the proliferation and migration of hypopharyngeal squamous cell carcinoma cells by activating the Akt/mTOR pathway, *Biotechnol Genet Eng Rev*, **40(2)**, 707-725 **(2024)**

23. Yang S., Ren X., Liang Y., Yan Y., Zhou Y., Hu J., Wang Z., Song F., Wang F., Liao W., Liao W., Ding Y. and Liang L., "KNK437 restricts the growth and metastasis of colorectal cancer via targeting DNAJA1/CDC45 axis, *Oncogene*, **39(2)**, 249-261 **(2020)**

24. Yang T., Fan Y., Bai G. and Huang Y., RFC4 confers radioresistance of esophagus squamous cell carcinoma through regulating DNA damage response, *American Journal of Physiology-Cell Physiology*, **328(2)**, C367-C380 **(2025)**

25. Yu L., Li J., Zhang M., Li Y., Bai J., Liu P., Yan J. and Wang C., Identification of RFC4 as a potential biomarker for pan-cancer involving prognosis, tumour immune microenvironment and drugs, *Journal of Cellular and Molecular Medicine*, **28(12)**, e18478 **(2024)**

26. Zhang Z., Ning M., Li L., Li Z., Wang Y. and Zhao J., Knockdown of ASF1B inhibits cell proliferation, migration, invasion and cisplatin resistance in gastric cancer through the Myc pathway, *Oncol Lett*, **25(6)**, 242 **(2023)**

27. Zhang Z.C., Liu Y.F., Xi P., Nie Y.C., Sun T. and Gong B.B., Upregulation of CENPM is associated with poor clinical outcome and suppression of immune profile in clear cell renal cell carcinoma, *Hereditas*, **160(1)**, 1 **(2023)**

28. Zheng C., Zhang T., Li D., Huang C., Tang H., Ni X.F. and Chen B., Upregulation of CENPM facilitates tumor metastasis via the mTOR/p70S6K signaling pathway in pancreatic cancer, *Oncol Rep*, **44(3)**, 1003-1012 **(2020)**

29. Zuo S., Wang H., Li L., Pan H. and Lu L., Thymidine Kinase 1 Drives Skin Cutaneous Melanoma Malignant Progression and Metabolic Reprogramming, *Front Onco*., **12**, 802807 **(2022)**.

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