Review Paper:

The Contribution of DNA Replication and Cell Cycle Genes in promoting Metastasis

Vardhan Nalla Reddy Harsha[1,2]*, Preetha K.[2], Radhu L.[2] and Reddy Aluru Ranganadha[1]
1. Department of Biotechnology, VFSTR deemed to be University, Guntur, 522213, INDIA
2. Department of Product and Process Development, NeoDx Biotech Labs Pvt. Ltd., Bangalore, 560099, INDIA
*harshavardhan.bio@gmail.com

Abstract

Metastasis, the dissemination of cancer cells to remote organs, is the primary cause of cancer-related mortality and is governed by complex disturbances in DNA replication and cell cycle regulation. This research identifies nine pivotal genes: CDC45, MCM5, ASF1B, RFC4, E2F1, TK1, CHTF18, CENPM and CDCA3, that are persistently overexpressed in metastatic tumours and significantly correlated with unfavourable clinical outcomes. CDC45 and MCM5 are essential constituents of CMG helicase complex that promotes the initiation of replication origins; their overexpression triggers replication stress and activates pro-metastatic signalling pathways.

E2F1, a key transcriptional regulator, modulates the expression of several other metastasis-associated genes including CDCA3, TK1, RFC4, ASF1B and CENPM. The dysregulation of these downstream pathways facilitates epithelial–mesenchymal transition (EMT), genomic instability and the preservation of cancer stem cell-like characteristics.

CHTF18 is pivotal in maintaining sister chromatid cohesion and facilitating tolerance to replication stress, both of which are vital for genomic integrity during accelerated cancer cell proliferation. Pan-cancer expression profiling underscores the collective prognostic and biomarker potential of these genes, with multigene analysis providing superior predictive value compared to individual indicators. Emerging therapeutic strategies targeting replication-associated pathways, such as inhibition of CMG helicase, ATR/CHK1 signaling and gene-specific inhibitors of RFC4, ASF1B and CDCA3, show promise but are challenged by tumor heterogeneity and drug resistance.

Future advances in single-cell genomics, structural biology and liquid biopsy technologies are expected to facilitate more precise and effective interventions against metastatic cancer.

Keywords: Cell-cycle regulation, DNA replication stress, CMG helicase, E2F1 transcription network, Cancer metastasis biomarkers.

Introduction

Cancer metastasis includes multiple stages in which malignant cells separate from the primary tumour and disseminate to other regions of the body[23]. The metastatic cascade starts when cancer cells leave the main tumour and invade the stroma. These cells then intravasate into the bloodstream or lymphatic vessels, enabling systemic dissemination. To successfully establish secondary tumors (metastatic lesions), cancer cells must navigate numerous biological barriers. The EMT is a crucial cellular reprogramming event that facilitates metastatic spread. During epithelial-mesenchymal transition (EMT), epithelial tumour cells lose cell–cell adhesion and apical–basal polarity, while acquiring mesenchymal characteristics such as enhanced motility, invasiveness, resistance to apoptosis and stem cell-like plasticity, traits crucial for metastatic potential[7] (Figure 1).

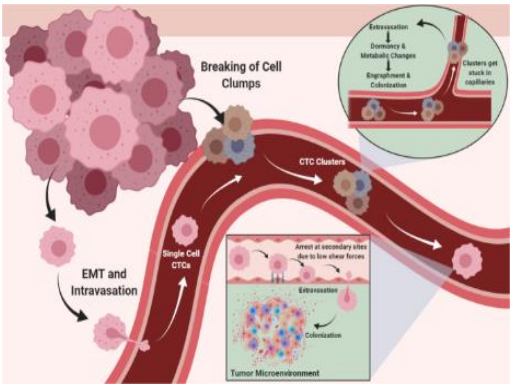

Figure 1: Molecular principles of metastasis[16]

A key subset of these migratory stem cell-like cells are circulating tumor cells (CTC), which enter the bloodstream following EMT. CTCs serve as primary mediators of metastasis, carrying the capacity to survive in the hostile circulatory environment, to evade immune surveillance and to colonize distant tissues. Studies show that EMT not only promotes the generation of CTCs but also contributes to their plasticity, allowing them to switch back via mesenchymal–epithelial transition (MET) to support colonization at metastatic sites[49]. Additionally, CTCs often express both epithelial and mesenchymal markers, a feature termed epithelial–mesenchymal plasticity (EMP) which supports their adaptability and survival[3]. Their detection and molecular characterization hold immense promise for non-invasive cancer diagnostics and personalized therapy. Targeting CTCs and their underlying EMT pathways is increasingly seen as a strategic approach to prevent metastatic progression[10,20].

EMT can be a vital biological process that enhances the migratory as well as invasive properties of epithelial cells, hence promoting metastasis. A characteristic of EMT is the downregulation of epithelial markers including E-cadherin, an essential mediator of tight cell–cell adhesion, combined with the overexpression of mesenchymal markers like N-cadherin and vimentin. These molecular alterations lead to the breakdown of intercellular junctions and augment cellular interactions with the extracellular matrix (ECM), a reprogramming that facilitates detachment, motility and tissue invasion.

This phenotypic switching, known as cellular plasticity, enables tumor cells to adapt to dynamic microenvironments and contributes significantly to tumor dissemination and resistance to therapy. Consequently, deciphering the molecular drivers of EMT and plasticity is essential for understanding the metastatic cascade and for identifying potential therapeutic targets.

In parallel, misregulation of cell-cycle and DNA replication machinery is another fundamental hallmark of cancer progression. Under physiological environments, the cell cycle is carefully regulated by following progression of G_1, S, G_2 and M phases, ensuring precise genome duplication. However, cancer cells often subvert these regulatory checkpoints, leading to uncontrolled proliferation, genomic instability and the emergence of mutator phenotypes that further fuel metastasis[5].

Key regulatory mechanisms such as replication licensing factors and cell cycle checkpoints become aberrantly activated in many cancers. This misregulation compromises genomic integrity, facilitates the accumulation of mutations and fosters conditions favorable to invasive and metastatic phenotypes.

Our latest integrative bioinformatics investigations found nine hub genes: CDC45, MCM5, ASF1B, RFC4, TK1, E2F1, CHTF18, CENPM and CDCA3[65]. Figure 2 illustrates factors that are often elevated in metastatic tumours and correlate with poor clinical outcomes[35,37]. These genes are functionally involved in:

Function	Gene
Initiating DNA replication	*CDC45, MCM5*
Chromatin assembly	*ASF1B*
Enhancing DNA polymerase processivity	*RFC4*
Transcriptional regulation of S-phase genes	*E2F1*
Thymidine metabolism and proliferation markers	*TK1*
Sister chromatid cohesion and segregation	*CHTF18, CENPM*
Cell cycle progression control	*CDCA3*

Objectives of the study

1. To elucidate the biological roles of nine pivotal genes—CDC45, TK1, CENPM, ASF1B, MCM5, RFC4, E2F1, CHTF18 and CDCA3 **[65]** with a specific focus on their involvement in DNA replication, chromatin dynamics, cell cycle progression and cancer-associated phenotypes.
2. To examine the impact of abnormalities in DNA replication machinery on metastatic capability and the attainment of stem cell-like characteristics in cancer cells, emphasising the essential function of the replication-plasticity axis in tumour progression.
3. To analyse the expression profiles of these hub genes across various tumour types (pan-cancer analysis), to examine their correlation with patient survival outcomes (prognostic significance) and to ascertain their biomarker potential by contrasting expression patterns between primary and metastatic lesions.
4. To explore the therapeutic implications of replication-metastasis interdependence by identifying druggable targets among the selected genes, evaluating the efficacy of existing inhibitors and proposing combination treatment regimens aimed at overcoming resistance and improving clinical outcomes in advanced cancers.

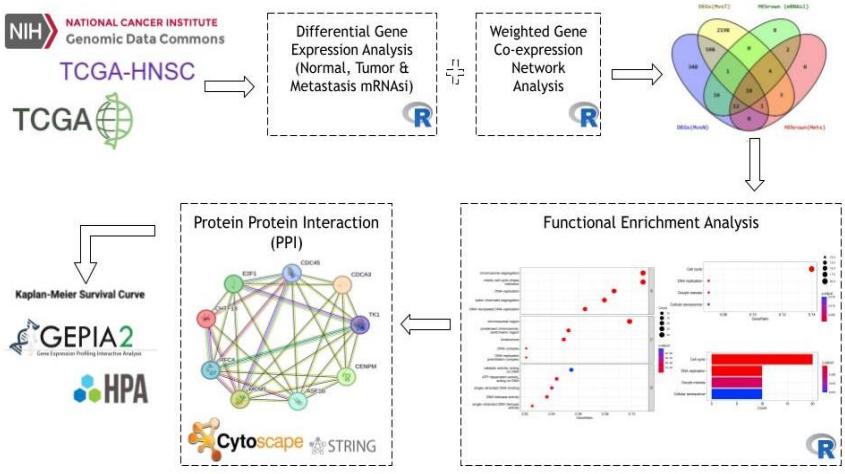

Figure 2: Integrated Bioinformatics Approach Unveils Genes linked to Metastasis in Head and Neck Squamous Cell Carcinoma[65]

DNA replication machinery and cancer metastasis

The Cell Cycle, Checkpoint Failure and Oncogenic Progression: In eukaryotic cells, the cell cycle has different phases—G1, S, G2 and M, each meticulously regulated to ensure precise DNA replication and systematic cell division[30]. This is controlled by cyclin-dependent kinases (CDKs) and checkpoint proteins that maintain genomic integrity by monitoring DNA damage and replication fidelity. Key regulatory checkpoints at G_1/S and G_2/M halt progression if the genome is damaged or incompletely replicated, allowing cells to activate DNA repair mechanisms before moving forward.

In cancer, these checkpoint mechanisms are frequently compromised when mutations occur in pivotal tumor suppressor genes that involve p53, ATM/ATR and CHK1/CHK2. Loss of p53 disrupts the transcriptional activation of DNA repair and apoptosis genes, while defective ATM/ATR signaling impairs damage sensing and checkpoint activation. This leads to impaired checkpoint arrest, unchecked CDK activity and premature S-phase entry, even under conditions of replication stress[50].

Simultaneously, disruption of RB pathway results in constitutive E2F activity, continuously promoting the transcription of S-phase genes. This deregulated E2F signaling not only accelerates cell cycle progression but also exacerbates genomic instability by bypassing critical control nodes[27], failure to stop at the G_2/M checkpoint under conditions of DNA damage enables mitotic entry despite replication stress, perpetuating chromosomal aberrations and mutation accumulation.

These cumulative defects foster accelerated proliferation, genomic instability and the evolution of clonal subpopulations with selective advantages including invasive and metastatic phenotypes. This process—driven by replication stress and checkpoint failure constitutes a central axis of tumorigenesis and metastatic progression. Notably, co-mutations in ATR and TP53 have been associated with significantly higher rates of metastasis and treatment resistance in breast cancer models[50].

DNA Replication role in Genomic Stability and Tumorigenesis: DNA replication is an essential biological process that ensures the precise duplicate of the genome prior to cell division. Regulating genomic stability during replication is crucial to prevent oncogenic transformation and tumor evolution. The S-phase of the cell cycle involves three sequentially coordinated steps of DNA replication:

1. **Origin Licensing:** The origin recognition complex (ORC1–6), Cdc6 and Cdt1 help load the MCM2–7 helicase complex onto DNA and select replication starting sites[4,63].
2. **Helicase Activation:** Dbf4-dependent kinase (DDK) and cyclin-dependent kinase 2 phosphorylate the MCM helicase to activate it. These events activate CDC45 and the GINS complex to unwind DNA[8,63].
3. **Polymerase Recruitment and Fork Progression:** After DNA unwinding, PCNA and RPA generate replication forks, allowing DNA polymerases to synthesise DNA with high fidelity[14].

Failures in any replication stage can cause fork collapse or stalling, resulting in double-strand breaks (DSBs) which are strongly linked to genomic instability and cancer development[68]. To improve replication fidelity, cells create the pre-replication complex (pre-RC) during late M- and G_1-phases, preparing them for precise S-phase entry[13]. However, in many cancers, replication licensing and activation are deregulated. For instance, overexpression of Cdc6 and Cdt1, often observed in tumors, can lead to re-replication, while reduced levels of geminin, a licensing inhibitor, permit aberrant origin firing. Likewise, CDC45 overexpression and aberrant DDK activity have been implicated in aggressive tumor phenotypes due to uncontrolled helicase activation[13]. Moreover, defective fork stabilization mechanisms such as PCNA ubiquitination failure or ATR–CHK1 pathway hyperactivation further contribute to replication stress, promoting mutation accumulation and clonal selection, both of which drive tumor heterogeneity and metastatic potential.

The CMG complex: central to replication and metastasis

Structure and function of the CMG complex: CMG helicase, comprised of CDC45, MCM2–7 and GINS subunits, is the main mechanism for DNA unwinding during eukaryotic cell replication[18,44]. It facilitates the unwinding of DNA at replication forks, an essential requirement for precise DNA synthesis[53].

Table 1
Overview of cell cycle phases, their regulators, checkpoints and common dysregulations in cancer[2,9].

Cell Cycle Phase	Key Regulators	Checkpoint Proteins	Dysregulation in Cancer
G_1	Cyclin D–CDK4/6, RB	p53, p21	RB loss; p53 mutations; p21 downregulation
S	Cyclin E–CDK2, Cyclin A–CDK2	ATR, CHK1	ATR/CHK1 inactivation; replication stress
G_2	Cyclin A–CDK1, Cyclin B–CDK1	ATM, CHK2	ATM/CHK2 mutations; bypass of G_2 arrest
M	APC/C, Cyclin B–CDK1	Spindle assembly checkpoint	Aneuploidy due to checkpoint override

Table 2
Stages of DNA replication, associated factors and their common alterations in tumorigenesis[13,17].

Replication Stage	Key Factors	Cancer-Associated Alterations
Origin licensing	ORC1–6, Cdc6, Cdt1	Overexpression of Cdc6/Cdt1; reduced geminin
Helicase activation	DDK (Cdc7–Dbf4), CDK2, CDC45, GINS	CDC45 upregulation; aberrant DDK activity
Fork progression	MCM2–7, RPA, PCNA	MCM overloading; PCNA ubiquitination deficiencies
Damage response	ATR–CHK1, ATM–CHK2	ATR/CHK1 hyperactivation; CHK2 loss in subsets

The MCM2–7 subunits form a ring-shaped hexamer that encircle the leading DNA strand and hydrolyze ATP to translocate along it. CDC45 associates with the MCM2/5 interface, stabilizing the helicase and regulating strand separation, while GINS acts as a structural bridge facilitating helicase activation[18,53]. Cryo-electron microscopy (cryo-EM) investigations have revealed that the CMG helicase complex possesses a divided structural configuration, wherein the N-terminal section constitutes the DNA entry channel and the C-terminal segment serving as the ATPase motor. This structural arrangement enables effective DNA unwinding at a rate of around 50 nucleotides per second and promotes coordinated contact with polymerases ε and δ during leading- and lagging-strand synthesis correspondingly[19,66].

CDC45 and MCM5 - Gatekeepers of DNA replication: CDC45 and MCM5 play essential roles in initiating and maintaining helicase activity to ensure accurate DNA replication [26]. CDC45 controls the controlled opening and closing of the interface between MCM2 and MCM5 subunits, hence modulating DNA entry and helicase conformational dynamics. MCM5, as part of the ATPase core, is pivotal in ATP binding and hydrolysis, facilitating mechanical movement along DNA strands. Misregulation of these components can induce replication stress and compromise fork stability[23,53]. The activation of the CMG complex necessitates phosphorylation by DDK and CDK2, which prepare the MCM helicase for origin firing. CDC45 is subsequently recruited to initiate unwinding, making its precise temporal regulation essential for replication fidelity.

Mechanisms linking CMG dysfunction to metastatic potential: Dysfunction in the CMG helicase, especially involving CDC45 or MCM5 induces replication stress, characterized by fork stalling and excessive origin firing. This activates checkpoint pathways such as ATR–CHK1 and NF-κB, initiating cellular responses that include enhanced survival, motility and invasion, all features of metastatic cells[24,64]. Prolonged replication stress triggers innate immune signaling through the cGAS–STING pathway which senses cytoplasmic DNA leakage from damaged replication forks. This leads to secretion of pro-metastatic cytokines like IL-6 and IL-8, creating a tumor-promoting inflammatory environment[25,36]. Persistent activation of this pathway facilitates tumor immune evasion, angiogenesis and metastatic niche formation[25].

CDC45 and MCM5 - crucial regulators of DNA replication and cancer progression

Role in CMG assembly and helicase activation: The CMG complex, that comprise of CDC45, the MCM2–7 helicase and GINS tetramer, serves as the primary replicative helicase in eukaryotic cells. It can be crucial for commencing DNA replication and facilitating replication fork advancement. CDC45 serves as a crucial connector, strengthening the association between the MCM helicase and GINS to form a fully operational CMG complex. This arrangement is essential for activating the helicase and facilitating effective DNA unwinding during S-phase of cell cycle[26].

The incorporation of CDC45 into the CMG complex results in an over fivefold enhancement of ATPase activity of MCM2–7 complex, hence providing the mechanical force necessary for DNA strand separation. MCM5, one of the six MCM subunits, is located at the ATP-binding interface between MCM2 and MCM3 and is crucial for effective ATP hydrolysis and translocation. GINS stabilises the interface between CDC45 and MCM, hence ensuring helicase processivity[56].

Overexpression in cancers and correlation with poor prognosis: High expression levels of CDC45 and MCM5 have been consistently reported in multiple human cancers based on integrative transcriptomic analyses from TCGA and GEO datasets[28]. In breast carcinoma, CDC45 and MCM5 show ~3.1-fold and ~2.8-fold upregulation, respectively, with CDC45 linked to shorter overall survival (OS) (Hazard ratios HR = 2.3), particularly in the basal-like subtype. Increased MCM5 has been associated to advanced clinical stage and lymph node metastases in colorectal cancer ($p < 0.01$). In hepatocellular carcinoma, both genes are related to vascular invasion and poor differentiation. This shows that CDC45 and MCM5 help cancer cells grow and spread.

Contribution to cancer stemness and metastatic behaviour: Beyond their enzymatic functions, CDC45 and MCM5 participate in transcriptional regulation of gene programs that support stemness and EMT. In glioblastoma stemcell-like cells, CDC45 has been shown to interact with chromatin modifiers to sustain the expression of pluripotency factors such as SOX2 and NANOG. CDC45 knockdown reduces neurosphere formation by over 60%[33], indicating a vital role in maintaining the stem-like phenotype.

Similarly, MCM5 supports EMT by cooperating with β-catenin to enhance transcription of SNAIL and TWIST, key

drivers of mesenchymal transformation, cell migration, as well as invasion[34,36]. These regulatory roles link CMG components to broader oncogenic programs that govern plasticity, invasiveness and metastasis.

Therapeutic targeting potential: Due to their tumor-specific overexpression and non-redundant essential roles in DNA replication, CDC45 and MCM5 represent attractive targets for anti-metastatic therapies. Recent preclinical efforts have focused on small-molecule inhibitors that disrupt the CDC45–MCM5 interaction, effectively disassembling the CMG helicase. Inhibition of CMG assembly halts DNA replication in proliferating tumor cells, while sparing quiescent normal cells, thereby minimizing off-target toxicity[56]. This targeted disruption of replication machinery presents a novel therapeutic avenue, particularly for aggressive and metastatic cancers with elevated CMG component expression[62].

E2F1 - master transcription factor in proliferation and metastasis: Control of G_1/S Transition and Replication Gene Expression. E2F1 is a key regulatory protein that increases DNA replication gene transcription to enhance S-phase[12]. It plays a pivotal role at the G_1/S transition, where it activates the transcription of over 200 genes required for origin licensing, helicase activation and DNA synthesis. In non-dividing cells, the retinoblastoma (RB) protein forms a compound with hypophosphorylated E2F1, blocking cell cycle progression [58]. Mitogenic stimulation sequentially activates cyclin D–CDK4/6 and cyclin E–CDK2, phosphorylating RB and releasing E2F1 to transactivate key replication factors such as Cyclin E, CDC6, MCM proteins and CMG complex components[37].

This process regulates DNA replication for optimal conditions. In numerous cancers, E2F1 becomes aberrantly activated, leading to persistent origin licensing and elevated replication stress. This, in turn, fosters genomic instability, tumor heterogeneity and enhanced cellular adaptability to stressful conditions[36,38].

Deregulation via RB Pathway and Oncogenic Consequences: Dysregulation of the RB–E2F axis is a hallmark of oncogenesis and occurs through several mechanisms:

- Loss-of-function mutations or deletions in RB1.
- Overexpression of cyclin D1, resulting in enhanced CDK4/6 activity.
- Inactivation of CDK inhibitors like p16^INK4a, normally suppress CDK4/6.

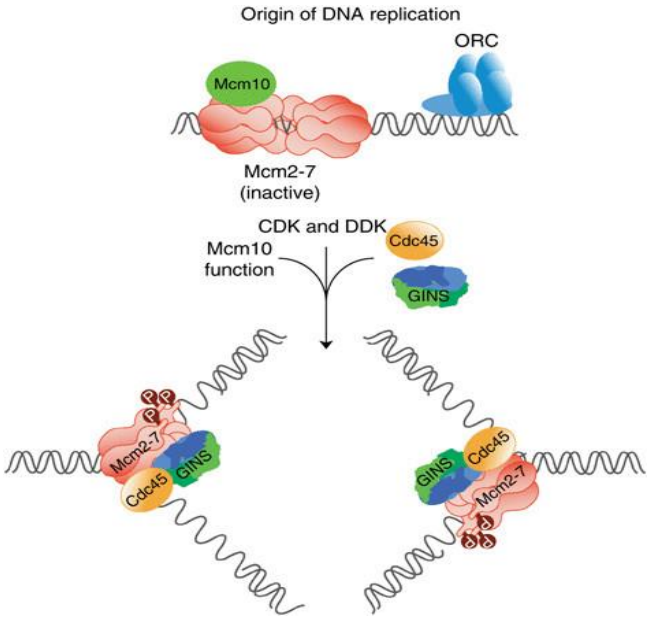

Figure 3: Model for the activation of Cdc45–MCM [11]

Table 3
Differential expression of CDC45 and MCM5 in selected cancers and their clinical correlations [28, 31].

Cancer Type	CDC45 Overexpression	MCM5 Overexpression	Clinical Correlation
Breast carcinoma	3.1fold ↑	2.8fold ↑	HR=2.3 for OS; associated with basal-like subtype[28]
Colorectal carcinoma	2.5fold ↑	2.1fold ↑	Correlates with lymph node involvement ($p<0.01$)[31]
Hepatocellular carcinoma	2.9fold ↑	2.6fold↑	Linked to vascular invasion and poor differentiation[28]

Table 4
Major categories of E2F1 target genes and their roles in replication and metastasis[28,42].

E2F1 Target Category	Representative Genes	Functional Outcome
DNA replication	CDC6, MCM2-7, CDC45	Origin licensing, helicase activation
Cell-cycle regulators	Cyclin E, Cyclin A, CDK1	Promotion of S-phase entry and progression
EMT and invasion	MMP2, SNAIL, ZEB1	Degradation of extracellular matrix and loss of adhesion
DNA damage response	BRCA1, RAD51	DNA repair and checkpoint activation

These alterations lead to uncontrolled E2F1 activation, allowing inappropriate S-phase entry and uncontrolled proliferation[40]. In addition to cell cycle drivers, E2F1 aberrantly induces genes involved in:

- Apoptosis (e.g. p73).
- DNA damage response and repair (e.g. BRCA1, RAD51).

The result is a cellular environment characterized by replicative stress and genomic instability, fueling clonal evolution and metastatic potential[41,69].

E2F1 as a Central Node Linking Replication and Metastasis: Beyond its canonical role in proliferation, E2F1 orchestrates transcriptional programs that promote invasion and metastasis. Genome-wide chromatin immunoprecipitation (ChIP-seq) studies have revealed that E2F1 binds promoter regions of genes involved in:

- Cell migration (e.g. MMP2, MMP9)
- Epithelial–mesenchymal transition (EMT) (e.g. SNAIL, ZEB1)
- Angiogenesis (e.g. VEGF)[42].

E2F1 also cooperates with chromatin modifiers to promote epigenetic remodeling, thereby enhancing transcription of pro-metastatic gene networks. This dual functionality positions E2F1 as a master regulator that bridges replication control with tumor progression and dissemination.

Downstream targets of E2F1 in metastasis

CDCA3 - Cell Cycle Progression and Metastasis Promotion: Cell Division Cycle Associated 3 (CDCA3), often referred to as Tome-1, is an F-box protein that promotes the ubiquitin-mediated degradation of WEE1 kinase, an essential inhibitor of the G_2/M transition. CDCA3 facilitates mitotic entrance by degrading WEE1, hence enhancing fast cell cycle advancement. E2F1 directly binds to the CDCA3 promoter, enhancing its transcriptional activation in highly proliferative and metastatic cancers[43].

In lung and gastric cancers, CDCA3 overexpression has been reported in gastric and lung cancers, where it correlates with lymph node metastasis and poor prognosis. Functionally, CDCA3 supports morphological transformation, facilitating cellular detachment and dissemination. The knockdown of CDCA3 leads to a marked decrease in proliferation and migration (about 40%), as well as improved sensitivity to DNA-damaging agents, suggesting its function as a contributor to tumour progression and therapeutic resistance[45,46].

TK1 - DNA Synthesis Enzyme and Proliferation Marker: Thymidine kinase 1 (TK1) serves a pivotal rate-limiting function in the nucleotide salvage pathway by phosphorylating thymidine to generate dTTP, a vital precursor for DNA replication. The transcription factor E2F1 upregulates its expression during the S-phase of the cell cycle, closely associating TK1 with cellular proliferation. Consequently, TK1 functions as a dependable indicator of cellular proliferation across many cancer types. Clinically, elevated TK1 levels are commonly associated with more aggressive tumors and are often utilized to assess tumor load and track therapeutic outcomes[1,47].

RFC4 - PCNA Loading and Replication Stress Amplifier: RFC4, an essential element of the "replication factor C (RFC)" clamp loader complex, operates by positioning PCNA onto DNA strands to ensure the rapidity and precision of DNA synthesis during both leading as well as lagging strand replication[6]. RFC4 is an established E2F1 target and its overexpression enhances replication dynamics, but at the cost of replication fork stalling and activation of ATR-mediated checkpoint signaling[15,51]. High RFC4 expression is common in metastatic tumors where it contributes to genomic instability and the replication stress phenotype often observed in aggressive cancers [52].

ASF1B - Chromatin Assembly and EMT Induction: ASF1B functions as a chaperone for histones H3 and H4, assisting in nucleosome deposition during DNA replication, hence promoting accurate chromatin reformation at replication forks[21]. ASF1B is increased in early S-phase under E2F1 regulation, synchronising histone availability with DNA synthesis. In prostate and ovarian malignancies, the overexpression of ASF1B has been related to the activation of EMT pathways, facilitating cell motility and invasion[22,32,64]. Loss of ASF1B slows replication fork progression (~30%) and inhibits mesenchymal transformation, underscoring its dual role in both replication and metastatic behavior[54].

CENPM - Chromosomal Instability and cGAS–STING Activation: CENPM, a constituent of the kinetochore complex, is crucial for precise chromosomal alignment and segregation during mitosis[48]. It is transcriptionally regulated by E2F1 during S and G_2 phases, with its overexpression observed in aggressive cancers such as hepatocellular carcinoma and melanoma[29,39]. Aberrant CENPM expression

promotes aneuploidy and micronuclei formation, which activate the cGAS–STING inflammatory pathway, a double-edged sword in metastasis, as it might either suppress tumors or may facilitate immune evasion and metastatic niche formation[55,70].

CHTF18 - cohesion and replication stress in cancer

Role in RFC-CTF18 Complex: CHTF18 is an essential element of the RFC–CTF18 clamp loader complex, a specialized variation of the classical RFC1 complex that functions in both DNA replication and sister chromatid cohesion. During the S phase of the cell cycle, RFC–CTF18 facilitates the loading of "PCNA (Proliferating Cell Nuclear Antigen)" onto DNA. PCNA operates as a sliding clamp that stabilises DNA polymerases, ensuring replication accuracy. In contrast to RFC1, RFC–CTF18 complex has a preferential interaction with cohesion complexes and their loaders, thereby coupling DNA synthesis with chromatid cohesion, a process essential for precise chromosomal segregation during anaphase.

Loss or dysfunction of CHTF18 disrupts these cohesion checkpoints, resulting in premature separation of sister chromatids as well as the development of aneuploidy, a chromosomal abnormality frequently associated with aggressive and metastatic tumors[57,59].

Contribution to Replication Fidelity and Genome Integrity: Beyond its role in chromatid cohesion, CHTF18 contributes to replication fork stability and genome maintenance. Together with RFC4, CHTF18 ensures the fidelity of replication under conditions of cellular stress. Elevated expression of CHTF18 and RFC4 has been seen in several rapidly proliferating cancers including breast and prostate carcinomas, suggesting a compensatory response to replication stress. CHTF18 depletion leads to: increased DNA damage markers (e.g., γH2AX foci), replication fork collapse and heightened sensitivity to PARP inhibitors. These findings underscore CHTF18's role as a replication stress mitigator, making it most critical for the survival of cancer cells which have high proliferative demand[60].

Co-Expression with RFC4 and Synergistic Role in Metastasis: Bioinformatic co-expression analyses have revealed strong transcriptional co-activation of CHTF18 and RFC4 in metastatic breast and prostate cancers. This co-expression correlates with enhanced invasive capacity and metastatic potential. Functional studies demonstrate that dual knockdown of CHTF18 and RFC4 induces synthetic lethality, drastically impairing tumor cell survival. Co-overexpression leads to a 2-fold increase in cellular invasion in trans well migration assays. This synergy supports the hypothesis that CHTF18 and RFC4 cooperatively maintain replication dynamics and chromatid cohesion, thereby facilitating both tumor growth and metastatic dissemination[61].

Targeting the Replication Machinery to Disarm Metastatic Cancer: The identification of a carefully controlled gene network CDC45, CENPM, ASF1B, E2F1, RFC4, MCM5, TK1, CHTF18 and CDCA3[65] which collectively sustain replication, chromatin architecture, checkpoint evasion and metastatic dissemination, provides multiple avenues for targeted intervention.

Table 5
Key downstream targets of E2F1 and their contributions to metastasis[29,45,47,52,64]

Gene	Function	Metastatic Role
CDCA3	F-box protein, G_2/M transition	Promotes detachment and invasion; correlates with advanced stages[45].
TK1	Thymidine salvage enzyme	Biomarker of proliferation; linked to poor survival[47].
RFC4	PCNA loader	Supports high fork speed; enables survival under stress[52].
ASF1B	Histone chaperone	Drives aberrant chromatin assembly; promotes EMT[64].
CENPM	Kinetochore component	Induces chromosomal instability; activates pro-metastatic inflammation[29].

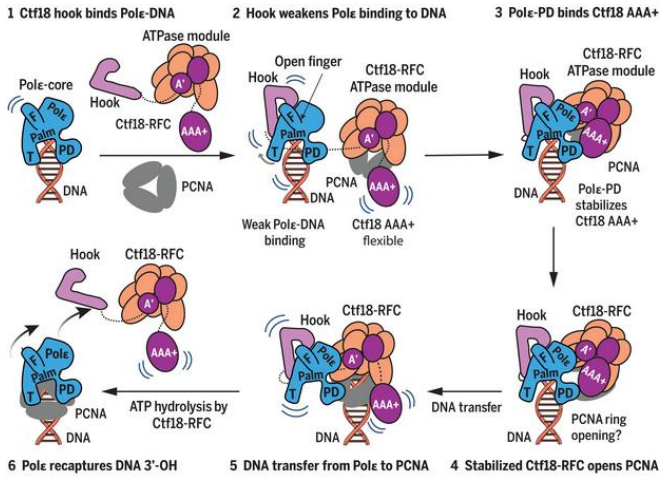

Figure 4: Mechanism of PCNA loading by Ctf18-RFC for leading-strand DNA synthesis [67].

Table 6
Comparison of canonical RFC and RFC-CTF18 complexes, their functions and cancer-associated alterations[57,61]

Complex	Subunits	Primary Function	Cancer-Associated Change
RFC1-RFC Loader	RFC1, RFC2–5	PCNA loading for replication and repair	RFC4 amplification; CHTF18 downregulation
RFC-CTF18 Alternate	CHTF18, DCC1, CTF8, RFC2–5	Sister-chromatid cohesion and replication stress response	Upregulation of CHTF18 and RFC4 in metastasis

The DNA replication apparatus, specifically elements of the CMG complex (CDC45–MCM–GINS) and PCNA loader systems (RFC4–CHTF18) and constitutes a critical weakness in rapidly proliferating, stress-adapted cancer cells.

Small Molecule Inhibitors of Replicative Drivers
CMG Complex Disruption: Pharmacological inhibition of CMG assembly halts S-phase progression by preventing helicase activation. This induces replication fork collapse, culminating in apoptosis in replication-dependent cancer cells, while sparing quiescent tissues. Small molecules targeting CDC45–MCM5 interaction interfaces are under early development as CMG disruptors.

Peptidomimetic Blockers of RFC4–PCNA Interaction: Peptidomimetics, synthetic analogs of peptide motifs, are being tested to interfere with the binding of RFC4 to PCNA, impairing clamp loading. This destabilizes the replication fork, enhances chemotherapeutic sensitization and disrupts cohesion machinery in hub gene-high tumors.

ASF1B Inhibitors and Fork Collapse–Driven Immunogenicity: Inhibiting ASF1B, a histone chaperone activated by E2F1, starves the replication fork of nucleosome components, triggering replication stress and chromatin instability. This not only impairs fork progression but also induces immunogenic cell death, especially when combined with immune checkpoint blockade (e.g. anti-PD1/PDL1 therapies), enhancing anti-tumor immunity.

CDCA3 Antagonists for Mitotic Control: Small-molecule CDCA3 inhibitors restore control over the G_2/M checkpoint by preventing degradation of WEE1 kinase, halting unscheduled mitotic entry. This induces mitotic arrest or senescence and has shown promise in models of basal-like breast cancer and colorectal carcinoma, where CDCA3 is overexpressed[39].

Future Perspectives

To effectively translate hub gene biology into clinical precision oncology, future strategies should include:

1. Development of Predictive Biomarkers: Identification of gene signatures (e.g., co-expression of CDC45 + RFC4 + ASF1B) as biomarkers of replication stress dependency, enabling patient stratification and personalized treatment.
2. Rational Combination Therapies: Dual inhibition of replication machinery (CMG + RFC complex) or hub gene plus immune modulator combinations to prevent resistance emergence and maximize synthetic lethality.
3. Integration with Immunotherapy: Replication stress inducers like ASF1B inhibitors and fork destabilizers can prime tumors for immune recognition, particularly in tumors with high cGAS–STING pathway activity due to chromosomal instability.
4. Single-cell and Spatial Transcriptomics: Profiling of intratumoral heterogeneity and identification of hub gene expression gradients across tumor compartments.
5. Structure-guided Drug Design: Targeting allosteric sites in CMG and RFC–CTF18 complexes to develop next-generation inhibitors with minimal off-target toxicity.
6. Liquid Biopsy Platforms: Incorporation of CDC45, TK1 and CDCA3 transcripts into CTC assays and cell-free RNA panels for early detection, monitoring and adaptive therapy planning.

The therapeutic exploitation of hub gene vulnerabilities including replication dependency, chromatin stress and checkpoint bypass offers a rational, systems-level approach to disrupt metastatic cancer programs.

By combining biomarker-driven precision medicine with novel inhibitors and immune strategies, future therapies may achieve durable control of replication-stressed tumors that are currently resistant to standard care.

Conclusion

This work thoroughly clarifies the functions of nine interrelated hub genes: CDC45, E2F1, CHTF18, MCM5, TK1, ASF1B, RFC4, CENPM and CDCA3 in regulating essential oncogenic processes, including DNA replication, chromatin assembly, cell cycle regulation and chromosome segregation. These genes constitute a closely integrated regulatory network that facilitates replication stress tolerance, checkpoint evasion and ultimately metastatic capability across various cancer types. A central finding is the feed-forward activation loop governed by E2F1, which transactivates multiple replication effectors such as CDC45, MCM5 and RFC4, leading to constitutive origin licensing and fork stress.

This stress, in turn, activates adaptive repair circuits (e.g. ATR–CHK1, cGAS–STING and NF-κB signaling) that allow tumor cells to continue proliferating despite genomic instability. The overexpression of these genes correlates with unfavourable prognosis, heightened invasion and resistance to conventional therapy, highlighting their significance as

biomarkers and therapeutic targets. Network analysis further reveals that this hub gene cluster exhibits high interdependence, suggesting that targeted disruption of even a single node may destabilize the entire metastatic program. These findings support a shift from monotherapies to multi-targeted inhibition strategies, tailored to exploit the tumor's reliance on replication-associated survival pathways.

References

1. Alegre M.M., Robison R.A. and O'Neill K.L., Thymidine kinase 1 upregulation is an early event in breast tumor formation, *J. Oncol*., **2012**, 575647 **(2012)**

2. Ansari M.A. et al, Exosome-based nanomedicine for cancer treatment by targeting inflammatory pathways: Current status and future perspectives, *Semin. Cancer Biol.*, **86(Pt 2)**, 678-696 **(2022)**

3. Balcik-Ercin P. et al, Epithelial-to-Mesenchymal Plasticity in Circulating Tumor Cell Lines Sequentially Derived from a Patient with Colorectal Cancer, *Cancers (Basel)*, **13(21)**, 5408 **(2021)**

4. Blow J.J. and Hodgson B., Replication licensing--defining the proliferative state?, *Trends Cell Biol.*, **12(2)**, 72-8 **(2002)**

5. Burkhart D.L. and Sage J., Cellular mechanisms of tumour suppression by the retinoblastoma gene, *Nat. Rev. Cancer*, **8(9)**, 671-82 **(2008)**

6. Chen Y., Wei L.N. and Muller J.D., Probing protein oligomerization in living cells with fluorescence fluctuation spectroscopy, Proc. Natl. Acad. Sci., USA, **100(26)**, 15492-7 **(2003)**

7. Corpet A. et al, Asf1b, the necessary Asf1 isoform for proliferation, is predictive of outcome in breast cancer, *EMBO J*., **30(3)**, 480-93 **(2011)**

8. De Jesus-Kim L. et al, DDK regulates replication initiation by controlling the multiplicity of Cdc45-GINS binding to Mcm2-7, *Elife*, **10**, e65471 **(2021)**

9. Del Re M. et al, Overexpression of TK1 and CDK9 in plasma-derived exosomes is associated with clinical resistance to CDK4/6 inhibitors in metastatic breast cancer patients, *Breast Cancer Res. Treat.*, **178(1)**, 57-62 **(2019)**

10. Deng C. et al, Pancancer Analysis of Neurovascular-Related NRP Family Genes as Potential Prognostic Biomarkers of Bladder Urothelial Carcinoma, *Biomed Res. Int.*, **2021**, 5546612 **(2021)**

11. Deursen F. et al, Mcm10 associates with the loaded DNA helicase at replication origins and defines a novel step in its activation, *The EMBO Journal*, **31**, 2195-206 **(2012)**

12. Dimova D.K. and Dyson N.J., The E2F transcriptional network: old acquaintances with new faces, *Oncogene*, **24(17)**, 2810-26 **(2005)**

13. Douglas M.E. et al, The mechanism of eukaryotic CMG helicase activation, *Nature*, **555(7695)**, 265-268 **(2018)**

14. Dyankova-Danovska T. et al, In and out of Replication Stress: PCNA/RPA1-Based Dynamics of Fork Stalling and Restart in the Same Cell, *Int. J. Mol. Sci*., **26(2)**, 667 **(2025)**

15. Fang Z. et al, E2F1 promotes cell cycle progression by stabilizing spindle fiber in colorectal cancer cells, *Cell. Mol. Biol. Lett.*, **27(1)**, 90 **(2022)**

16. Fares J. et al, Molecular principles of metastasis: a hallmark of cancer revisited, *Signal Transduct. Target. Ther.*, **5(1)**, 28 **(2020)**

17. Fernandes A.P. and Gandin V., Selenium compounds as therapeutic agents in cancer, *Biochim. Biophys. Acta*, **1850(8)**, 1642-60 **(2015)**

18. Gao Y. et al, CDC20 Holds Novel Regulation Mechanism in RPA1 during Different Stages of DNA Damage to Induce Radio-Chemoresistance, *Int. J. Mol. Sci.*, **25(15)**, 8383 **(2024)**

19. Glinsky G.V., Genomic models of metastatic cancer: functional analysis of death-from-cancer signature genes reveals aneuploid, anoikis-resistant, metastasis-enabling phenotype with altered cell cycle control and activated Polycomb Group (PcG) protein chromatin silencing pathway, *Cell Cycle*, **5(11)**, 1208-16 **(2006)**

20. Gostomczyk K. et al, Targeting circulating tumor cells to prevent metastases, *Hum. Cell*, **37(1)**, 101-120 **(2024)**

21. Groth A. et al, Regulation of replication fork progression through histone supply and demand, *Science*, **318(5858)**, 1928-31 **(2007)**

22. Han S. et al, E2F1 modulates RCCD1 expression to participate in the initiation and progression of EMT in colorectal cancer, *Pathol. Res. Pract.*, **260**, 155429 **(2024)**

23. Hanahan D. and Weinberg R.A., Hallmarks of cancer: the next generation, *Cell*, **144(5)**, 646-74 **(2011)**

24. Harding S.M. et al, Mitotic progression following DNA damage enables pattern recognition within micronuclei, *Nature*, **548(7668)**, 466-470 **(2017)**

25. Heidrich I. et al, Liquid biopsy for monitoring of tumor dormancy and early detection of disease recurrence in solid tumors, *Cancer Metastasis Rev.*, **42(1)**, 161-182 **(2023)**

26. Ilves I. et al, Activation of the MCM2-7 helicase by association with Cdc45 and GINS proteins, *Mol. Cell*, **37(2)**, 247-58 **(2010)**

27. Jonas D. et al, Novel path to apoptosis: small transmembrane pores created by staphylococcal alpha-toxin in T lymphocytes evoke internucleosomal DNA degradation, *Infect. Immun.*, **62(4)**, 1304-12 **(1994)**

28. Kasprzak A., Prognostic Biomarkers of Cell Proliferation in Colorectal Cancer (CRC): From Immunohistochemistry to Molecular Biology Techniques, *Cancers (Basel)*, **15(18)**, 4570 **(2023)**

29. Kassab A., Gupta I. and Moustafa A.A., Role of E2F transcription factor in oral cancer: Recent insight and advancements, *Semin. Cancer Biol.*, **92**, 28-41 **(2023)**

30. Keaton M.A., Morgan DO: The Cell Cycle: Principles of Control (Primers in Biology), *Cell Division*, **2(1)**, 27 **(2007)**

31. Kubota T. et al, Quantitative proteomic analysis of chromatin reveals that Ctf18 acts in the DNA replication checkpoint, *Mol. Cell. Proteomics*, **10(7)**, M110 005561 **(2011)**

32. Li J. et al, Prognostic role of E2F1 gene expression in human cancer: a meta-analysis, *BMC Cancer*, **23(1)**, 509 **(2023)**

33. Li Y. et al, CCT5 induces epithelial-mesenchymal transition to promote gastric cancer lymph node metastasis by activating the Wnt/beta-catenin signalling pathway, *Br. J. Cancer*, **126(12)**, 1684-1694 **(2022)**

34. Linhart C. et al, Deciphering transcriptional regulatory elements that encode specific cell cycle phasing by comparative genomics analysis, *Cell Cycle*, **4(12)**, 1788-97 **(2005)**

35. Liu Y. et al, CDC45 promotes the stemness and metastasis in lung adenocarcinoma by affecting the cell cycle, *J. Transl. Med.*, **22(1)**, 335 **(2024)**

36. Long Z.J. et al, cGAS/STING cross-talks with cell cycle and potentiates cancer immunotherapy, *Mol. Ther.*, **30(3)**, 1006-1017 **(2022)**

37. Lu H.P. et al, Expression of Cell Division Cycle Protein 45 in Tissue Microarrays and the CDC45 Gene by Bioinformatics Analysis in Human Hepatocellular Carcinoma and Patient Outcomes, *Med. Sci. Monit.*, **27**, e928800 **(2021)**

38. Ma D. et al, PER2 inhibits proliferation and stemness of glioma stem cells via the Wnt/beta-catenin signaling pathway, *Oncol. Rep.*, **44(2)**, 533-542 **(2020)**

39. Malki Fatiha, Alouache Ali and Meklat Atika, Synthesis of Heterocyclic Mesoionic Betaines Derivatives containing a Pyrimidine Ring for screening of their Biological Activities, *Res. J. Chem. Environ.*, **28(1)**, 43-47 **(2023)**

40. Masai H. et al, Phosphorylation of MCM4 by Cdc7 kinase facilitates its interaction with Cdc45 on the chromatin, *J. Biol. Chem.*, **281(51)**, 39249-61 **(2006)**

41. Mayer M.L. et al, Identification of RFC(Ctf18p, Ctf8p, Dcc1p): an alternative RFC complex required for sister chromatid cohesion in S. cerevisiae, *Mol. Cell*, **7(5)**, 959-70 **(2001)**

42. Mittal V., Epithelial Mesenchymal Transition in Tumor Metastasis, *Annu. Rev. Pathol.*, **13**, 395-412 **(2018)**

43. Moody C.A. and Laimins L.A., Human papillomavirus oncoproteins: pathways to transformation, *Nat. Rev. Cancer*, **10(8)**, 550-60 **(2010)**

44. Moyer S.E. et al, Isolation of the Cdc45/Mcm2-7/GINS (CMG) complex, a candidate for the eukaryotic DNA replication fork helicase, Proc. Natl. Acad. Sci., **103(27)**, 10236-10241 **(2006)**

45. Muller C. et al, A global view on carbapenem-resistant Acinetobacter baumannii, *mBio*, **14(6)**, e0226023 **(2023)**

46. Muller H. et al, Induction of S-phase entry by E2F transcription factors depends on their nuclear localization, *Mol. Cell. Biol.*, **17(9)**, 5508-20 **(1997)**

47. Nevins J.R., The Rb/E2F pathway and cancer, *Hum. Mol. Genet.*, **10(7)**, 699-703 **(2001)**

48. Norton L.E. and Layman D.K., Leucine regulates translation initiation of protein synthesis in skeletal muscle after exercise, *J. Nutr.*, **136(2)**, 533S-537S **(2006)**

49. Noubissi Nzeteu G.A. et al, Role of Epithelial-to-Mesenchymal Transition for the Generation of Circulating Tumors Cells and Cancer Cell Dissemination, *Cancers (Basel)*, **14(22)**, 5483 **(2022)**

50. Oropeza E. et al, Molecular portraits of cell cycle checkpoint kinases in cancer evolution, progression and treatment responsiveness, *Sci. Adv.*, **9(26)**, eadf2860 **(2023)**

51. Ouyang X. et al, ASF1B Serves as a Potential Therapeutic Target by Influencing Cell Cycle and Proliferation in Hepatocellular Carcinoma, *Front. Oncol.*, **11**, 801506 **(2021)**

52. Pekarek L. et al, An Overview of the Role of MicroRNAs on Carcinogenesis: A Focus on Cell Cycle, Angiogenesis and Metastasis, *Int. J. Mol. Sci.*, **24(8)**, 7268 **(2023)**

53. Petojevic T. et al, Cdc45 (cell division cycle protein 45) guards the gate of the Eukaryote Replisome helicase stabilizing leading strand engagement, Proc. Natl. Acad. Sci. USA, **112(3)**, E249-58 **(2015)**

54. Ronco C. et al, ATM, ATR, CHK1, CHK2 and WEE1 inhibitors in cancer and cancer stem cells, *Medchemcomm*, **8(2)**, 295-319 **(2017)**

55. Schuetz P. et al, Procalcitonin to initiate or discontinue antibiotics in acute respiratory tract infections, *Cochrane Database Syst. Rev.*, **10(10)**, CD007498 **(2017)**

56. Seo Y.S. and Kang Y.H., The Human Replicative Helicase, the CMG Complex, as a Target for Anti-cancer Therapy, *Front. Mol. Biosci.*, **5**, 26 **(2018)**

57. Sherley J.L. and Kelly T.J., Regulation of human thymidine kinase during the cell cycle, *J. Biol. Chem.*, **263(17)**, 8350-8 **(1988)**

58. Sherr C.J. and McCormick F., The RB and p53 pathways in cancer, *Cancer Cell*, **2(2)**,103-12 **(2002)**

59. Sherr C.J. and Roberts J.M., CDK inhibitors: positive and negative regulators of G1-phase progression, *Genes Dev.*, **13(12)**, 1501-12 **(1999)**

60. Shetty A., DNA replication licensing and cell cycle kinetics of normal and neoplastic breast, *Br J Cancer*, **93(11)**, 1295-300 **(2005)**

61. Singh G. et al, Targeting cell cycle regulators: A new paradigm in cancer therapeutics, *BIOCELL*, **48(12)**, 1639-1666 **(2024)**

62. Su W. et al, Bioinformatic analysis reveals hub genes and pathways that promote melanoma metastasis, *BMC Cancer*, **20(1)**, 863 **(2020)**

63. Ticau S. et al, Single-molecule studies of origin licensing reveal mechanisms ensuring bidirectional helicase loading, *Cell*, **161(3)**, 513-525 **(2015)**

64. Tsai J.H. and Yang J., Epithelial-mesenchymal plasticity in carcinoma metastasis, *Genes Dev.*, **27(20)**, 2192-206 **(2013)**

65. 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)**

66. Xiang S., Reed D.R. and Alexandrow M.G., The CMG helicase and cancer: a tumor "engine" and weakness with missing mutations, *Oncogene*, **42(7)**, 473-490 **(2023)**

67. Yuan Z. et al, Mechanism of PCNA loading by Ctf18-RFC for leading-strand DNA synthesis, *Science*, **385(6708)**, eadk5901 **(2024)**

68. Zeman M.K. and Cimprich K.A., Causes and consequences of replication stress, *Nat. Cell Biol.*, **16(1)**, 2-9 **(2014)**

69. Zhou Y. et al, Expanding Roles of the E2F-RB-p53 Pathway in Tumor Suppression, *Biology (Basel)*, **12(12)**, 1511 **(2023)**

70. Zierhut C., Potential cGAS-STING pathway functions in DNA damage responses, DNA replication and DNA repair, *DNA Repair (Amst)*, **133**, 103608 **(2024)**.

(Received 02nd September 2025, accepted 03rd November 2025)