

The Role and Mechanism of the Histone Methyltransferase G9a in Tumors: Update

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Abstract: Methylation-mediated gene silencing is closely related to the occurrence and development of human tumors. The euchromatic histone lysine methyltransferase 2 (EHMT2, also known as G9a) is highly expressed in many tumors and is generally considered to be an oncogene, which is associated with the poor outcome of many tumors. Combined immunotherapy and immune checkpoint blockade therapy also have good efficacy and certain safety. However, there are still many difficulties in the drugs targeting G9a, and the combined effect and safety of G9a with many drugs is still under study. This article aims to summarize the role and mechanism of G9a and its inhibitors in tumors in the past two years, and to understand the application prospect of G9a from the perspective of diagnosis and treatment.

Keywords: cancer, G9a, methyltransferase, function, mechanism

Introduction

Epigenetic regulation is an essential process in human growth and development. Epigenetic modifications allow differential expression of genes in different environments, altering gene functions without changing their sequence. Abnormal DNA methylation, chromatin modifications or remodeling, and dysregulated expression of non-coding RNAs(ncRNA) are hallmarks of cancer.¹ The epigenetic mechanism causes instability in the cancer genome, limits tumor suppressor genes, promotes oncogenic genes, and high methylation at CpG islands is a characteristic of the cancer genome. By adjusting these dysfunctions, tumor suppression can be achieved.² Chromatin, a large DNA and histones molecular complex, is a scaffold that packages the entire human genome. Chromatin-modifying enzymes substantially modify and remove DNA and histone modifications in a highly regulated manner.³ Chromatin remodeling signal transduction can affect DNA damage response and repair, DNA replication stress, aging, metastasis, angiogenesis, and tumor immunity, mainly through histone modification and ATP dependent remodeling agents to achieve its function. It can directly inhibit chromatin remodeling, DNA damage repair, or target genes, proteins, and even genetic defects in the genome.⁴

DNA forms chromatin in the eukaryotic cells' nucleus by associating with nuclear proteins. The nucleosome's fundamental structural unit of chromatin comprises 146 DNA base pairs wrapped around core histones H2A, H2B, H3, and H4.⁵ Chromatin structure is partially regulated by enzymes that post-translationally modify specific amino acids on histones. These post-translational modifications include acetylation, phosphorylation, poly-ADP-ribosylation, ubiquitination, and methylation.⁶ The epigenetic modifications-produced information is essential for all DNA-associated processes, including transcription, repair, and replication. Therefore, abnormal expression patterns or genomic alterations of chromatin regulatory factors may profoundly affect and promote the initiation and maintenance of various cancers.³ An important marker of epigenetic abnormalities is chronic DNA damage associated with cell stress, present in chronic inflammation and aging processes. This stress may transiently assemble transcriptional repressor complexes crucial for DNA repair at damaged sites.⁷ Over 50% of human cancers harbor mutations in enzymes involved in chromatin

organization. Tumor cells activate genetic and epigenetic changes and employ epigenetic processes as a routine mechanism to evade chemotherapy and host immune surveillance. Therefore, currently, the drug discovery research's primary target is epigenome, including DNA methylation and histone modifications.⁷ Epigenetic processes are also indicated to regulate immune cell function, mediating anti-tumor immunity. Compared to mono-immune-based therapies, co-treatment with epigenetic therapy may overcome certain limitations of current immune treatment strategies.⁸

ncRNA regulate gene expression by recruiting chromatin remodeling and modification complexes to specific sites. They can interact with chromatin modifying enzymes and affect chromosome structure by changing their histone modification status.⁹ DNMT and 10–7 translocase are the targets of ncRNAs, thereby affecting various pathological processes of tumors. Targeting a single ncRNA can regulate multiple cancer-related pathways, some miRNAs can be used in combination with chemotherapy drugs, and ncRNA can play a role in exosomes as a carrier, which has a good application prospect in the future.^{10,11} Heart failure is the outcome of a variety of cardiovascular diseases, in which chromatin remodeling is also involved, the most common one is DNA methylation, and ncRNA is also closely related to it. Many related ncRNA have been found, but little is known about its mechanism.¹²

Methylation is a critical regulatory factor for gene transcription, and DNA or RNA methylation alterations occur commonly in various tumors and during development. Increased methylation causing gene silencing has been extensively studied in the context of epigenetic regulation.¹³ Characteristic features of the cancer epigenome include alterations in the global DNA methylation and histone modification patterns. Typical features, such as overall DNA hypomethylation and promoter-specific hypermethylation, are mostly observed in benign tumors and early-stage cancers.¹⁴

Histone 3 lysine 9 methylation (H3K9me) mediated by DNA methylation is crucial for heterochromatin gene silencing, genome stability, and gene expression regulation. The internal loop within SUV39H (also called Clr4), the suppressor of variegation 3–9 homolog, inhibits its catalytic activity by blocking the H3K9 substrates binding pocket. Furthermore, the auto-methylation of specific lysine residues in this loop promotes a conformational switch, enhancing SUV39H H3K9me activity.¹⁵ Telomeres, or heterochromatic regions, form protective caps at eukaryotic chromosomes' ends and comprise TTAGGG repeat sequences bound to a series of specialized proteins. G9a is a primary enzyme comprising SET domain that catalyzes mono- and di-methylation of H3K9, heterodimerically complexed with G9a-like proteins.¹⁶ G9a is essentially associated with various developmental processes and determines cell fate by regulating H3K9me2 levels.¹⁷ The hominoid-specific gene TBC1 domain family member 3 interacts with G9a and promotes neural progenitor cells' generation and proliferation.¹⁸ G9a plays a role in many physiological or pathological processes, and its dysregulation results in pathological conditions (Figure 1). In the past few decades, comprehensive research on G9a has been conducted and has been functionally associated with many mechanisms. This investigation summarizes the tumor research progress in recent years to comprehensively understand G9a (Table 1).

Role and Mechanisms of G9a in Tumors

The dysregulation of G9a has been observed in many tumors and can cause tumor malignancy by silencing tumor suppressor and/or activating survival genes or the epithelial-mesenchymal transition (EMT) program, a potential reason for tumor aggressiveness.⁷³ Furthermore, it might also be linked with poorer overall survival (OS) and disease-free survival (DFS) (Figure 2A–D, this data comes from Gene Expression Profiling Interactive Analysis ((GEPIA), <http://gepia.cancer-pku.cn/detail.php?gene=EHMT2>)⁷⁴. The role and mechanism of G9a in tumors have been widely studied. It is believed that targeting G9a in cancer will lead to the re-expression of some important tumor suppressor genes, and the expression level of G9a is closely related to the occurrence and development of a variety of tumors.⁷⁴ For example: SLU7, a splicing regulator, is required for maintaining DNA methylation and interacts with DNA methyltransferase 1 (DNMT1), its adaptor protein ubiquitin-like with PHD and RING finger domain-1 (UHRF1), and the histone methyltransferase G9a at the chromatin level. DNMT1 is crucial for cancer cell proliferation and survival and, therefore, might be linked with tumor cells.⁷⁵ In cancer-associated fibroblasts (CAF) DNA, chromatin remodeling of C-X-C motif chemokine ligand 9 (CXCL9) and CXCL10 promoters occurs in a G9a and enhancer of zeste 2 polycomb repressive complex 2 subunits (EZH2) dependent manner. Transforming growth factor-beta 1 (TGF- β 1) deletion promotes inflammatory CAF infiltration, which reduces CXCL14 and increases CXCL9/10 production, and then recruit antigen-specific T cell, further limiting the efficacy of chemotherapy.⁷⁶ Cisplatin is widely used in clinical cancer chemotherapy but has severe ototoxic side effects, including

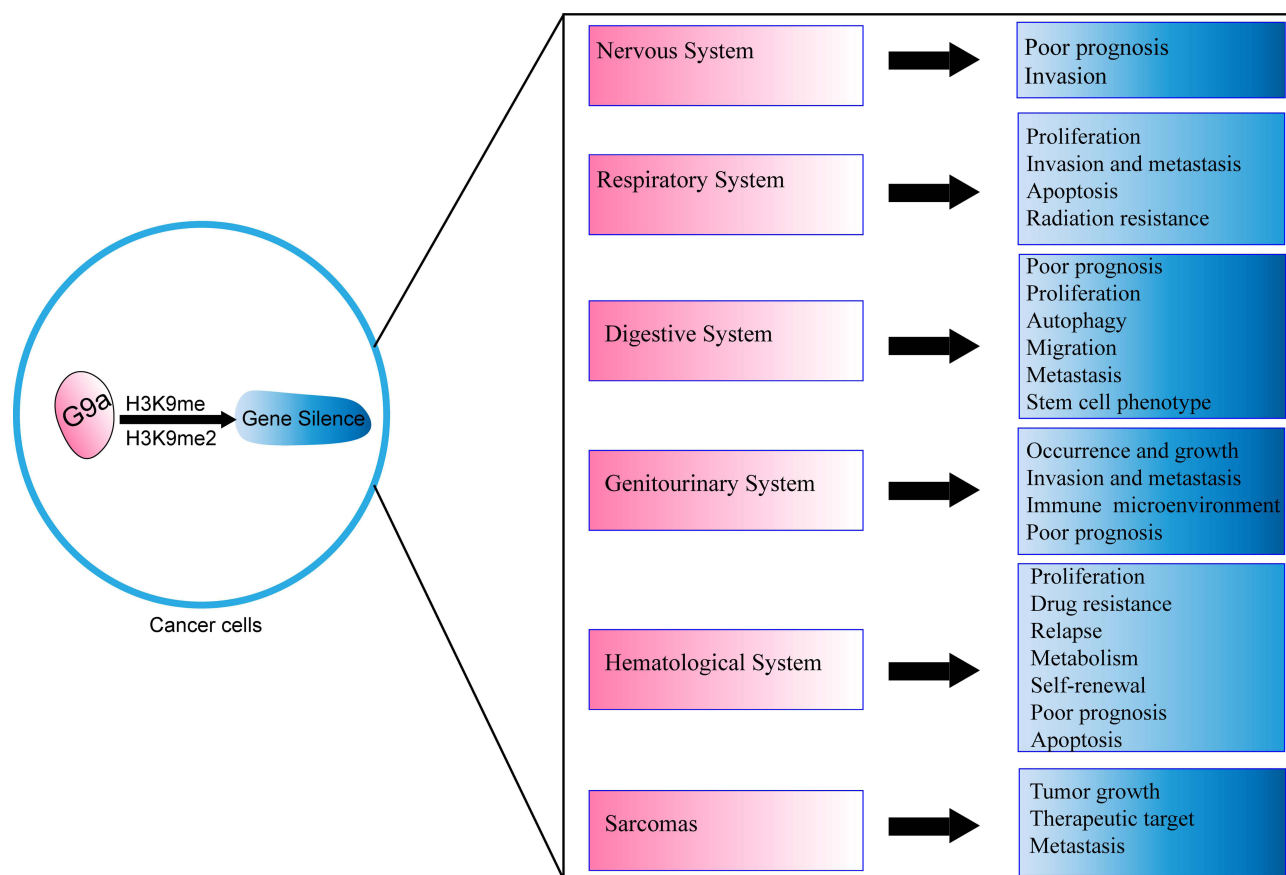


Figure 1 The role and pathways of G9a in various systemic tumors.

tinnitus and hearing impairment. Its treatment elevates H3K9me2 levels in cochlear hair cells, decreases forkhead box G1 (FOXG1) expression, reduces autophagy, accumulates reactive oxygen species (ROS), and causes cell death in cochlear hair cells. Inhibiting G9a can mitigate these toxic effects and alleviate hearing damage.⁷⁷ Fu et al found that deubiquitinases participate in cisplatin resistance in hepatocellular carcinoma (HCC) cells by stabilizing G9a via deubiquitination, providing evidence for cisplatin application.⁷⁸ The study of the mechanism of G9a regulation in tumors can be used to develop targeted drugs or diagnostic markers, or combined with the current standard of care for cancer patients.

Table 1 The Pathways and Function of G9a in Various System Tumors

Tumor Type	Pathway	Function
MB GBM	G9a/TP53 G9a-PARP3/Nfasc, Parvb, MLH1, Hif-2α, RUNX3, Ndr1, Ndr2, Ndr4	Poor prognosis ^{19,20} Invasion ²¹
Gliomas NSCLC LUAD	G9a/MHC-I, Fbxw7 G9a/NF-Kb/FAK AT2/G9a CNB/G9a, FOXO1 G9a/CCDC8 ROS/Snail/G9a G9a/NFYA/ALDH2	Poor prognosis ²² Invasion and metastasis ²³ Proliferation ²⁴ Regulating the proliferation, migration, and invasion of A549 cells and their apoptosis ²⁵ Radiation resistance ²⁶ EMT ²⁷ Mediation of resistance to Paclitaxel in vitro and in vivo ²⁸

(Continued)

Table I (Continued).

Tumor Type	Pathway	Function
EC	LSD1-G9a/ER stress pathway	Poor prognosis ²⁹
GC	Hypoxia/G9a/RUNX3	Proliferation ³⁰
	G9a/CA	Cellular autophagy ³¹
	SPINK1	Not yet clear ³²
	CALR/G9a/E-cadherin	Promote the migration of GC cells in vitro and in vivo ³³
HCC	G9a/miR-122	Poor prognosis ³⁴
	DNA damage/G9a/Bcl-2-p53	Occurrence of tumors ³⁵
	G9a/SLC7A2/PI3K/Akt/NF-κB/CXCL1	Inhibition metastasis ³⁶
	G9a/MMP2, MMP9, CD44v6, VEGF	Not yet clear ³⁷
	CDK9/G9a/MYC	Cancer growth and invasive ³⁸
HB	G9a/DNMT1/UHRF1	Cancer development ³⁹
CCA	G9a/ (MYC/Max heterodimer) / I5-PGDH	Promote CCA cell growth ⁴⁰
iCCA	KRASG12D/G9a/PHGDH	Increasing iCCA cell viability ⁴¹
PDAC	Tetraspanin1/FAM110A/ HIST1H2BK /G9a	Cancer progression ⁴²
CRC	Not yet clear	Driving cancer stem cell phenotype ^{43,44}
	G9a/ZNF518B/ RGS4, PADI3	Metastasis ⁴⁵
	G9a/IL8	Suppress cancer stemness and enhance chemosensitivity ^{46,47}
	Butyrate/ HECTD2/G9a	Promote CRC growth ⁴⁸
BCa	MYC/G9a/FTTH1	Promote occurrence and progression of cancer ^{49–51}
	G9a/BMP5/Smad	Regulate the growth and metastasis of BCa cells ⁵²
TNBC	Low-glucose/G9a	Invasion and migration ⁵³
HGSC	G9a and EZH2/CXCL10/CXCR3	Altering the immune microenvironment and tumor growth ⁵⁴
PCa	PAL1/G9a/PRC2	Poor prognosis ⁵⁵
	Paclitaxel/SPOP/G9a	Promote PCa cell growth ⁵⁶
	G9a/ERP29	Radioresistance ⁵⁷
RCC	G9a/ /p53	Carcinogenic effects ⁵⁸
AML	Destabilization of the complex composed of SUV39H1, GLP and G9a/IFN	Proliferation ⁵⁹
	Not yet clear	Drug resistance and relapse ⁶⁰
ALL	G9a/lysosome biogenesis/SES2/GSK-3	Impairs glycogen metabolism ⁶¹
CML	G9a/SOX6	Reduce self-renewal in CML ⁶²
MM	G9a, GLP/Mtor/ EIF4EBP1 /MYC/p38/MAPK9/JNK	Worse disease outcomes in newly diagnosed and relapsed ⁶³
	EZH2, G9a/IFN-stimulated genes/IRF4/MYC	Inhibiting cell growth ⁶⁴
MCL	Not yet clear	Cell cycle ⁶⁵
PTC	TESC/ALDH1A1/βIII-tubulin/PTEN	Promote tumor growth ⁶⁶
ARMS	NR4A1/G9a	Not yet clear ⁶⁷
	DDX5/G9a/AKT	Tumor growth ⁶⁸
	H3F3A/G9a/MCAM	Potential therapeutic target ⁶⁹
EWS	G9a/NEU1	Impairing in vitro and in vivo metastasis ⁷⁰
HNSCC	BRD4/G9a/MHC-I	Inhibiting anti-tumor immunity ⁷¹
OSCC	G9a/SPINK5/Wnt pathway	Invasion ⁷²

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ARMS, alveolar rhabdomyosarcoma; BCa, breast cancer; CCA, cholangiocarcinoma; CML, chronic myeloid leukemia; CRC, colorectal cancer; EC, esophageal cancer; EWS, ewing sarcoma; GBM, glioblastoma; GC, gastric cancer; HB, hepatoblastoma; HCC, hepatocellular carcinoma; HGSC, high-grade serous ovarian carcinoma; HNSCC, head and neck squamous cell carcinoma; iCCA, thioacetamide-induced intrahepatic CCA; LUAD, lung adenocarcinoma; MB, medulloblastoma; MCL, mantle cell lymphoma; MM, multiple myeloma; NSCLC, non-small cell lung cancer; OSCC, oral squamous cell carcinoma; PCa, prostate cancer; PDAC, pancreatic ductal adenocarcinoma; PTC, papillary thyroid carcinoma; RCC, renal cell carcinoma; TNBC, triple-negative BCa.

Nervous System Tumors

Medulloblastoma (MB) is a malignant brain tumor that affects children and is classified into four molecular subgroups. Compared to WNT tumors, the G9a gene expression is higher in sonic hedgehog (SHH), groups 3 and 4 MB subgroups.

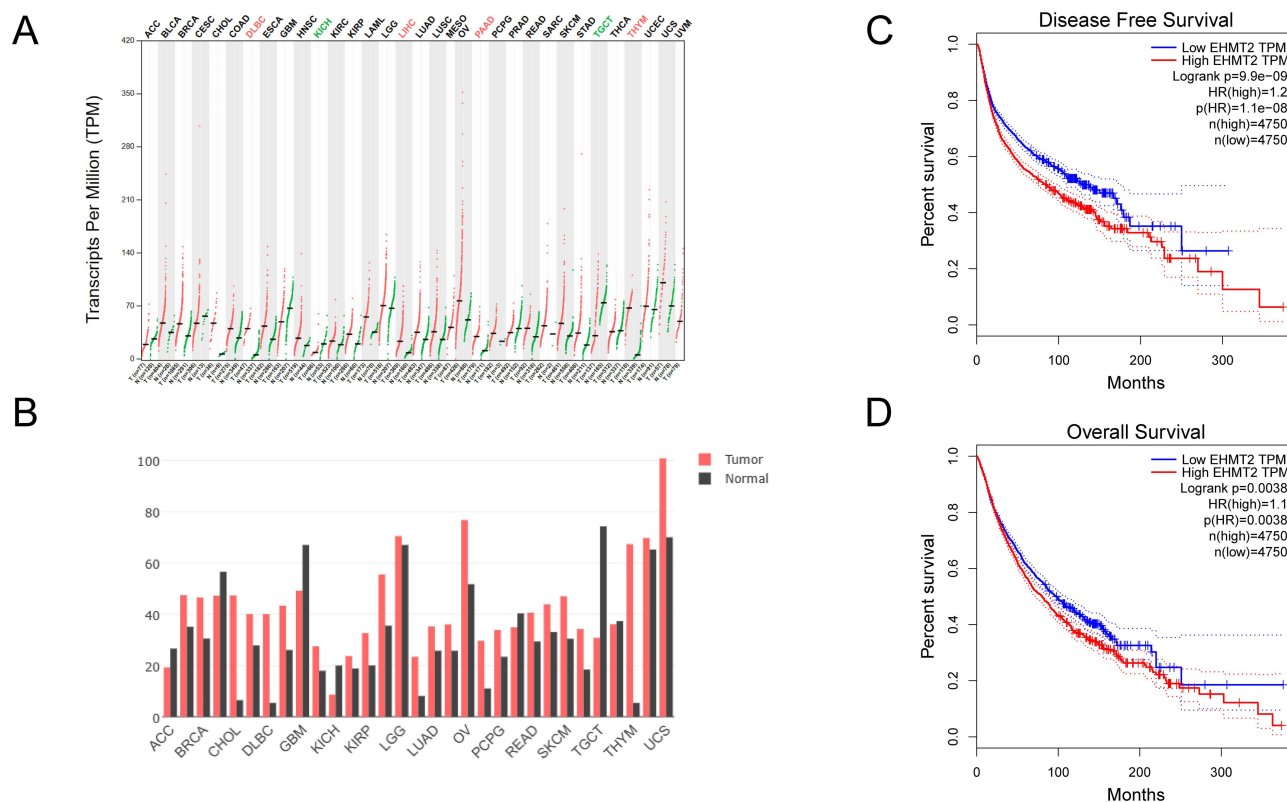


Figure 2 (A) The gene expression profile across all tumor samples and paired normal tissues (Dot plot), each dots represent expression of samples. **(B)** The gene expression profile across all tumor samples and paired normal tissues (Bar plot), the height of bar represents the median expression of certain tumor type or normal tissue. **(C)** Disease free survival rate of G9a in tumors. **(D)** Overall survival rate of G9a in tumors. Tang Z. et al (2017) GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res.* 10.1093/nar/gkx247.⁷⁹

In MB patients, high G9a expression is significantly associated with shorter OS and may serve as a prognostic factor for poor prognosis in SHH-MB patients, possibly related to TP53 mutations.^{19,20}

In glioblastoma (GBM), the expression of adhesion genes (neurofascin and parvin beta), hypoxia response genes [mutL homolog 1 (MLH1), endothelial PAS domain protein 1 (HIF- α), RUNX family transcription factor 3 (RUNX3), N-myc downstream regulated 1 (Ndr g1), Ndr g2, and Ndr g4] were synergistically regulated by G9a and poly (ADP-ribose) polymerase 3 (PARP3). Hypoxia is a hallmark of GBM invasion and is associated with significant cytoskeletal remodeling. G9a may enhance the vulnerability of the cytoskeleton microtubules and affect the sensitivity of GBM cells to microtubule destabilizers.²¹

The G9a is also studied in Gliomas. Its knockdown can reduce programmed cell death ligand 1(PD-L1) expression, increase major histocompatibility complex class I (MHC-I) expression, delay tumor growth in immunocompetent glioma mouse models, and prolong survival. G9a can interact with the Notch Inhibitor F-box and WD repeat domain containing 7 (FBXW7). Furthermore, it can methylate the FBXW7 promoter to inhibit gene transcription, thereby promoting stem cell characteristics and creating an immunosuppressive tumor microenvironment.²²

Respiratory System Tumors

Non-small cell lung cancer (NSCLC) is a common respiratory system tumor. Sun et al indicated that G9a could enhance NSCLC cells' ability to invade and metastasize by increasing the transcriptional activity of the nuclear factor of kappa light polypeptide gene enhancer in B cells 1 (NF- κ B), thereby significantly strengthening the expression of focal adhesion kinase (FAK) and activating its signaling pathway. NF- κ B transcriptional activity in NSCLC cells can be suppressed by targeting G9a to stabilize NF- κ B inhibitor α , exerting an anticancer effect.²³ Nuclear transcription factor Y subunit α (NFYA) is an important transcriptional activator of aldehyde dehydrogenase 2 (ALDH2). G9a increases

H3K9me2 levels on dense chromatin. Inhibiting G9a can promote the binding of NFYA to the ALDH2 promoter, activating the ALDH2 transcription function. This signaling axis mediates in-vitro and in-vivo resistance to paclitaxel.²⁸

In lung adenocarcinoma (LUAD), low G9a expression is associated with enhanced alveolar type 2 (AT2) gene expression and improved prognosis. G9a is a key regulator of the WNT signaling pathway and may be a potential target for cancer and other AT2-mediated lung pathologies.²⁴ Bufonis, an animal drug (called Chansu in China), is secreted by *Bufo gargarizans cantor* or *B. melanostictus Schneider*. Bufonis is a cardiac glycoside, a bufadienolide, with detoxification, anti-inflammatory, and analgesic effects. It can inhibit the proliferation, migration, and invasion of A549 cells and promote apoptosis by inhibiting G9a expression and activating the tumor suppressor gene FOXO1.²⁵

Furthermore, G9a might be associated with radio-resistance. G9a-controlled H3K9me3 interacts with the promoter of the tumor suppressor gene coiled-coil domain containing 8 (CCDC8) and suppresses its expression, promoting radio-resistance in lung cancer cells.²⁶ Nagaraja et al indicate that in epithelial cells, X-ray radiation can alter the global histone methylation levels, mediating the expression of radiation-induced EMT markers in lung cancer cells through the ROS/Snail/G9a pathway.²⁷

Digestive System Tumors

Compared to healthy tissues, LSD1 and G9a are upregulated in esophageal cancer (EC) tissues and this increased expression is significantly associated with poor prognosis. Mechanistically, inhibiting histone methyltransferase eraser LSD1 and writer G9a induces cell death by S-phase arrest and apoptosis, targeting the endoplasmic reticulum (RS) stress pathway, thereby increasing this in-vitro and in-vivo effect.²⁹

Hypoxia significantly increases the G9a protein level, which interacts with the Runt domain of RUNX3, increasing its methylation at K129 and K171, promoting cell cycle progression or division, and enhancing the growth of gastric cancer (GC) cells. G9a also inhibits immune responses and cell apoptosis, promoting early tumor growth.³⁰ Cinnamaldehyde (CA) is a bioactive natural flavonoid acquired from *Cinnamomum cassia* and is a potent anticancer compound. Flavonoids such as luteolin, quercetin, and CA exert their effects by inhibiting histone deacetylases (HDAC). Kim et al indicated that G9a inhibition can regulate CA-induced autophagic cell death in GC cells, showing potential for clinical application.³¹ The study by Wang et al found that the expression of SPINK1 and G9a in GC tissues is positively correlated and is closely related to tumor infiltration, TNM staging, and lymph node metastasis but independent of patient age, sex, differentiation degree, and tumor location. However, the specific interaction mechanisms between them remain unclear.³² G9a is also linked with GC metastasis. GC tissues have increased expression of Calreticulin (CALR), which is associated with lymph node metastasis and poor patient prognosis. It can promote the migration of GC cells in-vitro and in-vivo. CALR can mediate DNA methylation of the E-cadherin promoter by interacting with G9a, the knockdown of which can restore CDH1 expression and block the stimulating effect of CALR on GC cell migration.³³

Increased mRNA and protein expression of G9a are associated with poor OS and DFS in HCC patients. Mechanistically, G9a can target liver-specific miR-122, suppress its expression, and promote colony formation and invasion of HCC cells.³⁴ Nakatsuka et al suggested that DNA damage recruits G9a to the p53 response element of the pro-apoptotic Bcl-2 family member Bcl-G gene, impairing p53 enrichment in that region and reducing Bcl-G expression. Conversely, G9a downregulation enables p53 recruitment and upregulates Bcl-G expression, indicating that G9a silences Bcl-G to allow DNA-damaged liver cells to escape p53-induced apoptosis.³⁵ Interestingly, Xia et al revealed that G9a-mediated H3K9me2 silences the expression of solute carrier family 7 member 2 (SLC7A2). SLC7A2 decrease upregulates CXCL1 via the phosphatidylinositol 3-kinase/serine/threonine kinase 1 (AKT)/NF- κ B pathway. This pathway recruits myeloid-derived suppressor cells and exerts tumor immune-suppressive effects, thereby inhibiting HCC metastasis.³⁶ Furthermore, Bai et al indicated that G9a elevates liver cancer cells' malignant behaviors, such as proliferation, invasion, and metastasis, by affecting the expression of matrix metalloproteinase 2 (MMP2), MMP9, CD44V6, and vascular endothelial growth factor (VEGF).³⁷ Hepatoblastoma (HB) is the most frequently occurring liver cancer in children. A significant positive correlation exists between the mRNA expression levels of G9a, DNMT1, and UHRF1 in HB tissues. G9a is significantly upregulated in malignant tumor epigenetic and transcriptomic features, and inhibiting G9a significantly counteracts MYC-driven transcriptome in HB cells, reducing tumor development.³⁹ This association was also observed in HCC in a study by Thng et al, which demonstrated that the interaction between MYC

and G9a cooperatively regulates MYC-dependent gene suppression. Furthermore, G9a stabilizes MYC to promote cancer development and contributes to the growth and invasive capacity of HCC. The combined therapy targeting the synthetic lethal targets of G9a and MYC, cyclin-dependent kinase 9 (CDK9), has shown potent efficacy in MYC-driven HCC patients.³⁸

Cholangiocarcinoma (CCA) is a disease with increased mortality and no effective treatment. Zhang et al discovered that NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH) inhibits CCA cell growth in-vitro and in xenograft models. Furthermore, it was revealed that G9a is recruited to the 15-PGDH gene promoter via protein–protein interaction with the E-box bound MYC/Max heterodimer. Recruited G9a enhances H3K9 methylation, silencing the 15-PGDH gene. The 15-PGDH and G9a expression indicated a negative correlation between human and murine CCA.⁴⁰ Additionally, G9a expression was increased in thioacetamide-induced intrahepatic CCA (iCCA). EGFR signaling and mutant KRAS can activate interleukin 6 (IL6) production in CCA cells. Moreover, in human iCCA, G9a expression also correlated with phosphoglycerate dehydrogenase (PHGDH), the rate-limiting enzyme in the serine-glycine pathway, which was upregulated during iCCA. KRAS promotes PHGDH expression in a G9a activity-dependent manner, diverting glucose towards serine synthesis and increasing CCA cell viability.⁴¹

The incidence and mortality rates of pancreatic tumors are increasing and 80–85% of patients present unresectable advanced disease, necessitating the urgent need for effective therapeutic approaches. Member A of the family with sequence similarity 110 member A (FAM110A) plays a role in cell cycle-related biological processes and has been implicated in the pancreatic ductal adenocarcinoma (PDAC) pathogenesis. Huang et al discovered that the Tetraspanin1/FAM110A/Histone Cluster 1 H2B Family Member K (HIST1H2BK)/G9a pathway regulates PDAC progression. This pathway was validated through RNA-sequencing, Western blotting, luciferase reporter gene, and tumor phenotype rescue experiments, providing new prognostic and therapeutic strategies for PDAC treatment.⁴²

Colorectal cancer (CRC) is the fourth leading cause of cancer-related deaths worldwide with approximately 9 million deaths annually. The G9a expression level is associated with adverse CRC outcomes, and pharmacological G9a inhibition can effectively stimulate the differentiation of CRC stem cells and limit their self-renewal ability and tumorigenicity. The WNT/ β -catenin pathway, extracellular matrix organization, and EMT may constitute crucial networks regulated by G9a, suggesting that it could affect the phenotype of cancer stem cells.^{43,44} Gimeno et al indicated that G9a is associated with the human zinc finger protein 518B (ZNF518B) gene, and interacts with the regulator of G protein signaling 4 (RGS4) and peptidyl arginine deiminase 3 (PADI3) in a ZNF518B-dependent manner, both of which are implicated in CRC.⁴⁵ Furthermore, Ichikawa et al discovered that G9a inhibits cancer stemness and enhances chemosensitivity in CRC via IL8 modulation, offering a novel prognostic marker for predicting CRC patients' prognosis. G9a inhibition can suppress cancer stem cell activity in human CRC and synergize with 5-fluorouracil in inhibiting CRC proliferation.^{46,47} The human microbiome plays a significant role in the body's immune system, food digestion, and protection against harmful intestinal tract bacterial invasion. For instance, butyrate promotes proteasomal degradation of G9a by upregulating the HECT domain E3 ubiquitin ligase 2 (HECTD2), inhibiting CRC growth.⁴⁸

Genitourinary System Tumors

Breast cancer (BCa) is one of the most extensively studied malignancies worldwide. The MYC gene dysregulation plays a ubiquitous role in human cancers and has been associated with G9a. Inhibiting G9a either through drugs or gene targeting can suppress the growth of MYC-dependent BCa.⁴⁹ Furthermore, targeting the MYC/G9a/Ferritin heavy chain (FTH1) axis could also inhibit the occurrence and progression of BCa.⁵⁰ FTH1 is part of the iron storage protein and interacts with MYC and G9a genes, thereby altering cellular iron availability. Silencing FTH1 promotes BCa cell growth and migration and is associated with increased expression of MYC and G9a.⁵¹ In Luminal A-type BCa, G9a knockdown significantly increases the expression of bone morphogenetic protein 5 (BMP5), inducing SMAD proteins' phosphorylation (the TGF- β family's main mediators). SMAD proteins serve as intracellular signaling mediators for BMP5, therefore BMP5 could be a novel target of G9a and may regulate BCa cells' ability to grow and metastasize.⁵² Triple-negative BCa (TNBC) is the subtype with the highest recurrence and mortality rates. Aftab et al revealed that under low glucose conditions, the expression of EMT structural proteins and regulatory molecules is downregulated in MDA-MB-231 TNBC cells, while that of G9a, Snail, fructose-1,6-bisphosphatase, MMP13, and pyruvate kinase M1/2 is upregulated.

This MDA-MB-231 cancer cells' resistance mechanism activation allows them to respond to the stress of the tumor microenvironment, thereby increasing cancer invasion and migration.⁵³

The prognosis of high-grade serous ovarian carcinoma (HGSC) is directly related to the presence of intratumoral lymphocytes. Epigenetic immune stimulatory genes' silencing is associated with immune evasion in HGSC. These genes' re-expression promotes tumor immune clearance, whereas G9a and EZH2 inhibition stimulates the CXCL10-CXCR3 axis and increases effector lymphocytes and natural killer cells tumor infiltration, altering the immune microenvironment and reducing tumor growth.⁵⁴

Prostate cancer (PCa) is the most common male malignancy in Western countries and is also linked with G9a-related regulatory mechanisms. PALI1 is a newly identified accessory protein of the Polycomb repressive complex 2 (PRC2) that catalyzes H3K27 methylation. It has been indicated to interact with G9a and generate a unique G9a-PALI1-PRC2 super-complex, which occupies a subset of G9a target genes and mediates dual H3K9/K27 methylation and gene repression. Many of these genes are developmental regulatory factors required for cell differentiation, and their loss in PCa signifies poor prognosis. These genes promote in-vitro PCa cell proliferation and invasion and in-vivo xenograft tumors' growth.⁵⁵ CULLIN3-RBX1 E3 ubiquitin ligase complex (SPOP) normally binds and promotes the polyubiquitination and degradation of methyltransferases and DNMT interactor euchromatic histone lysine methyltransferase 1 (GLP). In PCa cells, SPOP knockdown increases the GLP-G9a complex expression. SPOP mutations can enhance PCa cell growth, but this tendency can be blocked by paclitaxel.⁵⁶ G9a can inhibit endoplasmic reticulum protein 29 (ERP29) transcription and promote radioresistance in PCa cells by regulating the methylation levels.⁵⁷

Furthermore, G9a expression has been observed to be significantly increased in renal cell carcinoma (RCC) than in adjacent tissues, and its higher expression is closely associated with advanced stages of RCC. In RCC, G9a interacts with the promoter region of SPINK5 and affects its expression, thereby inhibiting tumor suppressor gene p53 expression and exerting carcinogenic effects.⁵⁸

Hematological System Tumors

The inhibition of SUV39H1 is necessary for the development of acute myeloid leukemia (AML), and its loss activates the interferon (IFN) pathway, which mechanistically occurs by destabilization of the complex composed of SUV39H1 and two methyltransferases (G9a and GLP).⁵⁹ Furthermore, AML patients indicate increased expression of G9a and lysine demethylase 2B (KDM2B), associated with detrimental genomic alterations. In response to induction chemotherapy, G9a and KDM2B are significantly higher in patients with drug resistance and relapse than in complete remission patients.⁶⁰ G9a has also been associated with acute lymphoblastic leukemia (ALL), and its inhibition promotes lysosome biogenesis, which inhibits the metabolic sensor sestrin 2 (SESN2), represses glycogen synthase kinase-3 (GSK-3)-related autophagic degradation, and ultimately impairs glycogen metabolism.⁶¹ Moreover, G9a targets the tumor suppressor factor SRY-box transcription factor 6 (SOX6), therefore, targeting G9a to upregulate SOX6 can inhibit self-renewal ability in chronic myeloid leukemia (CML).⁶²

The overexpression of G9a has been reported in several cancers, such as multiple myeloma (MM), in which it is associated with disease progression, metastasis, and poor prognosis. According to a study by Smedt et al, in newly diagnosed and relapsed MM patients, high G9a RNA levels were linked with worse disease outcomes. Targeting G9a/GLP promotes autophagy-related cell apoptosis by inactivating the mechanistic target of rapamycin kinase (mTOR)/eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1) pathway. Furthermore, reducing MYC levels activates p-38 and mitogen-activated protein kinase 9 (MAPK9)/MAPK8 signaling, and sensitizes MM cells to proteasome inhibitors such as bortezomib and carfilzomib.⁶³ Consistently, Smedt et al and Kazuya et al revealed that inhibition of EZH2 and G9a significantly upregulates IFN-stimulated genes and inhibits the IFN regulatory factor 4 (IRF4)-MYC axis in MM cells. Therefore, this approach could reduce H3K9/K27 methylation and increase the expression of endogenous retrovirus genes.⁶⁴

Lymphomas can affect any organ in the body and present multiple symptoms. In the study by Wang et al, 68.57% (24/35) of mantle cell lymphoma (MCL) patients indicated G9a expression; however, it was not expressed in any reactive hyperplasia. Knockdown of G9a regulates numerous genes and enrichment of relevant signaling pathways, enhancing apoptosis and inducing G0/G1 arrest in MCL cells. Suggesting, that G9a might be associated with cell cycle protein.⁶⁵

Sarcomas

The G9a gene-silencing promotes tumorigenesis in various tumor types, including alveolar rhabdomyosarcoma (ARMS). ARMS indicates overexpression of the orphan nuclear receptor 4A1 (NR4A1), which exhibits oncogenic activity. The NR4A1/Sp1 (a transcription factor) complex interacts with the GC-rich region in the G9a promoter to regulate G9a gene expression.⁶⁷ Gualtieri et al suggested that DEAD-box helicase 5 (DDX5) directly interacts with G9a mRNA, regulating its stability and subsequent protein expression. Furthermore, DDX5 acts upstream of the G9a/AKT survival signaling pathway and slows ARMS growth in a xenograft model.⁶⁸ Moreover, Karthik et al found that the histone variant H3.3 is overexpressed in ARMS, and knockdown of the H3.3-encoding gene H3F3A significantly impairs ARMS cells' migration and invasion ability. G9a is an upstream H3F3A regulator, whereas melanoma cell adhesion molecule (MCAM/CD146) is a direct downstream target of H3.3 and a potential therapeutic target.⁶⁹

In Ewing sarcoma (EWS) tumors, G9a overexpression is associated with poor prognosis. The expression of G9a is significantly higher in metastatic EWS tumors than in primary or recurrent tumors. During EWS metastasis, G9a is directly targeted and affected by Sialidase (NEU1), the overexpression of which impairs migration, invasion, and clonogenicity of EWS cell lines. Inhibition of G9a leads to NEU1 overexpression, impairing in-vitro and in-vivo metastasis.⁷⁰

Other Tumors

In head and neck squamous cell carcinoma (HNSCC), bromodomain containing 4 (BRD4) acts as a transcriptional repressor and recruits G9a to suppress MHC-I expression, thereby inhibiting anti-tumor immunity.⁷¹ One of the important prognostic biomarkers of oral squamous cell carcinoma (OSCC) is serine protease inhibitor kazal-type 5 (SPINK5); G9a binds to the SPINK5 promoter and inhibits SPINK5 expression through methylation. Overexpression of SPINK5 can reverse the stimulatory effect of G9a on the invasiveness of OSCC cells by weakening the WNT/ β -catenin pathway and inhibiting tumor growth in-vivo.⁷²

In papillary thyroid carcinoma (PTC), tescalcin (TESC) is a potential radiation-induced biomarker, which is activated by the overexpression of long non-coding RNA ROR by inhibiting G9a recruitment and H3K9me on the TESC promoter, subsequently activating the TESC/ALDH1A1/ β III-tubulin/tensin homolog (PTEN) axis and suppressing intratumoral PTEN to promote tumor growth.⁶⁶

Research on G9a Inhibitors

There have been multiple studies on G9a inhibitors (Table 2), for example, CM-272, a dual inhibitor of DNMT and G9a, has shown promising results in preventing tumor-related bone loss and reducing tumor burden in a mouse model of MM, thus reversing MM-associated bone disease.⁸⁰ CM-272 is a novel, selective, reversible chemical probe that can be used for in vitro treatment of hematological diseases, inhibiting cell proliferation and promoting cell apoptosis, inducing interferon stimulated gene and immunogenic cell death.⁸¹ CM-272 can reduce the growth, migration, and invasion ability

Table 2 The Cancer Type and Function of G9a Inhibitor in Various System Tumors

Inhibitor	Cancer	Function
CM-272	MM HCC CCA HB	Reversing MM-associated bone disease Inhibiting cancer progression Reduce progression of CCA cells both in vivo and in vitro Regulating the expression and half-life period of c-MYC genes
UNC0642	Melanoma BCa, cancer, melanoma Melanoma Melanoma	Inducing tumor cell cycle arrest and apoptosis in vitro Induces gene expression related to RS stress and ROS production Inducing melanoma cell death

Abbreviations: BCa, breast cancer; CCA, cholangiocarcinoma; HB, hepatoblastoma; HCC, hepatocellular carcinoma; MM, multiple myeloma.

of CCA cells both *in vivo* and *in vitro*. However, it does not affect the organoid activity established by healthy human bile duct cells. The organoid activity established by healthy human bile duct cells is not affected, and it can also reduce liver injury markers to a certain extent. Its immunosuppressive microenvironment is alleviated to a certain extent, and it has a synergistic effect with cisplatin and lapatinib.⁸² Similar effects were also observed in HB cells by regulating the expression and half-life period of c-MYC genes, which are crucial in HB cells.³⁹ The epigenetic mechanism can affect the development of gastric cancer. It has been proven that depletion of G9a effectively reduces histone methylation levels and triggers GC cell apoptosis. CM-272's dual targeting of G9a and DNMT1 can affect glucose metabolism, which is crucial for gastric cancer, and may also regulate immune therapy unresponsive GC through epigenetic regulation. In the future, studying the diagnosis and treatment of GC from an epigenetic perspective is also a research with great potential.⁸³ The combined inhibition of G9a and EZH2 induces gene expression related to RS stress and ROS production. IL24, a common target of G9a and EZH2, is crucial for inducing tumor cell death. Loss of function of G9a and EZH2 activates the IL24-ER stress axis and increases apoptosis in cancer cells without affecting normal cells.⁸⁴ There are also studies that have shown that UNC0642 can exert an effect on the target gene LC3B of G9a, inducing melanoma cell death.⁸⁵ In the study by De Beck et al, inhibition of G9a and DNMT-induced tumor cell cycle arrest and apoptosis through targeted epigenetic reprogramming *in-vitro*. Consistent with a transient delay in tumor growth and enhanced IFN-I response in immunocompetent mice, improving the efficacy of T-cell receptor redirected T and dendritic cell vaccines, thereby increasing the overall survival of tumor-bearing mice.⁸⁶ Furthermore, there are 3D-QSAR pharmacophore-based studies presenting novel and safer lead compounds targeting G9a, which have reduced the toxicity and increased the value of G9a inhibitors in therapeutic applications.⁸⁷

Discussion

The G9a catalyzes H3 lysine residues, recruiting additional epigenetic factors and inhibiting transcription. It is highly expressed in many tissues and directly regulates key tumor pathways, affecting tumor phenotype, and is related to autophagy, chemoradiotherapy response, cancer metabolism, and tumor microenvironment alterations. Therefore, many inhibitors targeting G9a have been developed to suppress the occurrence and development of various tumors, and significant outcomes have also been achieved. However, G9a has various functions, and many tumor-related studies have been conducted since its discovery. This article mainly summarizes recent data on G9a as a regulator of tumor-related pathways to promote related biological processes and aims to provide some new diagnostic and prognostic markers for clinical diagnosis.

Recently, immunotherapy has been a hot topic in tumor treatment. Inhibitors targeting histone methyltransferase, such as G9a, also have considerable synergistic effects with immunotherapy against different cancers, including bladder cancer, HGSC, and other tumors.^{54,73,88} Immunotherapy involves the transcriptional activation of immune-stimulating genes, increasing effector lymphocytes, Natural killer cells, and other mechanisms. The immune system activation and gene methylation are two strongly related factors. G9a can inhibit and activate genetic programs associated with a variety of immune responses, many of which have been mentioned previously. In bladder cancer, treatment with CM-272 induces apoptosis and immunogenic cell death, in combination with PD-L1 can turn cold tumors into hot tumors, and in melanoma, G9a can induce immunosuppression by mediating the WNT/ β -catenin pathway.^{73,88,89} G9a expression can also be a predictive biomarker of immune checkpoint blockade response. Dual blockade of G9A and EZH2 was also found in HGSC to reprogram the immune tumor microenvironment and activate transcription in the immune network both *in vitro* and *in vivo*, indicating that G9a is an important regulator in the immune microenvironment.⁵⁴ However, clinically, patients with advanced cold tumors are relatively rare and have a small sample size, which is also a major challenge for research; animal models of the human immune microenvironment might meet the research needs.

The G9a has various activities in tumors and interacts with many common oncogenes or tumor suppressors, such as the WNT pathway, TP53, and the MYC gene as mentioned earlier.^{20,73} Since G9a has a broad role in cancer, it could be presumed to serve as a drug or marker. At present, many molecule drugs have been developed that target the G9a mechanisms with acceptable therapeutic effects; however, these have not entered clinical trials as they do not have optimized physicochemical and pharmacokinetic properties.^{84,90} More targets could be developed for its mechanism of action, for instance, via gene therapy because histone methyltransferases are more likely to demethylation a certain site of

the target gene; it acts through the level of post-transcriptional translation. Some researchers suggest that if the dysregulation of histone modification is the underlying cause of some human diseases, then reprogramming this epigenetic dysregulation could cure or alleviate these diseases or abnormalities.¹⁶ If a site of G9a or its downstream target is cut or corrected, the therapeutic effect may be better.

Conclusion

Histone methyltransferase G9a is a promising tumor marker and therapeutic target, with significant efficiency against various tumors and synergistic effects with immunotherapy. Drugs targeting G9a are also under development and are expected to be used clinically in the future.

Ethical Approval

The manuscript does not contain clinical studies or patient data.

Informed Consent

Informed consent is not required for this type of study.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no potential conflicts of interest in this work.

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