STATE-OF-THE-ART REVIEW

EHMT1/EHMT2 in EMT, cancer stemness and drug resistance: emerging evidence and mechanisms
Alamelu Nachiyappan¹, Neelima Gupta¹ and Reshma Taneja¹,²

¹ Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
² Healthy Longevity Translational Research Program, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Keywords
cancer stem cells; drug resistance; EMT; G9a; GLP; metabolism; metastasis; methylation; therapeutics

Correspondence
R. Taneja, Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Singapore
Tel: +65 6515 3236
E-mail: phsrt@nus.edu.sg

(Received 3 October 2021, revised 25 November 2021, accepted 23 December 2021)
doi:10.1111/febs.16334

Introduction

Cellular reprogramming mediated by a combination of genetic and epigenetic changes contributes to tumour progression by inducing plasticity and drug resistance [1]. Histones are subject to post-translational modifications that alter their interaction with proteins, culminating in chromatin reorganisation and altered gene regulation. Histone 3 and 4 methylation occurring at lysine (K) and arginine (R) residues are among the most prominent modifications in cancer [2]. Lysine methyltransferases (KMTs) catalyse histone

Abbreviations
5-FU, 5-fluorouracil; 53BP1, p53-binding protein 1; ABCG2, ATP-binding cassette transporter G2; ABL, ABL proto-oncogene, non-receptor tyrosine kinase; ADP, adenosine diphosphate ribose; AKT, Ak strain transforming, protein kinase B; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; ANGPTL4, angiopoietin like 4; Anti-CTLA-4, anti-cytotoxic T-lymphocyte-associated protein 4; Anti-PD, anti-programmed cell death protein 1; APC2, adenomatous polyposis coli protein 2; ATM, ataxia-telangiectasia mutated (serine/threonine kinase); AURKB, aurora kinase B; Bcl-G, proapoptotic member of Bcl-2 family; BCR, BCR activator of Rho GEF and GTPase; BLBC, basal-like breast cancer; BRCA1, breast cancer type 1 susceptibility protein; CaloGR, cervical cancer cell line; CASP1, caspase 1; CBX-3, chromobox family protein, component of PRC1 complex; CDC42, cell division control protein 42 homolog; CDH, cadherin; CDKN1A, cyclin-dependent kinase inhibitor 1A; CDYL, chromodomain on Y-like 2; CHK-1, checkpoint kinase 1; circEHMT1, EHMT1 circular RNA; CLBC, claudin-low breast cancer; CRC, colorectal cancer; CSC, cancer stem cell; CXCL, chemokine (C-X-C motif) ligand 1; DCIS, ductal carcinoma in situ; dCK, deoxycytidine kinase; DHCR7, 7-dehydrocholesterol reductase; DNMT, DNA methyltransferases; DPP4, dipeptidylpeptidase IV; DSB, double-stranded break; DSC3, Desmocollin 3; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; EHMT1, euchromatin histone methyltransferases 1; EHMT1/2, EHMT1/EHMT2 EMT-TF, epithelial-to-mesenchymal transition-inducing transcription factor; EHMT2, euchromatin histone methyltransferases 2; EMT, epithelial-to-mesenchymal transition; EpCAM, epithelial cell adhesion molecule; ER, estrogen receptor; ERK, extracellular signal-regulated kinase (serine/threonine protein kinase).
metylation by transferring a methyl group from S-adenosyl methionine (SAM) on to a specific lysine residue [3]. Histone modification-mediated promoter methylation affects chromatin conformation and promoter accessibility, thereby modulating tissue-specific gene expression [4]. Since the initial discovery of suppressor of variegation 3-9 homolog 1 (SUV39H1/ KMT8) in 2000, numerous others KMTs have been discovered and characterized based on their structure and substrate specificity. Depending upon the substrate, lysine residue and degree of methylation (mono-me1/di-me2/tri-me3), KMTs can impart either active or repressive marks. A majority of KMTs are SET domain-containing proteins that methylate histone and non-histone proteins, thus contributing to transcriptional regulation, genome stability, DNA repair and RNA processing.

Among the SET domain-containing enzymes, EHMT1 and EHMT2 exhibit diverse biological roles and their deregulation is associated with various cancers [5–8]. Multiple studies have demonstrated elevated EHMT2 (also known as G9a) expression in different cancers including breast, melanoma, ovarian, prostate, lung, bladder, colon, cervical, gastric, head and neck, neuroendocrine, hepatocellular, multiple myeloma and haematological malignancies [9,10]. EHMT1 (also known as GLP) is also elevated in a few cancers including gastric, lung, breast, ovarian and oesophageal squamous cell carcinoma [10]. The overexpression of EHMT1 and EHMT2 has been attributed to high

Abbreviations Continued
ESA, epithelial-specific antigen; ESCC, esophageal squamous cell carcinoma; EZH2, enhancer of zeste homolog 2; FAK, focal adhesion kinase; FBP-1, fructose-1,6-bisphosphatase; FOXP1, forkhead Box P1; GBM, glioblastoma; GC, gastric cancer; GCLC, glutamate cysteine ligase catalytic subunit; GEM, gemcitabine; GLI, GLI family zinc finger 1; GLP, G9a-like protein; GLUT1, glucose transporter 1; GM-CSF, granulocytemacrophage colony-stimulating factor; GR, glucocorticoid receptor; GSEA, gene-set enrichment analysis; GSH, glutathione; H3K9, histone 3 Lysine 9; HDAC, histone deacetylases; HEN1, human equilibrative nucleoside transporter 1; HEPH, hephaestin; HER2, human epidermal growth factor receptor 2; HGSOC, high-grade serous ovarian carcinoma; Hh, hedgehog; HKII, hexokinase 2; HMLE, homeobox protein Hox-C8; HP-1, heterochromatin protein 1; HPDE, pancreatic ductal epithelial cells; HSCs, haematopoietic stem cells; IDC, invasive ductal carcinoma; IFNγ, interferon beta; IHC, immuno-histochemistry analysis; IL-8, Interlekin-8; ING, Inhibitor of growth; ITGB, integrin β; kixβ, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; JIB, JmjC family of lysine methylase inhibitor; JPH3, junctophilin 3; K, lysine; KAP-1, KRAB domain-associated protein 1; KDM, lysine demethylase; KMT, lysine methyl transferases; KRAS, KRAS proto-oncogene, GTPase; LDHA, lactate dehydrogenase-A; Lgr5, leucine-rich repeat-containing G-protein coupled receptor 5; IncRNAs, long non-coding RNAs; LSH, lymphocyte-specific helicase; MASPIN, mammary serine protease inhibitor; MCP-1, monocyte chemoattractant protein 1; me1, monomethylation; me2, dimethylation; me3, trimethylation; MEK, mitogen-activated protein kinase kinase; MEKi, mitogen-activated protein kinase kinase inhibitor; MEK1, mouse epidermal growth factor receptor; MEK2, mitogen-activated protein kinase 2; MM, multiple myeloma; MMP, matrix metallopeptidase; MSK, mitogen- and stress-activated kinase; MTH 1h, metallothionein 1h; MYC, MYC proto-oncogene, BHLH transcription factor; Nalm6, B-cell precursor leukemia cell line; NANOsiRNA, homebox protein; NEAT1, nuclear paraspeckle assembly transcript 1; NF-κB, nuclear factor kappa light polypeptide gene enhancer in B-cells inhibitor, kappa; NHEJ, homologous recombination (HR) and non-homologous end joining; NSCC, non-small cell lung cancer; NSCLC, Non-small cell lung cancer; OCT3/4, octamer-binding transcription factor 3/4; O-GlcNAc, O-linked β-N-acetylgalactosamine; OGT, O-linked N-acetylglucosamine transferase; OLIG2, oligodendrocyte transcription factor 2; OXPHOS, oxidative phosphorylation; PAN1, pancreatic cancer cells; PAN1-C-R, gemcitabine-resistant pancreatic cancer cell line; PARP1, poly(ADP-ribose) polymerase; PARPi, PARP inhibitor; PCL3, polyclub like 3; PDAC, pancreatic ductal adenocarcinoma; PD-L1, anti-programmed cell death ligand 1; POX, patient-derived xenografts; PERK, protein kinase R (PKR)-like endoplasmic reticulum kinase; PGHD, phosphoglycerate dehydrogenase; PI3K/mTOR, phosphatidylinositol 3-kinase/ mechanistic target of rapamycin; PKM2, pyruvate kinase M2; PKP3, plakophilin 3; Pou5f2, Pou domain, class 3, transcription factor 2; PP2A, protein phosphatase 2 A; PR, progesterone receptor; PRC2, polycomb repressive complex 2; PSAT1, phosphoserine aminotransferase 1; PSFH, phosphoserine phosphatase; PTEN, phosphatase and tensin homolog deleted on chromosome 10; R, arginine; RARRES3, retinoic acid receptor responder protein 3; RB, retinoblastoma protein; Reg IV, regenerating protein IV; RNF168, ring finger protein 168; ROBO1, missense mutation in human receptor roundabout 1; ROS, reactive oxygen species; RPA, replication protein A; SALL2, sal-like protein 2; SAM, S-adenosyl methionine; SAPK/JNK, c-Jun-N-terminal kinases; SATB2, special AT-rich sequence-binding protein 2; SC, stem cell antigen-1; SDF, stromal cell-derived factor; Serpin E1, serine proteinase inhibitor; SETD5, SET domain containing 5; SHMT2, serine hydroxymethyltransferase 2; SOX2, sex-determining region Y-box 2; SP, specificity protein 1 (transcription factor); SREBF2, sterol regulatory element-binding protein 2; STAMP1, STAM-binding protein like 1; STAT3, signal transducer and activator of transcription 3; SUV39H1, suppressor of variegation 3-9 homolog 1; TCA, citric acid cycle; TCGA, the cancer genome atlas; TERT, telomerase reverse transcriptase; TIC, tumour-initiating Cells; TIGAR, TP53-induced glycolysis and apoptosis regulator; TIMP, tissue inhibitor of metalloproteinases; TLRs, toll-like receptor inhibitors; TNA1, Talin1; TME, tumour microenvironment; TNBC, triple-negative breast cancer; TPC, tumour propagating cells; TPR, transforming growth factor; TPR53, tumour suppressor; TRERNA1, translation regulatory long non-coding RNA 1; TSPAN15, tetraspanin 15; u-PA, plasminogen activator, urokinase; VEGF, vascular endothelial growth factor A; VLA-4, integrin α4β1 or very late antigen-4; Wnt, wingless-related integration site; ZEB, zinc finger E-box-binding homeobox 1 or 2; α-KG, α-ketoglutarate; γ-H2AX, H2A histone family member X.
copy number, transcriptional dysregulation and, to a lesser extent, mutational changes. Collectively, studies thus far have shown that EHMT1 and EHMT2 primarily possess oncogenic roles, although a few studies have highlighted tumour-suppressive roles as well.

Deregulated EHMT1 and EHMT2 expression is associated with advanced tumour stage, metastasis and poor prognosis and is thus of clinical relevance. The aberrant epigenetic landscape mediated by EHMT1/EHMT2 (EHMT1/2) is linked with cancer-promoting events including cellular proliferation, metabolic adaptation, EMT, metastasis, stress tolerance/response and stem cell maintenance [10–12]. We discuss EHMT1/2-dependent mechanisms involved in EMT, stemness, drug resistance and metabolism.

Cancer metastasis

Cancer cells adapt to multiple constraints posed by the tumour microenvironment by altering the genetic and epigenetic landscape. Metastasis is associated with acquisition of mesenchymal characteristics and loss of epithelial traits. EMT is a dynamic, reversible process which involves transition of epithelial cells to mesenchymal-like cells by gaining characteristics including motility, invasiveness and resistance to apoptosis, while losing cell–cell adhesion and apical–basal polarity [13]. These changes are apparent by the downregulation of epithelial markers such as E-cadherin, and upregulation of mesenchymal markers such as N-cadherin and vimentin. EMT is associated with invasion, migration, stroma formation, metastasis, cellular plasticity and drug resistance [14]. In this section, we discuss the targets and mechanisms underlying EHMT1/2 in driving EMT and metastasis (Fig. 1 and Table 1) in distinct cancers.

Breast cancer

Breast cancer is highly heterogeneous and is classified into multiple subtypes based on molecular differences (hormone receptor and gene expression pattern) [15]. Interestingly, transforming growth factor beta (TGF-β)-induced EMT in normal breast epithelial cell lines is associated with EHMT1/2-mediated H3K9me2 repression mark at the E-cadherin promoter. EMT was apparent by the induction of Snail, the downregulation of epithelial markers E-cadherin and claudin-3 and -7, and acquisition of mesenchymal markers vimentin and N-cadherin [11].

EHMT2 is overexpressed in breast cancer. High EHMT2 expression is associated with uncontrolled proliferation, metastasis and recurrence [16]. Triple-negative breast cancers (TNBCs) are deficient in oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2) and are characteristically highly aggressive and recurrent [17]. Treatment with the EHMT2 inhibitors (UNC0638 and BIX-01294) or siRNA-mediated inhibition of EHMT2 reduced metastasis in TNBC cell lines. Suppression of metastasis was accompanied by reduced expression of EMT transcription factors (EMT-TFs), Snail-1 and Snail-2/Slug and mesenchymal markers (N-cadherin and vimentin), and increased expression of epithelial markers (E-cadherin and claudin) and E-cadherin promoter activity. Furthermore, EHMT2 knockdown in TNBC cell lines resulted in altered cell adhesion, microtubule formation and depolymerisation of gene signatures. Mechanistically, EHMT2 suppressed E-cadherin by regulating the expression and activity of MSK1, which transcriptionally upregulates Snail-1 and promotes metastasis in TNBC [18,19]. Apart from EMT markers, EHMT2-associated H3K9me2 and DNA methyltransferases (DNMT)-mediated DNA methylation were pivotal in the transcriptional repression of desmocolin 3 (DSC3) and mammary serine protease inhibitor (MAS-PIN), which are anti-metastatic and tumour suppressor genes respectively [20]. EHMT2 knockdown exhibited similar changes in EMT and metastasis in claudin-low breast cancer (CLBC) subtype. In addition, activation of signalling pathways including TGF-β and β-catenin suppressed tumour growth and lung colonisation. Mechanistically, Snail-mediated recruitment of EHMT2 and subsequent association with DNMTs were responsible for E-cadherin promoter methylation and its transcriptional silencing [11].

Hypoxia influences tumour growth and metastasis. Under hypoxic conditions, EHMT2 was stabilised through reduced proline hydroxylation, which impairs its proteasomal degradation. The increased EHMT2 levels under hypoxic conditions downregulated genes involved in tumour suppression, leading to increased motility and survival of breast cancer cells. In addition, EHMT2 transcriptionally silenced CDH10, a type II cadherin, leading to breast cancer metastasis [21,22].

Leptin is associated with obesity and tumour progression in various cancers [23,24] and contributes to breast cancer progression by activating signal transducer and activator of transcription 3 (STAT3). STAT3 was found to recruit EHMT2, resulting in suppression of miR-200c-3p and leading to EMT in MCF ER+ breast cancer cells and tumour aggressiveness in vivo. Interestingly, enrichment of STAT3-EHMT2 at the miR-200c-3p promoter was higher in TNBC as compared to the luminal subtype [25]. The recruitment and interaction of EHMT2 with Chromodomain on Y-like 2 (CDYL) transcriptionally repressed miR124. The
downregulation of miR124 is associated with invasive, migratory and stemness of breast cancer cells through activation of NF-κB and STAT3 signalling [26].

EHMT2 is a mediator of hypoxic response in breast cancer and it is apparent that EHMT2 is overexpressed in different breast cancer subtypes and exerts epigenetic...
Table 1. Metastasis-related genes that are regulated by EHMT2. In multiple cancers, E-cadherin expression is regulated by EHMT2. In addition, high-throughput sequencing and molecular studies have revealed that several additional metastasis genes are regulated by EHMT2. A list of EHMT2-regulated genes, their function and effect on metastasis is listed. ↑Upregulated; ↓Downregulated.

| Cancer, HNSCC, Endometrial, Gastric, Cervical, Pancreatic, Ovarian, HCC, NSCLC and Osteosarcoma | Genes regulated by EHMT2 | Gene function | Effect on metastasis | References |
|---|---|---|---|---|
| Breast | ↓E-cadherin (CHD1) | Calcium-dependent transmembrane protein – Epithelial cell adhesion | Promotes | [11,18,32,33,38,43,54,55,58,65] |
| | ↑E-cadherin (CHD1) | Calcium-dependent transmembrane protein – Epithelial cell adhesion | Promotes | [11,18,32,33,38,43,54,55,58,65] |
| | ↓Claudin1 (CLDN1) | Transmembrane protein – Epithelial or endothelial Cell – Cell adhesion | Promotes metastasis | [18,19] |
| | ↑Vimentin (VIM) | Class III intermediate filaments in mesenchymal cells – Stabilises cytoskeletal interaction | Promotes metastasis | |
| | ↑Fibronectin (FN1) | Glycoprotein – Cell adhesion and Migration | Promotes metastasis | |
| | ↑Mitogen- and stress-activated kinase (MSK) | Nuclear protein phosphorylates Histone 3 | Promotes metastasis | |
| | ↓Cadherin 10 (CDH10) | Calcium-dependent integral membrane protein – Epithelial cell adhesion | Promotes metastasis | [21] |
| | ↓Desmocollin 2 (DSC2) | Glycoprotein – Cell–Cell adhesion | Promotes metastasis | [20] |
| | ↓MASPIN (SERPINB5) | Protease inhibitor – ECM degradation | Promotes metastasis | |
| | ↓CEACAM7 | Glycoprotein – Cell–Cell adhesion | Promotes metastasis | [22] |
| | ↓miR-200c-3p (miR-200c) | Non-coding RNA – Tumour suppressor | Promotes metastasis | [25] |
| | ↓miR124 | Non-coding RNA – Post-translational regulation of gene expression | Promotes invasion and migration | [26] |
| | ↑Integrin-β3 (ITGB3) | Integral cell surface protein – Cell adhesion and signalling | Promotes metastasis | [37] |
| Cervical | ↑Angiogenin (Ang) | RNAase superfamily – Angiogenesis | Associated with metastasis | [39] |
| | ↑Interleukin-8 (IL-8) | CXC chemokine family – Angiogenesis; proinflammation | Associated with metastasis | |
| | ↑CXC motif chemokine ligand 16 (CXCL16) | Chemotactic factor – Angiogenesis | Associated with metastasis | |
| | ↑Tissue inhibitor of metalloproteinases 1 & 4 (TIMP1&4) | Inhibitor of MMP – ECM degradation | Promotes metastasis | |
| | ↑Thrombospondin (THBS1) | Glycoprotein – Cell–Cell/ECM interaction | Induces metastasis | |
| | ↑Serpine 1 | Serine protease inhibitor – tumour progression | Facilitates metastasis | |
| | ↑Vascular endothelial growth factor (VEGF) | Growth factor–vascular endothelial migration/ angiogenesis | Associated with metastasis | |
| | ↑Matrix Metalloproteinase 9 (MMP9) | Breakdown of ECM | Promotes metastasis | |
| Ovarian | ↑Type 11 and 12 collagen (COL11A1 and COL12A1) | Structural component of ECM | Promotes invasion | [43] |
| | ↑Elastin Microfibril Interface located protein 2 (EMILIN2) | ECM glycoprotein – Cell–ECM interaction | Promotes invasion | |
| | ↑Collagen triple-helix repeat containing 1 (CTHRC1) | Exact function is unknown | Promotes metastasis | |
| | ↑Integrin α2 (ITGA2) | Transmembrane receptor – Cell–ECM interaction | Promotes invasion | |

The FEBS Journal 289 (2022) 1329–1351 © 2022 Federation of European Biochemical Societies.
| Cancer     | Genes regulated by EHMT2                                                                 | Gene function                                      | Effect on metastasis | References |
|------------|----------------------------------------------------------------------------------------|---------------------------------------------------|----------------------|------------|
| HCC        | ↑Neural cell adhesion molecule L1 (L1CAM)                                               | Axonal glycoprotein – Cell–Cell adhesion          | Promotes invasion & migration |            |
|            | ↑Dachsous cadherin-related 1 (DCHS1)                                                    | Calcium-dependent Cell adhesion                   | Promotes migration    |            |
|            | ↑Melanoma adhesion molecule (MCAM)                                                      | Cell–Cell/ECM adhesion                            | Promotes metastasis   |            |
|            | ↑Desmocollin 2 (DSC2)                                                                   | Calcium-dependent glycoprotein – Cell–Cell adhesion| Promotes invasion & migration |            |
|            | ↑Dual specificity phosphatases (DUSP1, DUSP3 and DUSP9)                                 | Tumour suppressor – Negative regulator of MAPKinase| Promotes invasion     |            |
|            | ↑Epithelial cell adhesion molecule (EpCAM)                                               | Calcium-dependent transmembrane protein – Epithelial cell adhesion | Promotes metastasis | EMT/metastasis |
|            | ↑GADD34 (PPP1R15A)                                                                      | Tumour suppressor – Induces growth arrest          | Promotes metastasis   |            |
|            | ↑Sprouty4 (SPRY4)                                                                       | Inhibitor of Ras/MAPK signalling cascade           | Promotes metastasis   |            |
|            | ↑Regulator of G-protein signalling 2 (RGS2)                                               | GTPase involved in GPCR signalling; prognostic marker downregulated in metastatic tumours |            |            |
|            | ↓S100A14                                                                                | Cell migration by modulating MMP2 levels           | Promotes metastasis   |            |
|            | ↑Vav guanine nucleotide exchange factor 3 (VAV3)                                         | Rho guanine nucleotide exchange factor – Integrin-mediated cell adhesion | Promotes invasion and metastasis |            |
|            | ↓MASPIN (SERPINB5)                                                                      | Serine Protease Inhibitor – Tumour suppressor     | Promotes invasion and metastasis | [43,45]    |
|            | ↓Desmocollin 3 (DSC3)                                                                   | Transmembrane glycoprotein – Tumour suppressor, Cell–Cell adhesion | Promotes metastasis |            |
|            | ↑Tetraspanin 15 (TSPAN1)                                                                | Cell surface protein involved in growth and motility | Promotes invasion, migration and metastasis | [45]       |
|            | ↓Cadherin 11 (CDH11)                                                                    | Calcium-dependent Transmembrane protein – Epithelial cell adhesion | Promotes metastasis |            |
|            | ↑Plakophilin 3 (PKP3)                                                                   | Cellular desmosome-dependent adhesion             | Promotes invasion and metastasis |            |
| Lung       | ↑Retinoic acid receptor responder protein 3 (RARRES3)                                   | Tumour suppressor gene                            | Promotes metastasis   | [50]       |
|            | ↑NFκB inhibitor α – (κBx)                                                                | Inhibitor of NFκB transmembrane glycoprotein – Cell–Cell interaction/ adhesion; Biomarker in NSCLC | Promotes metastasis | [57]       |
|            | ↑Desmocollin 3 (DSC3)                                                                   | Cell–ECM interaction – Angiogenesis               | Promotes metastasis   | [58]       |
|            | ↑Angiopoietin like 4 (ANGPTL4)                                                          | Cell–ECM interaction – Angiogenesis               | Promotes metastasis   |            |
|            | ↑Urokinase-type plasminogen activator (u-PA)                                             | ECM remodelling – Cell–ECM interaction             | Promotes invasion and metastasis |            |
|            | ↓Junctophilin (JPH3)                                                                    | Cell–Cell interaction                             | Promotes invasion and migration |            |
|            | ↓Roundabout guidance receptor 1 (ROBO1)                                                 | Transmembrane protein – Receptor for SLIT1 and Tumour suppressor Cell–Cell adhesion | Promotes metastasis |            |
|            | ↑Talin 1 (TLN1)                                                                         | Cell–Cell adhesion                                | Promotes metastasis   |            |
regulation of multiple targets culminating in the induction of EMT. Hence, it may be a therapeutic target or a potential biomarker to stratify breast cancer patients on the basis of response to EHMT2 inhibitors.

The role of EHMT1 in breast cancer appears distinct from EHMT2. Analysis of TCGA database revealed lower expression of EHMT1 in the highly metastatic basal-like breast cancer (BLBC) when compared to non-basal subtypes [27,28]. The expression of EHMT1 circular RNA (circEHMT1) is suppressed in breast cancer. circEHMT1 reduced matrix metalloproteinase-2 (MMP-2) expression and metastasis by downregulating miR-1233-3p [29]. In addition, miR-210 correlated with poor prognosis and the transition from ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC). EHMT1 was one among the major genes [breast cancer type 1 susceptibility protein (BRCA1), RB1, CDH1 and PARP1] that was downregulated in IDC when compared to DCIS [30]. These studies suggest a tumour suppressor role for EHMT1 which requires further investigations.

**Head and neck squamous cell carcinoma and endometrial cancer**

In head and neck squamous cell carcinoma (HNSCC), elevated EHMT2 expression is correlated with poor prognosis [31]. EHMT2, in association with Snail-1, transcriptionally repressed E-cadherin via deposition of H3K9me2 marks at its promoter. In addition, EHMT2-facilitated EMT in the presence of TGF-β. Depletion of EHMT2 activity with BIX-01294 prevented metastasis, reduced EMT markers (N-cadherin and vimentin) and reversed EMT in HNSCC [32]. In endometrial cancer cell lines, elevated EHMT2 expression was correlated with deep myometrial invasion and poor prognosis alongside negative correlation with E-cadherin. EHMT2-mediated recruitment of DNMT1 repressed E-cadherin, leading to invasion and migration. Although Snail was associated with the repressor complex, inhibition of EHMT2 did not affect the recruitment of Snail to E-cadherin promoter but resulted in a drastic reduction in DNMT1 and H3K9me2 marks [33].

**Gastric cancer**

EHMT1 and EHMT2 overexpression in gastric cancer is associated with late tumour stage, advanced tumour growth (lymph node and peritoneal metastasis) and poor prognosis [34–36]. EHMT1 knockdown induced E-cadherin expression, resulting in reduced peritoneal metastasis in vitro and in vivo [34]. Regenerating protein IV (Reg IV) was found to upregulate EHMT2 expression, leading to activation of integrin-β3 (ITGB3), a member of the integrin family involved in metastasis by promoting the adherence of gastric cancer cells to the peritoneum [37]. EHMT2 served as a scaffolding protein to form an activator complex with glucocorticoid receptor (GR) and p300, leading to expression of the GR target gene ITGB3 in methyltransferase activity-independent manner.

**Cervical cancer**

Human cervical cancer cell lines (CaloGR) exhibit high levels of EHMT2 as compared to cervical epithelial cells. Loss-of-function studies indicated a reduction in cell adhesion and invasive capacities through downregulation of E-cadherin [38]. Pharmacological and genetic inhibition of EHMT2 impeded angiogenesis, invasion and migration both in vitro and in vivo, with a reduction in the expression of angiogenic genes.

---

**Table 1.** (Continued).

| Cancer                       | Genes regulated by EHMT2 | Gene function                                   | Effect on metastasis | References |
|------------------------------|--------------------------|-------------------------------------------------|----------------------|------------|
| [Epithelial cell adhesion molecule (EpCAM)] ↓ CASP1 | Calcium-dependent transmembrane protein – Epithelial Cell adhesion Cysteine-aspartic acid protease; Component of inflamasome complex | Promotes EMT/metastasis Facilitate invasion and migration | [62] |
| Prostate ↑ Matrix metalloproteinase 9 & 13 (MMP 9 &13) ↑ Colony-stimulating factor 2 (CSF2) ↑ CXC motif chemokine ligand 16 (CXCL-16 or SDF-1) ↑ Cystatin (CST7) | Breakdown of ECM Cytokine – Stimulates growth and differentiation Chemotactic factor; Angiogenesis Cysteine protease associated with malignant tumours | Promotes metastasis Associated with metastasis Associated with metastasis Enhances metastasis | [63] |

---

The FEBS Journal 289 (2022) 1329–1351 © 2022 Federation of European Biochemical Societies.
O-GlcNac transferase (OGT), an enzyme responsible for the addition of single O-linked N-acetylgalactosamine (O-GlcNAc) to serine and threonine residues of proteins, is upregulated and correlated with poor prognosis in cervical cancer. EHMT1 and other histone modifiers were identified in the OGT interactome. Notably, Snail1 and ING were identified as targets of OGT, suggesting that OGT and histone modifiers may cooperate in regulation of EMT-TFs. As EHMT2 is involved in EMT through association with multiprotein repressor complexes involving Snail-1 and DNMT, it is possible that in cervical cancer EHMT1 and OGT act as a complex to regulate EMT-related genes [40].

**Pancreatic cancer**

EHMT2 overexpression in pancreatic cancer cell lines and pancreatic ductal epithelial cells promotes EMT. Similar effects were seen in a gemcitabine (GEM)-resistant pancreatic cancer cell line. The EHMT2 inhibitor UNC0638 prevented invasion of tumours in orthotopic xenograft mouse models. EHMT2 mediated H3K9me2 marks at the E-cadherin promoter and also upregulated the expression of PCL3 (component of PRC2 complex) to promote enhancer of zeste homolog 2 (EZH2) recruitment, leading to E-cadherin silencing. Furthermore, EHMT2 downregulated lysine demethylase 7A (KDM7A) expression which mediates H3K27 demethylation [41,42]. However, no correlation was noted between EHMT2 expression and tumour metastasis in patient tissues.

**Ovarian cancer**

EHMT2 expression is elevated in high-grade serous-type ovarian cancer (HGSOC) and metastatic lesions compared to primary tumours. Inhibition of EHMT2 reduced invasion, migration and anoikis resistance in cell lines, and attenuated metastasis to abdominal organs and ascites in a peritoneal metastasis model. EHMT2 regulates many metastasis genes, including E-cadherin, by promoter methylation. However, overexpression studies did not reveal a marked increase in metastasis suggesting that other epigenetic regulators may control metastasis [43].

EHMT1/2 levels are induced by hypoxia and exert tumorigenic effects via methylation of a wide range of histone and non-histone substrates [44]. Consistently, recruitment of EHMT1/2 and concomitant H3K9me2/me3 at the E-cadherin promoter were drastically increased during hypoxia. Gene set enrichment analysis (GSEA) of 478 ovarian cancer tissues revealed a negative correlation between the expression of EHMT2 and anti-metastatic gene sets. Specially, ‘aigner-ZEB1-target’ gene set which included genes involved in epithelial differentiation and cell adhesion (CDH1, CDH11, TSPAN15, CLDN7, MASPIN and DSC3) was observed [45].

**Hepatocellular carcinoma (HCC)**

Activation of TGF-β and Hedgehog signalling induced EMT in hepatocytes [46,47]. Initially, although interaction between Snail and EHMT2 was detected, there was no association with TGF-β-induced EMT in HCC [48]. However, much like other cancers, overexpression of EHMT2 in HCC was associated with aggressiveness and poor prognosis [49]. EHMT2 was upregulated due to gene amplification and loss of miR-1 (a negative regulator of EHMT2). Depletion of EHMT2 through RNAi/CRISPR/CAS9 and pharmacological inhibition reduced proliferation and metastasis both in vitro and in vivo through repression of RARRES3, a tumour suppressor gene [50]. Transcriptomic analysis in HCC cell line (HepG2) after treatment with CM-272 indicated a difference in genes involved in metastasis. Furthermore, treatment with CM-272 restored E-cadherin expression by mechanisms that are unclear [49]. Translation regulatory long non-coding RNA 1 (TRERNA1), which promotes cancer metastasis in several cancers [51–53], induced metastasis in HCC by recruiting EHMT2 to a Snail-1 repressor complex and/or by increasing H3K9me2 at the E-cadherin promoter. Surprisingly, other EMT markers (except vimentin and E-cadherin) were significantly unaltered upon TRERNA1 suppression [54]. In various cancers, the association of Snail-1 and EHMT2 is well documented in controlling E-cadherin expression but in HCC, in addition to Snail-1, Snail-2 was also associated with EMT. Furthermore, Snail-2 mediated recruitment of histone deacetylases (HDAC 1, 2 and 3), while EHMT2 was essential for the removal and deposition of H3K56ac/H3K4ac and H3K9me2 marks, respectively, to negatively regulate E-cadherin. Indeed, pharmacological inhibition of EHMT2 and HDAC attenuated metastasis by reactivating E-cadherin in HCC cell lines [55]. Thus, EHMT2 mostly works in association with other epigenetic regulators to alter EMT in HCC.

**Lung cancer**

Overexpression of EHMT2 was identified in non-small cell lung cancer (NSCLC) as compared to normal lung
EHMT2 contributes to various stages of malignancy by regulating different signalling pathways. For instance, EHMT2 together with heterochromatin protein 1α (HP-1α) and DNMT1 transcriptionally suppress the wingless-related integration site (Wnt) inhibitor adenomatous polyposis coli protein 2 (APC2), thereby promoting Wnt signalling and tumour growth [56]. Recently, EHMT2 was reported to promote invasiveness through upregulation of the focal adhesion kinase (FAK) pathway, wherein EHMT2 represses IκBα through H3K9me2, leading to activation of NF-κB and its downstream target FAK [57]. In addition, EHMT2 knockdown modulated the expression of HP-1α, APC2, ANGPTL4, u-PA, jPH3, TLN1, ABCG2 and TERT, which are involved in growth, adhesion, angiogenesis, apoptosis and hypoxia. Similar to HCC, in lung cancer cells, a repressor complex of Snail-2/EHMT2/HDAC promoted EMT by repressing E-cadherin expression [58]. In NSCLC, EHMT2 silences caspase 1 (CASP1), a component of the inflammasome complex, thereby facilitating invasion and migration, colony formation and tumour growth [59]. The tumour suppressor SATB2 prevented invasion/EMT in NSCLC via downregulation of EHMT2 and associated histone marks (H3K9me3, H3K27me3 and H3K27me3). Furthermore, SATB2 upregulated expression of epithelial markers (E-cadherin and β-catenin) and downregulated expression of EMT-TFs Slug and Snail and mesenchymal markers (vimentin). However, it is not clear whether EHMT2 is indispensable for SATB2-dependent effects [60].

EHMT2 is overexpressed in highly metastatic lung cancer cell lines [61]. As in other cancers, high EHMT2 expression is correlated with advanced tumour stage, lymph node metastases and poor prognosis. Tissue microarray of lung cancer patients indicated an inverse correlation between EHMT2 and epithelial cell adhesion molecule (EpCAM). Depletion of EHMT1 and EHMT2 prevented invasion, which is an early phase of metastasis, in lung cancer both in vitro and in vivo. EHMT1 was shown to be essential for EHMT2-mediated promotion of cell migration through its ability to stabilise the latter. Mechanistically, EHMT2 along with HP1, DNMT and HDAC prevented transactivation of Ep-CAM by SP-1 [62].

Prostate cancer

EHMT2 in association with Runx2 positively regulated the expression of genes (MMP9, MMP13, PGC, CSF2, SDF-1 and CST7) involved in prostate cancer growth, invasion and metastasis [63]. In contrast, interaction between EHMT1 and metallothionein 1h (MT1h) was necessary for histone methylation and tumour suppressor activity. Knockdown of EHMT1 or disruption of the binding with MT1h reduced tumour suppressor function. This study indicates a tumour suppressor role of EHMT1, with MT1h as a cofactor, in suppressing metastasis in PCa [64].

Osteosarcoma

NEAT1, a long non-coding RNA (LncRNA) which is overexpressed in osteosarcoma, is associated with distant metastasis and poor prognosis. In osteosarcoma cell lines, NEAT1 is present in a repressor complex with EHMT2-Snail-DNMT1, resulting in repression of E-cadherin. Furthermore, NEAT1 reduced expression of epithelial markers (E-cadherin and α-catenin) and increased expression of mesenchymal markers (vimentin and N-cadherin) [65].

Other cancers

In oesophageal squamous cell carcinoma (ESCC), EHMT1 and EHMT2 were shown to be overexpressed compared to normal oesophageal tissue. The expression of EHMT1 and EHMT2 correlated with the magnitude of invasion, lymph node metastasis and tumour stage [66,67]. Similarly, EHMT2 expression was associated with distant metastasis in colorectal cancer (CRC) [68]. EHMT2 correlated with the expression of integrin subunit alpha-4 (VLA-4) in acute lymphoblastic leukaemia (ALL) which facilitated ALL cells to migrate. However, depletion or pharmacological inhibition of EHMT2 with BIX-01294 did not affect VLA-4 expression but modulated cell migration, suggesting a distinct mechanism by which EHMT2 regulates ALL cell motility.

Cancer stemness

In concordance with their role in maintenance of pluripotency in ES cells [69–71], EHMT1/2 have been shown to be critical regulators of stem-like transcriptional signatures in cancer cells (Fig. 2 and Table 2).

In acute myeloid leukaemia (AML) mouse models, EHMT1/2 was shown to induce rapid progression towards end-stage AML. EHMT2-deficient mice showed reduced self-renewing leukaemia stem cell (LSC) frequency, with no adverse effect on haematopoietic stem cells (HSCs). Moreover, ablation of EHMT2 and treatment with EHMT1/2 inhibitor (UNC0638) both in vivo and in vitro showed a significant increase in differentiated myeloid cells, emphasising the importance of EHMT1/2-dependent methyltransferase activity in LSC-mediated AML progression [72]. While elevated
EHMT2-mediated H3K9me2 mark was correlated with increased cellular differentiation, EHMT2 overexpression in AML appears to augment LSC frequency.

In contrast, EHMT2 exhibited an inhibitory role in self-renewal of glioma cancer stem cells (CSC). CD133+ glioma CSCs were H3K9me2 negative in contrast to CD133- cells, which immunostained positively for H3K9me2. Moreover, enhanced tumorsphere formation in EHMT2 inhibitor (BIX-01294)-treated glioma CSCs confirmed that EHMT2 obstructs self-renewal. Consistent with its role as a transcriptional repressor, EHMT2 directly repressed CD133 and SOX2 transcriptionally by methylating the promoter and enhancer. In glioma CSCs, EHMT2-mediated H3K9me2 serves as a repressive switch for self-renewal that is seen in ES cells as well [73]. Similarly, tumour repopulating cells (TRC) in melanoma exhibited low levels of H3K9me2 and elevated expression of SOX-2. EHMT2, FAK or CDC42 silencing increased proliferation/self-renewal capacity of melanoma cells through elevated SOX-2 expression [74,75]. Likewise, in lung adenocarcinoma, EHMT2 inhibited tumour progression by functioning as a suppressor of tumour propagating cells (TPC). EHMT2 inhibition in lung adenocarcinoma cells promoted stemness and accelerated tumour progression through upregulation of KRAS- and ECM-associated genes including MMP10. Indeed, depletion of Kdm3a suppressed CSCs, suggesting it as a potential target for lung adenocarcinoma [76]. Although this study was limited to analysis of TPCs expressing stem cell antigen-1 (Sca-1) and CD24, it nonetheless highlighted the importance of tumour heterogeneity, the cancer-type-dependent role of EHMT2 and the detrimental effects of inhibiting its activity in lung cancer. In contrast, knockdown of EHMT1, which is significantly elevated in lung cancers, reduced formation and aggregation of 3D spheroids via upregulation of CDKN1A [77].

Inhibition of EHMT2 in GEM-resistant pancreatic cancer cell line PANC-1-R reduced expression of CD133, Nestin and Lrg5, decreased tumorsphere

**Fig. 2.** EHMT2 regulates cancer stemness. The illustration demonstrates the role of EHMT2 in the progression of CSCs. In lung adenocarcinoma and glioma stem cells, EHMT2 inhibits the propagation of CSCs by repressing stemness markers (CD24, Sca1 and CD133). In contrast, in breast cancer, CDYL2 recruits EHMT2 to microRNA genes to regulate stemness and metastasis via p65/NF-kB and STAT3 pathways. In pancreatic cancer, EHMT2 regulates IL-8/CXCR1/2-mediated autocrine and paracrine signalling resulting in the induction of stemness and drug resistance. These studies highlight the context-dependent specific role of EHMT2 in various cancers.
formation and increased sensitivity to GEM. Furthermore, EHMT2-overexpressing PANC-1-R stimulated pancreatic stellate cells through activation of IL-8/CXCR1/2 autocrine and paracrine signalling [42]. The study reveals a distinct mechanism of EHMT2-dependent regulation of stemness in pancreatic cancer. In addition to IL-8, several other cytokines, namely CXCL5 and CXCL1, were modulated; the role of these cytokines needs further exploration.

HCC cell lines exhibited high expression of EHMT2 and its inhibition markedly reduced sphere-forming ability [49]. Interestingly, repression of miR124 by CDYL2-EHMT2 activated NF-κB and STAT3 signalling, resulting in stemness in addition to invasion and migration [26]. In addition, inhibition of EHMT2, which attenuated EMT in HNSCC, also reduced tumourspheres and the expression of CD44 [32], establishing a link between EHMT2-dependent regulation of EMT and stemness.

In colon cancer, EHMT2 regulates the CD133+ ESA+ (epithelial-specific antigen) CSCs. Indeed, EHMT2 knockdown reduced CD133+ cells, hindered migration and sphere formation [78]. Furthermore, the reduction in stem cell phenotype and metastatic properties was associated with its ability to alter DNA damage response through protein phosphatase 2A–replication protein A (PP2A-RPA) axis [79].

In non-small cell lung cancer (NSCLC), EHMT2 expressed in TICs was shown to induce CSC markers, sphere formation and growth both in vitro and in vivo. Genome-wide analysis of methylation changes upon EHMT2 knockdown in patient-derived TICs revealed upregulation of a set of tumour suppressor genes (CDYL2, DPP4, SP5, FOXP1, STAMBPL1 and ROBO1). This study established the role of EHMT2 in altering DNA methylation patterns in these target genes, leading to maintenance of TICs which facilitate metastasis and drug resistance [80].

In BCR-ABL+ CML, leukaemia stem cells (LSCs) lie at the core of relapse and drug resistance. Tyrosine kinase inhibitors (TKIs) fail to eliminate LSCs. Similar to other cancers such as HNSCC [32], colon cancer [79] and pancreatic cancer [42], where EHMT2 has been reported to drive stemness and confer chemoresistance, EHMT2 is overexpressed in LSCs. Both in vitro and in vivo, EHMT2 inhibition resulted in significantly impaired self-renewal, increased apoptosis and prolonged survival. RNA sequencing analysis identified the tumour suppressor SOX6 as target of EHMT2 in CML LSCs [81]. As EHMT2 has a limited effect on normal haematopoiesis, its selective role in LSC sustenance suggests it is a promising therapeutic target for CML.

### Drug resistance

CSCs contribute to drug resistance [82]. Given the importance of EHMT2 in maintenance of CSCs, it is not surprising that EHMT2 is involved in drug resistance by sustaining stemness. High EHMT2 expression is associated with drug resistance through its ability to modulate transcriptional regulation of tumour suppressor genes, drug metabolism, DNA repair and cell survival pathways (Fig. 3 and Table 3).

### DNA damage

EHMT1/2 protects and supports the growth of cancer cells by preventing chromosome disruption, recruiting DNA repair factors [p53-Binding protein 1 (53BP1), BRCA1 and RNF168] at double-stranded breaks (DSBs) and inducing DNA repair mechanisms (homologous recombination (HR) and non-homologous end joining (NHEJ)). Indeed, EHMT2 knockdown disrupted chromatin organisation at centromeres leading to instability [83], impaired DNA repair pathways in a p53-independent manner and prevented HR and NHEJ through reduced H3K9me1/2. Furthermore, inhibition of EHMT2 in osteosarcoma sensitised cells to irradiation [84]. EHMT2 inhibitors in combination with chemotherapeutic drugs sensitised cancer cells to DNA DSB and enhanced cell death in U2OS cells.

| Cancer        | CSC/EMT features | Stemness | References |
|--------------|-----------------|----------|------------|
| Glialoma      | CD133↑ ↓        | Suppressor | [73]       |
| HNSSC        | CD44↑ ↑         | Activator | [32]       |
| Pancreatic    | CD133↑↑, Nestin and Lgr5 ↑ | Activator | [42]       |
| Breast        | CD24/CD44↑      | Activator | [25]       |
| Lung          | CD24↓ ↓         | Suppressor | [76]       |
| Melanoma      | SOX-2↓ ↓        | Suppressor | [74-75]    |
| NSCLC         | CD133↑↑         | Activator | [80]       |
| Colorectal    | CD133↑, CD44↑, Lgr5 ↑ | Activator | [78,79]    |
| CML           | LSC Self-renewal ↑ | Activator | [81]       |
[85]. Similarly, in glioma cell lines, loss of EHMT2 led to impaired DNA DSB repair signalling (HR and NHEJ pathways) and increased sensitivity to ionising radiation. EHMT2 inhibition reduced activation of ataxia-telangiectasia mutated (serine/threonine kinase) (ATM) kinase and tat interactive protein (Tip60), consequently decreasing the phosphorylation of KRAB domain-associated protein 1 (KAP-1, repressor protein) and increasing radiosensitivity [86]. Recently, EHMT2 expression was shown to initiate cancer in DNA damaged hepatocytes both in vitro and in vivo. Mechanistically, EHMT2 negatively regulated the expression of Bcl-G (proapoptotic member of Bcl-2 family) by blocking p53 in these cells. Treatment of hepatoma cell lines with EHMT2 inhibitor sensitised them to DNA damage inducers ( irradiation and hydrogen peroxide) and enhanced cytotoxicity [87]. While most studies have shown the involvement of EHMT2 in DNA repair, depletion of EHMT2 induced DNA DSB, chromosomal aberrations and senescence in CRC. EHMT2 inhibitors in combination with topoisomerase I inhibitor elevated γ-H2A histone family member X (γ-H2AX) expression and led to CRC cell death [88]. High expression of EHMT2 conferred cancer stemness to colon cancer cells, eventually causing resistance to radiation treatment. Knockdown of EHMT2 increased chemo-radio sensitivity and promoted DNA damage through transcriptionally upregulating PP2A in HT-29 colon cancer cells. PP2A impaired the phosphorylation of checkpoint kinase 1 (CHK-1) and RPA, thereby enhancing replication-linked DSB [79]. Interestingly, in HGSOC, PARP inhibitor (PARPi) resistance in patient-derived xenografts (PDX) and PARPi-resistant ovarian cancer cell lines exhibited high levels of H3K9me2 and EHMT1/2. Inhibition of EHMT1/2 (using UNC0642) restored sensitivity to PARP inhibitors through induction of DNA damage and altered cell cycle regulation. Furthermore, RNA-seq analysis revealed alterations in survival pathway including mTOR, PI3K and Ak strain transforming, protein kinase B (AKT) [89].

Survival signalling pathways
EHMT1/2 imparts drug resistance by upregulating survival signalling pathways in tumour cells. Pharmacological inhibition of EHMT2 augmented anti-tumour efficacy of temozolomide (TMZ), a therapeutic agent used as first line of treatment in glioblastoma (GBM). Pre- and post-treatment with the EHMT2 inhibitor BIX-01294 sensitised GBM cells to TMZ and restored apoptosis. In addition, EHMT2 inhibitors induced autophagic cell death and enhanced the anti-tumour effect of TMZ. However, the mechanisms underlying apoptosis and autophagy-related cell death need further investigation [90]. In multiple myeloma, combinatorial treatment with EHMT1/2 inhibitors and proteasome inhibitors induced autophagy cell death and tumor reduction in vitro and in vivo respectively. Mechanistically, the combinatorial treatment upregulated SAPK/JNK and p38 signalling while inhibiting mTOR signalling and cMyc levels in MM cells [91]. In the pancreatic ductal adenocarcinoma (PDAC) cell line (PANC-1), suppression of EHMT2 upregulated p27 and G1 cell cycle arrest, leading to sensitisation to PI3K/mTOR inhibitors BEZ235 and Cay10626 [92]. In fact, overexpression of EHMT2 and associated H3K9me2 led to the repression of coiled-coil domain-containing protein 8 (CCDC8), a tumour suppressor that is correlated with aggressiveness in radio-resistant lung cancer cell lines. Treatment with EHMT2 inhibitors sensitised resistant cells A549/IR and XWLC-05/IR to radiotherapy [93]. Epidermal growth factor receptor (EGFR)-induced expression of HER3 and activation of the PI3K/akt signalling pathway contributes to therapy resistance in lung cancer. To identify potential targets against EGFR+ lung cancer cells, 172 therapeutic agents that target stemness were screened. Among these, STAT3 inhibitor BBI608 was found to reduce viability of EGFR+ cells. The inhibition of STAT3 in EGFR-TKI-resistant lung cancer cell lines downregulated EHMT2 and suppressed HER3 through miR-145-59, thereby preventing HER3-mediated EGFR-TKI resistance. In addition, the EHMT2 inhibitor UNC0642 cotreated with afatinib significantly reduced tumorspheres in TKI-sensitive EGFR+ cell lines [94].

Immunotherapies
In melanoma patients, EHMT2 is inversely correlated with T-cell signatures. Administration of EHMT1/2 inhibitor UNC0642 in combination with anti-programmed cell death protein 1 (anti-PD1) and anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4) significantly regressed the tumours in a syngeneic melanoma mouse model [95]. Similarly, in bladder cancer, EHMT2 was associated with tumour recurrence and poor outcome. A combination of EHMT2 inhibitor with cisplatin and/or immune checkpoint inhibitor anti-programmed cell death ligand 1 (PD-L1) regressed tumours and prevented metastasis in aggressive transgenic mouse models. The inhibition of EHMT2 sensitised bladder cancer cells to PD-L1 and converted cold tumours (non-inflamed) to hot tumours (inflamed) through its ability to enhance...
Fig. 3. EHMT2-associated drug resistance and mode of EHMT2 inhibitors in drug sensitisation. In a tumour population, cells overexpressing EHMT2 (indicated by a green asterisk) remain unaffected by standard treatment regimens (radiotherapy, chemotherapy, targeted therapy and immune therapy) and increase response to treatment. On the other hand, sensitive cells on exposure to drug acquire resistance through expression of EHMT2. EHMT2 regulates various functional properties in these cells eventually conferring drug resistance. Treatment with EHMT2/EHMT1 inhibitors sensitise cancer cells to standard drug therapies.
Table 3. Effect of EHMT2 inhibition on drug sensitisation in various cancers. EHMT2 inhibition in combination with chemo/radiation therapy sensitises cancer cells to treatment regimens. The signalling pathways through which EHMT2 functions are listed. *Increased; ↓Decreased.

| Cancer Model                        | Treatment combination | Treatment outcome                                                                 | Cellular process/ Signalling pathway | References |
|-------------------------------------|-----------------------|----------------------------------------------------------------------------------|--------------------------------------|------------|
| Osteosarcoma Cell line – U2OS       | shEHMT2/ UNC0638 + IR | Increased cellular sensitivity to IR                                              | ↓ DNA repair – ↓S3BP1 and BRCA1 recruitment | [84]       |
|                                     | UNC0638/A-366 + Etomycin/ Phelomycin | Sensitised the cancer cells to DNA DSB-inducing agent and increased the cytotoxic effect | ↓NHEJ DNA repair                     | [85]       |
| CRC Cell lines – HT29, SW620        | shEHMT2 or UNC0638 + CPT or SN-38 | Synergistic increase in DNA damage response (↑γH2AX)                           | ↑Chromosomal aberration rate, ↑DNA double-strand break and ↑cellular senescence | [88]       |
|                                     | HT 29 shEHMT2 or UNC0638 or BIX-01294 + CPT / Etomycin | Enhanced DNA damage                                                               | ↓Tumorsphere formation ↓PP2A resulting in dephosphorylation of RPA and enhanced DSB | [79]       |
| Glioblastoma (GBM) Cell lines – LN382, T989, U343, U373, LN428, LN827, U118 and U87 | BIX-01294 + Radiation therapy | Increased radiosensitivity to IR                                                  | ↓DSB repair signalling factors (ATM signalling, phosphorylation of KAP-1 and Tip60 acetyltransferase activation) and pathway (NHEJ and HR) | [86]       |
|                                     | LN18, U251 BIX-01294 + TMZ | Sensitised cancer cells to TMZ and augmented cell death                        | ↑Autophagy cell death                | [90]       |
| Ovarian Resistant cell lines (PEO1-OR) | UNC0642/ UNC0638 + Olaparib | Sensitised PEO1-OR to Olaparib. Induced DNA damage (↑γH2AX)                   | ↑DNA damage and deregulated cell cycle. ↓Expression of PI3K/ AKT signalling | [89]       |
| Multiple myeloma Cell lines – OPM-2 and X-G7; Syngeneic immunocompetent murine 5TMM models. | Bortezomib + BIX-01294 | Sensitised MM cells to proteosome inhibitors (bortezomib and carfilzomib). Reduced tumour growth and prolonged survival | ↑Autophagy ↓mTOR signalling – 4EBP1 and c-MYC. ↑Cell cycle arrest at G1 phase | [92]       |
| Lung Cell line – A549/IR and XWLC-05/IR | shEHMT2 + Radiotherapy | Sensitised radioreistant cell lines to radiotherapy                          | ↑CCDC8 (tumour suppressor protein) | [93]       |
| HCC827 Cell line – A549/IR and XWLC-05/IR | UNC0642 + Afinatinib | Significantly reduced tumourspheres                                             | ↑miR-145-5p and ↓HER3                | [94]       |
| Pancreatic ductal adenocarcinoma (PDAC) Cell line – PANC-1 and orthotopic mouse model | shEHMT1 + BEZ235 or Cay10626 (PI3K/mTOR inhibitor) | Sensitised cells to PI3K/mTOR inhibitor and enhanced cytotoxicity | ↑p27 and induced cell cycle arrest at G1 phase | [92]       |
|                                   | GEM + UNC0638 | Reduced stem cell property and sensitised cells to GEM | ↑Transcriptional level of IL-8 | [42]       |
|                                   | Trametinib + UNC0638 + RGFP966 (HDAC3 inhibitor) | Inhibited cell proliferation and regressed tumours                              | ↓Drug resistance genes involved in GSH metabolism and OXPHOS | [101]      |
immune response regulation (IFNα, IFNβ and TNFα) [96]. Altogether, these findings suggest an immunoprotective role of EHMT2 in the tumour microenvironment, correlate its overexpression with immune therapy resistance and highlight the potential of EHMT2 inhibitors in augmenting the efficacy of immune checkpoint inhibitors.

**Gemcitabine sensitivity**

The anti-tumour activity of GEM and resistance development is related to the expression of genes involved in membrane transport (human equilibrating nucleoside transporter 1, hENT1) and drug metabolism (deoxycytidine kinase, dCK). In cervical cancer, EHMT2-mediated H3K9me was shown to be responsible for the inactivation of genes hENT and hcdk, which was associated with drug resistance. Depletion of EHMT2 restored GEM sensitivity in a resistant CaloGR. Further treatment with hydralazine reduced the expression and activity of EHMT2, consequently modulating the expression of hENT and hcdk in CaloGR [97]. Additionally, EHMT2 overexpression enhanced proliferation, metastasis and GEM resistance in pancreatic cancer through increased production of IL-8, which promotes stromal–cancer cell interaction and ECM deposition associated with drug resistance. Inhibition of EHMT2 reduced stemness and sensitised GEM-resistant cell lines (PANC-1-R) both in vitro and in vivo [42]. Cisplatin-resistant HNSCC cells exhibited

---

**Table 3. (Continued).**

| Cancer         | Model                                      | Treatment combination | Treatment outcome                                                                 | Cellular process/Signalling pathway | References |
|----------------|--------------------------------------------|-----------------------|------------------------------------------------------------------------------------|------------------------------------|------------|
| Melanoma       | Anti-PD1-resistant B16F10 mouse melanoma model | UNC0642 + anti-PD1    | Augmented the efficacy of checkpoint inhibitor blockade and resulted in tumour regression | ↑ MAP1LC3B and autophagy cell death. | [95]       |
| Bladder        | Immunocompetent quadruple KO (Pten<sup>lox/lox</sup>, Trp53<sup>lox/lox</sup>, Rb<sup>11</sup><sup>lox/lox</sup>) | CM-272 + Cisplatin    | Increased sensitivity to anti-PD-1, tumour regression.                             | ↑ TNFα, IFNα and γ                 | [96]       |
| Cervical       | Resistant cell line – Calo-GR               | siEHMT2 + GEM         | Restored sensitivity to GEM and enhanced cytotoxicity                              | ↑ Drug metabolic genes (DCK) and membrane transporter gene (ENT) | [97]       |
|                |                                             |                       |                                                                                    | ↓ Transcriptional levels of glutamate cysteine ligase catalytic subunit (GCLC) and ultimately GSH levels | [99]       |
| HNSCC          | Resistant cell line – SAS-CR                | shEHMT2/UNC0638 + Cisplatin | Sensitised resistant cells to cisplatin and enhanced cytotoxicity                  |                                    |            |
| NSCLC          | Resistant cell lines – PC9/ER and HCC827/ER; Xenograft model injected with PC9/ER | UNC0642 + Erlotinib   | Sensitised cells to TKIs. Induced apoptosis and reduced tumour growth.            |                                    |            |
| B-ALL          | NALM-6                                     | Dexamethasone + AZD2811 (AURKB inhibitor) | Inhibition of AURKB reduced EHMT2/EHMT1 phosphorylation, increased drug sensitivity and GC regulated cell death | ↓ GC regulated cell death effector genes. | [102]      |
| ALL            | Cytarabine-resistant cell line – U937/AR    | BIX-01294 + PERK siRNA or GSK260641 | Restored sensitivity to GC-induced cell death.                                   | Mutation in the methylation site of EHMT2/EHMT1 failed to enhance GC-induced cell death | [103]      |
|                |                                            |                       |                                                                                   |                                    | [104]      |

The FEBS Journal 289 (2022) 1329–1351 © 2022 Federation of European Biochemical Societies.
high levels of EHMT2. In addition, overexpression of EHMT2 was responsible for multidrug resistance (oxaliplatin, 5-FU and bleomycin) in HNSCC. EHMT2-mediated H3K9me1 protected the HNSCC cells by transcriptionally upregulating the expression of glutamate cysteine ligase catalytic subunit (GCLC), one of the enzymes involved in glutathione (GSH) biosynthesis. GSH is involved in cancer progression and drug resistance in various cancers [98]. Depletion of EHMT2 expression and activity sensitised cells to cisplatin by reducing GSH content and promoting apoptosis [99].

**Acquired drug resistance**

The evidence summarised so far correlate EHMT2 overexpression in tumour cells with their intrinsic drug resistance ability. On the other hand, overexpression of EHMT2 is also indicated in acquired drug resistance following therapy administration. For instance, overexpression of EHMT2 was associated with erlotinib (EGFR-TKI) resistance in NSCLC. The inhibition of EHMT2 led to the transcriptional activation of PTEN and reduced AKT signalling, which attenuated aggressiveness and sensitised NSCLC cells to TKI. Indeed, a combination of erlotinib and UNC0638 significantly reduced cancer growth and induced apoptosis in resistant cells *in vitro* and *in vivo* [100]. In PDAC, treatment with the MEK inhibitor (MEKi) resulted in resistance and SETD5 was identified as a major driver of acquired targeted therapy resistance. SETD5 lacks enzymatic activity and forms a corepressor complex either with EHMT2 or HDAC3 to exhibit therapy resistance. Transcriptomic analysis of resistant cell lines revealed that the loss of SETD5 or EHMT2 downregulated genes involved in GSH metabolism and the cytochrome P450 pathway, leading to cellular reprogramming and improved response to MEKi. Therefore, combined pharmacological inhibition of MEK1, EHMT2 and HDAC3 reduced tumour growth in a relapse PDX model [101]. EHMT2 overexpression in GEM-resistant cell line was reported to promote EMT through its ability to negatively regulate E-cadherin [41].

In B-cell ALL (B-ALL), glucocorticoid-induced cell death depends on the coactivator complex chromobox family protein, CBX-3 and EHMT1/2. Upregulation of aurora kinase B (AURKB) resulted in phosphorylation of EHMT1/2, thereby preventing expression of genes responsible for glucocorticoid-induced cell death, conferring drug resistance. Inhibition of AURKB potentiated cytotoxic effects of glucocorticoid in NALM6 cells [102]. On the other hand, automethylation of EHMT1/2 enhances recruitment of HP-1 and coactivator complex formation to induce GR target genes. In line with the role of EHMT1/2 and its methylation in glucocorticoid-mediated regulation, treatment with JmJc family of lysine demethylase inhibitor (JIB-04) prevented EHMT2 demethylation and resorted sensitivity to glucocorticoid causing cell death in Nalm-6 cells [103]. Therefore, contrary to other cancers, methylation of EHMT1/2 is shown to be important for drug sensitisation in B-ALL cancer.

Nonetheless, cancer cells also develop resistance to EHMT2 inhibitors. In AML, treatment with BIX-01294 led to the upregulation of phosphorylated PERK and eIF2α, causing endoplasmic reticulum stress. Genetic suppression of PERK sensitised ALL cells to BIX-01294-induced apoptotic cell death. However, the mechanism involved in PERK inhibition and sensitivity of AML cells to BIX-01294 is unclear [104].

**Metabolic alterations**

Cellular metabolism yields metabolites which function as cofactors and substrates for various epigenetic regulators. Abnormal levels of metabolites (oncometabolites) lead to epigenetic dysregulation, and conversely, deregulation of the epigenetic landscape affects expression of metabolic enzymes that contribute to cancer progression [105–107]. Uprogulation of glycolysis is observed both in primary and metastatic tumours. Increased uptake of glucose and altered glucose metabolism (glycolytic enzyme activation, impaired OXPHOS and suppressed gluconeogenic enzymes) are correlated with cancer metastasis and poor prognosis [108]. Consistent with this notion, in BLBC cells, EHMT2 associated with Snail and DNMT to suppress the expression of fructose-1,6-bisphosphatase (FBP1). The decrease in FBP1 had pleotropic effects, including increased glycolysis, reduced mitochondrial complex I activity contributing to lower oxygen consumption, increased lactate production and reactive oxygen species generation that collectively contributed to breast tumorigenicity [109]. Similarly in HCC, dual inhibition of EHMT2 and DNMT by CM-272 impaired metabolic adaptation of cancer cells to hypoxic conditions by rewiring glucose metabolism (increased glyconeogenesis and decreased glycolysis) and reactivating the expression of FBP1 through reduced promoter methylation [49].

The serine–glycine biosynthesis pathway is involved in cancer cell proliferation and survival [110,111]. In response to serine levels, EHMT2-mediated H3K9me1 upregulated the expression of multiple enzymes involved in serine synthesis pathway including phosphoglycerate...
dehydrogenase (PHGDH), phosphoserine aminotransferase 1 (PSAT1), phosphoserine phosphatase (PSPH) and serine hydroxy methyltransferase 2 (SHMT2). Inhibition of the same caused nutrient deprivation leading to autophagy-mediated cell death in various cancer cell lines (neuroblastoma, colorectal, cervical, sympathetic nervous system, breast and bone cancer) [112]. Furthermore, EHMT2-dependent overexpression of PSAT1 upregulated the citric acid cycle (TCA) through increased production of α-ketoglutarate (α-KG) in CRC [113].

Cooperation between EHMT2- and DNMT-mediated methylation of histones and DNA is evident in several cancers. In cholangiocarcinoma, treatment with CM272 (EHMT2/DNMT1 inhibitor) induced differentiation and quiescence. Microarray analysis revealed reduction in serine–glycine pathway (PHGDG, FSG and SHMT2), cholesterol biosynthesis (SQLE and DHCR7) and glycolytic enzymes (GLUT1, HKII and LDHA), while enzymes involved in cholesterol catabolism (CYP7A1) and gluconeogenesis (FBP1) were upregulated upon treatment with CM272 [114]. Surprisingly, in NSCLC and pancreatic cancer, activation of cholesterol biosynthesis pathway upon treatment with BIX-01294 exhibited anti-tumour effects [115]. Inhibition of EHMT2 activated SREBF2 (sterol regulatory element-binding protein 2), resulting in autophagy-induced cell death and cholesterol biosynthesis [116].

Abnormal levels of TCA intermediates influence pluripotency and metastasis leading to cancer progression. In nasopharyngeal carcinoma, lymphocyte-specific helicase (LSH) in combination with EHMT2 suppressed fumarate hydratase (FH) expression. Reduced FH levels increased the ratio of α-KG to fumarate, resulting in metastasis by upregulation of NF-κB signalling and EMT gene expression [117].

Reprogramming of iron metabolism facilitates cancer cell survival and metastasis [118,119]. EHMT2 in complex with HDAC1 and YY1 can alter iron metabolism by transcriptionally repressing the expression of iron homeostasis regulator ferrooxidase hephasatin (HEPH), thereby leading to accumulation of cellular iron in breast cancer cells [120]. Also, suppression of EHMT2-induced expression of TP53-induced glycolysis and apoptosis regulator (TIGAR) and pyruvate kinase M2 (PKM2) alters ROS and autophagy [121].

**Conclusion**

In the last few decades, major progress has been made in the management of cancers, which includes early detection and treatment of disease. Thus, mortality has declined for some common epithelial malignancies such as breast cancer and prostate cancer. Despite such advances, metastasis, drug resistance and recurrence pose a major challenge in the effective treatment of high-risk cancers. There is growing evidence for epigenetic dysregulation contributing to EMT and drug resistance. Given that these alterations are reversible, targeting epigenetic modulators that mediate these changes, in combination with standard treatment of care, is gaining momentum.

Manifestation of EMT or a partial EMT programme (expression of both epithelial and mesenchymal markers) is different in distinct cancer types. Indeed, modulation of any EMT-TF can affect the process as they are functionally non-redundant. Given the plasticity of cells undergoing EMT, in addition to alterations associated with morphology, EMT is also associated with stem cell properties, as well as proliferation and survival of cancer cells.

Collectively, studies suggest a strong association between EHMT1/2 with EMT dynamics that has an impact on all the above-mentioned phenotypes (Fig. 4). However, there are many questions which remain to be addressed. For instance, EHMT2 either directly associates with EMT-TFs or modulates their expression to facilitate EMT. However, it remains unclear how EHMT2 is recruited and chooses its interacting partners to regulate different signalling pathways during multiple stages of cancer development. Moreover, apart from EMT-related genes, transcriptional analysis in several cancers reveal that EHMT2 regulates a wide spectrum of genes associated with metastasis (Table 1). Further investigations are needed to delineate these regulatory networks and their functions in metastasis. Second, the mechanisms/molecular pathways through which EHMT1/2 promote cellular plasticity and drug resistance are not fully understood. Moreover, not all cancers which metastasise exhibit EMT. Therefore, understanding other pathways that lead to metastasis are also of importance. Third, while the association of EHMT1/2 with EMT is established, their role in mesenchymal-to-epithelial transition (MET) which is needed for distant organ colonisation of tumours remains to be defined. Fourth, EHMT2 provides a survival advantage to cancer cells by meeting energy demands and resisting cell death. The advancements in high-throughput sequencing have shed light on gene signatures and pathways affected by EHMT2 in tissue and cancer subtype-specific manner. Further mechanistic studies would shed insights into mechanisms by which EHMT1/2 link metabolism with CSC maintenance. While some insights have been gleaned into mechanisms by which EHMT1 and EHMT2 are overexpressed in distinct cancers, the
upstream regulators are largely uncharacterised. Identifying these regulatory pathways may provide relevant therapeutic targets. Moreover, as EHMT1 exhibits functions independent of EHMT2, further characterisation of its targets in cancer progression would provide avenues for selectively targeting its network.

Depending upon the cancer type, EHMT2 can either positively or negatively regulate metastasis in a methylation-dependent or -independent manner. These differences in its function have to be considered while using EHMT2 inhibitors to prevent metastasis. In this context, the development of highly selective EHMT1 and EHMT2 inhibitors and degraders is important given their distinct cancer-type-dependent functions.

**Acknowledgements**

Work in the RT laboratory is supported by grants from the National Medical Research Council (CBRG/0105/2016). We thank Nandini Karthik for proofreading the review. Figures in the review were created with Biorender.com

**Conflict of interest**

The authors declare that they have no conflict of interest.

**Author contributions**

All authors wrote the review.

**References**

1. Easwaran H, Tsai H-C, Baylin SB. Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance. *Mol Cell*. 2014;54:716–27.

2. Husmann D, Gozani O. Histone lysine methyltransferases in biology and disease. *Nat Struct Mol Biol*. 2019;26:880–9.

3. Lee JE, Kim M-Y. Cancer epigenetics: past, present and future. *Semin Cancer Biol*. 2021. doi: 10.1016/j.semcancer.2021.03.025

4. Kristensen LS, Raynor MP, Candiloro I, Dobrovic A. Methylation profiling of normal individuals reveals mosaic promoter methylation of cancer-associated genes. *Oncotarget*. 2012;3:450–61.

5. Scheer S, Zaph C. The lysine methyltransferase G9a in immune cell differentiation and function. *Front Immunol*. 2017;8:429.

6. Shankar SR, Bahirvani AG, Rao VK, Bharathy N, Ow JR, Taneja R. G9a, a multipotent regulator of gene expression. *Epigenetics*. 2013;8:16–22.

7. Kramer JM. Regulation of cell differentiation and function by the euchromatin histone methyltransferases G9a and GLP. *Biochem Cell Biol*. 2016;94:26–32.

8. Benevento M, van de Molengraft M, van Westen R, van Bokhoven H, Kasri NN. The role of chromatin repressive marks in cognition and disease: a focus on the repressive complex GLP/G9a. *Neurobiol Learn Mem*. 2015;124:88–96.

9. Jan S, Dar MI, Wani R, Sandey J, Mushtaq I, Lateef S, et al. Targeting EHMT2/G9a for cancer therapy: progress and perspective. *Eur J Pharmacol*. 2020;173827.

10. Rahman Z, Bazaz MR, Devabattula G, Khan MA, Godugu C. Targeting H3K9 methyltransferase G9a and its related molecule GLP as a potential therapeutic strategy for cancer. *J Biochem Mol Toxicol*. 2021;35: e22674.

11. Dong C, Wu Y, Yao J, Wang Y, Yu Y, Rychahou PG, et al. G9a interacts with Snail and is critical for Snail-mediated E-cadherin repression in human breast cancer. *J Clin Investig*. 2012;122:1469–86.
12 Chen H, Yan Y, Davidson TL, Shinkai Y, Costa M. Hypoxic stress induces dimethylated histone H3 lysine 9 through histone methyltransferase G9a in mammalian cells. Can Res. 2006;66:9009–16.
13 Bhatia S, Wang P, Toh A, Thompson EW. New insights into the role of phenotypic plasticity and EMT in driving cancer progression. Front Mol Biosci. 2020;7:71.
14 Celià-Terrassa T, Bastian C, Liu DD, El B, Aiello NM, Wei Y, et al. Hysteresis control of epithelial-mesenchymal transition dynamics conveys a distinct program with enhanced metastatic ability. Nat Commun. 2018;9:1–12.
15 Bandypadhyay S, Ali-Fehmi R. Breast carcinoma: molecular profiling and updates. Clin Lab Med. 2013;33:891–909.
16 Mabe NW, Garcia NMG, Wolery SE, Newcomb R, Meingasner RC, Vilona BA, et al. G9a promotes breast cancer recurrence through repression of a pro-inflammatory program. Cell Rep. 2020;33:108341.
17 Voduc KD, Cheang MC, Tyldesley S, Gelmon K, Nielsen TO, Kennecke H. Breast cancer subtypes and molecular profiling and updates. Clin Lab Med. 2018;49:62.
18 Liu XR, Zhou LH, Hu JX, Liu LM, Wan HP, Zhang XQ, UNC0638, a G9a inhibitor, suppresses epithelial-mesenchymal transition-mediated cellular migration and invasion in triple negative breast cancer. Mol Med Rep. 2018;17:2239–44.
19 Kim K, Son M-Y, Jung C-R, Kim D-S, Cho H-S. EHMT2 is a metastasis regulator in breast cancer. Biochem Biophys Res Commun. 2018;496:758–62.
20 Wozniak RJ, Klimecki WT, Lau SS, Feinstein Y, Futscher BW. 5-Aza-2′-deoxycytidine-mediated reductions in G9A histone methyltransferase and histone H3 K9 di-methylation levels are linked to tumor suppressor gene reactivation. Oncogene. 2007;26:77–90.
21 Casciolo F, Al-Ejeh F, Miranda M, Kelly G, Baxter E, Windloch K, et al. G9a-mediated repression of CDH10 in hypoxia enhances breast tumour cell motility and associates with poor survival outcome. Theranostics. 2020;10:4515.
22 Casciolo F, Al-Ejeh F, Kelly G, Brennan DJ, Ngio SF, Young A, et al. G9a drives hypoxia-mediated gene repression for breast cancer cell survival and tumorigenesis. Proc Natl Acad Sci USA. 2017;114:7077–82.
23 Hu X, Juneja SC, Muhlle NJ, Cleary MP. Leptin—a growth factor in normal and malignant breast cells and for normal mammary gland development. J Natl Cancer Inst. 2002;94:1704–11.
24 Ligibel J. Obesity and breast cancer. Oncology. 2011;25:994.
25 Chang C-C, Wu M-J, Yang J-Y, Camarillo IG, Chang C-J. Leptin–STAT3–G9a signaling promotes obesity-mediated breast cancer progression. Can Res. 2015;75:2375–86.
26 Siouda M, Dujardin AD, Barbollat-Boutrand L, Mendoza-Parra MA, Gibert B, Ouzounova M, et al. CDYL2 epigenetically regulates MIR124 to control NF-xB/STAT3-dependent breast cancer cell plasticity. Oncience. 2020;23:101141.
27 Weigelt B, Mackay A, A’hearn R, Natrajian R, Tan DSP, Dowsett M, et al. Breast cancer molecular profiling with single sample predictors: a retrospective analysis. Lancet Oncol. 2010;11:339–49.
28 Liu L, Kimball S, Liu H, Holowatyj A, Yang Z-Q. Genetic alterations of histone lysine methyltransferases and their significance in breast cancer. Oncotarget. 2015;6:2466.
29 Li K-C, Hua K-T, Lin Y-S, Su C-Y, Ko J-Y, Hsiao M, et al. Inhibition of G9a induces DUSP4-dependent autophagic cell death in head and neck squamous cell carcinoma. Mol Cancer. 2014;13:1–14.
30 Liu S, Ye D, Guo W, Wu Y, He Y, Hu J, et al. G9a is essential for EMT-mediated metastasis and maintenance of cancer stem cell-like characters in head and neck squamous cell carcinoma. Oncotarget. 2015;6:6887.
31 Hsiao S-M, Chen M-W, Chen C-A, Chien M-H, Hua K-T, Hsiao M, et al. The H3K9 methyltransferase G9a represses e-cadherin and is associated with myometrial invasion in endometrial cancer. Ann Surg Oncol. 2015;22:1556–65.
32 Yang Y, Shen J, Yan D, Yuan B, Zhang S, Wei J, et al. Euchromatic histone lysine methyltransferase1 regulates cancer development in human gastric cancer by regulating E-cadherin. Oncol Lett. 2018;15:9480–6.
33 Zhang C, Wei S, Hu J, Xiong Z. Upregulated expression of G9a is correlated with poor prognosis of gastric cancer patients. Medicine. 2019;98:e18212.
34 Lin X, Huang Y, Zou Y, Chen X, Ma X. Depletion of G9a gene induces cell apoptosis in human gastric carcinoma. Oncol Rep. 2016;35:3041–9.
35 Hu L, Wang H-X, Zhang B-G, Wang Z-Q, Fan Z-Y, Hu W, et al. G9A promotes gastric cancer metastasis by upregulating ITGB3 in a SET domain-independent manner. Cell Death Dis. 2018;9:1–14.
36 Chen G, Yu X, Zhang M, Zheng A, Wang Z, Zuo Y, et al. Inhibition of euchromatic histone lysine methyltransferase 2 (EHMT2) suppresses the
proliferation and invasion of cervical cancer cells. *Cytogenet Genom Res.* 2019;158:205–12.
39 Chen R-J, Shun C-T, Yen M-L, Chou C-H, Lin M-C. Methylation transferase G9a promotes cervical cancer angiogenesis and decreases patient survival. *Oncotarget.* 2017;8:62081.
40 Gao J, Yang Y, Qiu R, Zhang K, Teng X, Liu R, et al. Proteomic analysis of the OGT interactome: novel links to epithelial–mesenchymal transition and metastasis of cervical cancer. *Carcinogenesis.* 2018;39:1222–34.
41 Pan M-R, Hsu M-C, Chen L-T, Hung W-C. G9a orchestrates PCL3 and KDM7A to promote histone H3K27 methylation. *Sci Rep.* 2015;5:1–8.
42 Pan M-R, Hsu M-C, Luo C-W, Chen L-T, Shan Y-S, Hung W-C. The histone methyltransferase G9a as a therapeutic target to override gemcitabine resistance in pancreatic cancer. *Oncotarget.* 2016;7:61136.
43 Hua K-T, Wang M-Y, Chen M-W, Wei L-H, Chen C-K, Ko C-H, et al. The H3K9 methyltransferase G9a is a marker of aggressive ovarian cancer that promotes peritoneal metastasis. *Mol Cancer.* 2014;13:1–13.
44 Chopra A, Cho WC, Willmore WG, Biggar KK. Hypoxia-inducible lysine methyltransferases: G9a and GLP hypoxic regulation, non-histone substrate modification, and pathological relevance. *Front Genet.* 2020;11:579636.
45 Kang J, Shin S-H, Yoon H, Huh J, Shin H-W, Chun Y-S, et al. FIH is an oxygen sensor in ovarian cancer for G9a/GLP-driven epigenetic regulation of metastasis-related genes. *Can Res.* 2018;78:11844–99.
46 Caja L, Bertran E, Campbell J, Fausto N, Fabregat I. The transforming growth factor-beta (TGF-β) mediates acquisition of a mesenchymal stem cell-like phenotype in human liver cells. *J Cell Physiol.* 2011;226:1214–23.
47 Omenetti A, Porrello A, Jung Y, Yang L, Popov Y, Choi SS, et al. Hedgehog signaling regulates epithelial-mesenchymal transition during biliary fibrosis in rodents and humans. *J Clin Invest.* 2008;118:3331–42.
48 Yokoyama M, Chiba T, Zen Y, Oshima M, Kusakabe Y, Noguchi Y, et al. Histone lysine methyltransferase G9a is a novel epigenetic target for the treatment of hepatocellular carcinoma. *Oncotarget.* 2017;8:21315.
49 Bárcena-Varela M, Caruso S, Llerena S, Álvarez-Sola G, Uriarte I, Latasa MU, et al. Dual targeting of histone methyltransferase G9a and DNA-methyltransferase 1 for the treatment of experimental hepatocellular carcinoma. *Hepatology.* 2019;69:587–603.
50 Wei L, Chiu DK-C, Tsang FH-C, Law C-T, Cheng CL-H, Au SL-K, et al. Histone methyltransferase G9a promotes liver cancer development by epigenetic silencing of tumor suppressor gene RARRES3. *J Hepatol.* 2017;67:758–69.
51 Miller CR, Ruppert AS, Coombes K, Lehman AM, Blachly JS, Lucas DM, et al. The aberrantly expressed long noncoding RNA, TRERNA1, predicts for aggressive disease in chronic lymphocytic leukemia. *Blood.* 2015;126:2911.
52 Malgulwar PB, Namibirajan A, Singh M, Suri V, Sarkar C, Sharma MC. Expression and Clinical Significance of Translation Regulatory Long Non-Coding RNA 1 (TRERNA1) in Ependymomas. *Pathol Oncol Res.* 2020;26:1975–81.
53 Wu H, Hu Y, Liu X, Song W, Gong P, Zhang K, et al. LncRNA TRERNA1 function as an enhancer of SNAI1 promotes gastric cancer metastasis by regulating epithelial-mesenchymal transition. *Mol Ther Nucleic Acids.* 2017;8:291–9.
54 Song W, Gu Y, Lu S, Wu H, Cheng Z, Hu J, et al. LncRNA TRERNA1 facilitates hepatocellular carcinoma metastasis by dimethylating H3K9 in the CDH1 promoter region via the recruitment of the EHMT2/SNAI1 complex. *Cell Prolif.* 2019;52:e12621.
55 Hu Y, Zheng Y, Dai M, Wang X, Wu J, Yu B, et al. G9a and histone deacetylases are crucial for Snail2-mediated E-cadherin repression and metastasis in hepatocellular carcinoma. *Cancer Sci.* 2019;110:3442–52.
56 Guo Y, Zhao Y-R, Liu H, Xin Y, Yu J-Z, Zang Y-J, et al. EHMT2 promotes the pathogenesis of hepatocellular carcinoma by epigenetically silencing APC expression. *Cell Biosci.* 2021;11:1–16.
57 Sun T, Zhang K, Panenji RP, Wu J, Li W, Du Y, et al. G9a promotes invasion and metastasis of non-small cell lung cancer through enhancing focal adhesion kinase activation via NF-κb signaling pathway. *Mol Cancer Res.* 2021;19:429–40.
58 Hu Y, Zheng Y, Dai M, Wu J, Yu B, Zhang H, et al. Snail2 induced E-cadherin suppression and metastasis in lung carcinoma facilitated by G9a and HDACs. *Cell Adh Migr.* 2019;13:284–91.
59 Huang T, Zhang P, Li W, Zhao T, Zhang Z, Chen S, et al. G9a promotes tumor cell growth and invasion by silencing CASP1 in non-small-cell lung cancer cells. *Cell Death Dis.* 2017;8:e2726.
60 Mu Y-N, Zhang H-Y, Fei L-R, Zhang M-Y, Wang C-C, Luo Y, et al. SATB2 suppresses non-small cell lung cancer invasiveness by G9a. *Clin Exp Med.* 2018;18:37–44.
61 Wu P-C, Lu J-W, Yang J-Y, Lin I-H, Ou D-L, Lin Y-H, et al. H3K9 histone methyltransferase, KMT1E/SETDB1, cooperates with the SMAD2/3 pathway to suppress lung cancer metastasis. *Can Res.* 2014;74:7333–43.
62 Chen M-W, Hua K-T, Kao H-J, Chi C-C, Wei L-H, Johansson G, et al. H3K9 histone methyltransferase G9a promotes lung cancer invasion and metastasis by silencing the cell adhesion molecule Ep-CAM. *Can Res.* 2010;70:7830–40.
A. Nachiyappan et al.

EHMT1/2 in EMT and Stemness

63 Purcell DJ, Khalid O, Ou CY, Little GH, Frenkel B, Baniwal SK, et al. Recruitment of coregulator G9a by Runx2 for selective enhancement or suppression of transcription. *J Cell Biochem*. 2012;113:2406–14.

64 Han YC, Zheng ZL, Zuo ZH, Yu YP, Chen R, Tseng GC, et al. Metallothionein 1 h tumour suppressor activity in prostate cancer is mediated by euchromatin methyltransferase 1. *J Pathol*. 2013;230:184–93.

65 Li Y, Cheng C. Long noncoding RNA NEAT1 promotes the metastasis of osteosarcoma via interaction with the G9a-DNMT1-Snail complex. *Am J Cancer Res*. 2018;8:81.

66 Guan X, Zhong X, Men W, Gong S, Zhang L, Han Y. Analysis of EHMT1 expression and its correlations with clinical significance in esophageal squamous cell cancer. *Mol Clin Oncol*. 2014;2:76–80.

67 Zhong X, Chen X, Guan X, Zhang H, Ma Y, Zhang S, et al. Overexpression of G9a and MCM 7 in esophageal squamous cell carcinoma is associated with poor prognosis. *Histopathology*. 2015;66:192–200.

68 Qin J, Zeng Z, Luo T, Li Q, Hao Y, Chen Y. Clinicopathological significance of G9A expression in colorectal carcinoma. *Oncol Letters*. 2018.

69 Leitch HG, McEwen KR, Turp A, Encheva V, Carroll T, Grabole N, et al. Naive pluripotency is associated with global DNA hypomethylation. *Nat Struct Mol Biol*. 2013;20:311–6.

70 Ikegami K, Iwati M, Suzuki M, Tachibana M, Shinkai Y, Tanaka S, et al. Genome-wide and locus-specific DNA hypomethylation in G9a deficient mouse embryonic stem cells. *Genes Cells*. 2007;12:1–11.

71 Boroviak T, Loos R, Bertone P, Smith A, Nichols J. The ability of inner-cell-mass cells to self-renew as embryonic stem cells is acquired following epiblast specification. *Nat Cell Biol*. 2014;16:516–28.

72 Lehnerz B, Pabst C, Su L, Miller M, Liu F, Yi L, et al. The methyltransferase G9a regulates HoxA9-dependent transcription in AML. *Genes Dev*. 2014;28:317–27.

73 Tao H, Li H, Su Y, Feng D, Wang X, Zhang C, et al. Histone methyltransferase G9a and H3K9 dimethylation inhibit the self-renewal of glioma cancer stem cells. *Mol Cell Biochem*. 2014;394:23–30.

74 Tan Y, Wood AR, Jia Q, Zhou W, Luo J, Yang F, et al. Soft matrices downregulate FAK activity to promote growth of tumor-repopulating cells. *Biochem Biophys Res Comm*. 2017;483:456–62.

75 Tan Y, Tajik A, Chen J, Jia Q, Chowdhury F, Wang L, et al. Matrix softness regulates plasticity of tumour-repopulating cells via H3K9 demethylation and Sox2 expression. *Nat Commun*. 2014;5:4619.

76 Rowbotham S, Li F, Dost A, Louie S, Marsh B, Pessina P, et al. H3K9 methyltransferases and demethylases control lung tumor-propagating cells and lung cancer progression. *Nat Commun*. 2018;9:1–13.

77 Lee J, Kim K, Ryu TY, Jung CR, Lee MS, Lim JH, et al. EHMT1 knockdown induces apoptosis and cell cycle arrest in lung cancer cells by increasing CDKN1A expression. *Mol Oncol*. 2021;15(11):2989–3002.

78 Bergin CJ, Zugagga A, Haebe JR, Masibag AN, Desrochers FM, Reiley SY, et al. G9a controls pluripotent-like identity and tumor-initiating function in human colorectal cancer. *Oncogene*. 2021;40:1191–202.

79 Luo CW, Wang JY, Hung WC, Peng G, Tsai YL, Chang TM, et al. G9a governs colon cancer stem cell phenotype and chemoradioresistance through PP2A-RPA axis-mediated DNA damage response. *Radiother Oncol*. 2017;124:395–402.

80 Pangeni RP, Yang L, Zhang K, Wang J, Li W, Guo C, et al. G9a regulates tumorigenicity and stemness through genome-wide DNA methylation reprogramming in non-small cell lung cancer. *Clin Epigenet*. 2020;12:1–17.

81 Zhou M, Zhang X, Liu C, Nie D, Li S, Lai P, et al. Targeting protein lysine methyltransferase G9A impairs self-renewal of chronic myelogenous leukemia stem cells via upregulation of SOX6. *Oncogene*. 2021;40:3564–77.

82 Ishii H, Iwatsuki M, Ieta K, Ohta D, Haraguchi N, Mimori K, et al. Cancer stem cells and chemoradiation resistance. *Cancer Sci*. 2008;99:1871–7.

83 Kondo Y, Shen L, Ahmed S, Bounber Y, Sekido Y, Haddad BR, et al. Downregulation of histone H3 lysine 9 methyltransferase G9a induces centrosome disruption and chromosome instability in cancer cells. *PLoS One*. 2008;3:e2037.

84 Ginjala V, Rodriguez-Colon L, Ganguly B, Gangidi P, Gallina P, Al-Hraiwash H, et al. Protein-lysine methyltransferases G9a and GLP1 promote responses to DNA damage. *Sci Rep*. 2017;7:1–12.

85 Agarwal P, Jackson SP. G9a inhibition potentiates the anti-tumour activity of DNA double-strand break inducing agents by impairing DNA repair independent of p53 status. *Cancer Lett*. 2016;380:467–75.

86 Gursoy-Yuzugullu O, Carman C, Serafin RB, Myronakis M, Valente V, Price BD. Epigenetic therapy with inhibitors of histone methylation suppresses DNA damage signaling and increases glioma cell radiosensitivity. *Oncotarget*. 2017;8:24518.

87 Nakatsuka T, Tateishi K, Kato H, Fujisawa H, Yamamoto K, Kudo Y, et al. Inhibition of histone methyltransferase G9a attenuates liver cancer initiation by sensitizing DNA-damaged hepatocytes to p53-induced apoptosis. *Cell Death Dis*. 2021;12:1–13.

88 Zhang J, He P, Xi Y, Geng M, Chen Y, Ding J. Down-regulation of G9a triggers DNA damage response and inhibits colorectal cancer cells proliferation. *Oncotarget*. 2015;6:2917.
89 Watson ZL, Yamamoto TM, McMellen A, Kim H, Hughes CJ, Wheeler LJ, et al. Histone methyltransferases EHMT1 and EHMT2 (GLP/G9A) maintain PARP inhibitor resistance in high-grade serous ovarian carcinoma. Clin Epigenet. 2019;11:1–16.

90 Ciechomksa IA, Marciniak MP, Jackl J, Kaminaska B. Pre-treatment or post-treatment of human glioma cells with BIX01294, the inhibitor of histone methyltransferase G9a, sensitizes cells to temozolomide. Front Pharmacol. 2018;9:1271.

91 De Smedt E, Devin J, Muylaert C, Robert N, Requirand G, Vlummens P, et al. G9a/GLP targeting in MM promotes autophagy-associated apoptosis and boosts proteasome inhibitor-mediated cell death. Blood Adv. 2021;5:2325–38.

92 Tian Y-F, Wang H-C, Luo C-W, Hung W-C, Lin Y-H, Chen T-Y, et al. Preprogramming therapeutic response of P13K/mTOR dual inhibitor via the regulation of EHMT2 and p27 in pancreatic cancer. Am J Cancer Res. 2018;8:1812.

93 Li Y, Chen Z, Cao K, Zhang L, Ma Y, Yu S, et al. G9a regulates cell sensitivity to radiotherapy via histone H3 Lysine 9 trimethylation and CCDC8 in lung cancer. Onco Targets Ther. 2021;14:3721.

94 Chang Y-F, Lin K-H, Chiang Y-W, Sie Z-L, Chang J, Ho A-S, et al. STAT3 induces G9a to exacerbate HER3 expression for the survival of epidermal growth factor receptor-tyrosine kinase inhibitors in lung cancers. BMC Cancer. 2019;19:1–14.

95 Kelly GM, Al-Ejeh F, McCuaig R, Casciello F, Kamal NA, Ferguson B, et al. G9a inhibition enhances checkpoint inhibitor blockade response in melanoma. Clin Cancer Res. 2021;27:2624–35.

96 Segovia C, San Jose-Eneriz E, Munera-Maravilla E, Martinez-Fernandez M, Garate L, Miranda E, et al. Inhibition of a G9a/DNMT network triggers immuno-mediated bladder cancer regression. Cancer Cell. 2019;35:147–59.

97 Reina-Campos M, Diaz-Meco MT, Moscat J. The complexity of the serine glycine one-carbon pathway in cancer. J Cell Biol. 2020;219:1351–70.

98 Jiang Y, Eom J-I, Jeung H-K, Seol S-Y, Chung H, Kim YY, et al. Endoplasmic reticulum stress signaling comprises a G9a inhibitor tolerance pathway and PERK inhibition increases anti-leukemia activity of G9a inhibitor in leukemia cells and leukemia stem-like cells. Blood. 2018;132:1360.

99 Keating ST, El-Osta A. Epigenetics and metabolism. Circ Res. 2015;116:715–36.

100 Wang Z, Hausmann S, Lyu R, Li T-M, Lofgren SM, Flores NM, et al. SETD5-coordinated chromatin reprogramming regulates adaptive resistance to targeted pancreatic cancer therapy. Cancer Cell. 2020;37:834–849.e13.

101 Poulard C, Kim HN, Fang M, Kruth K, Gagneux C, Gerke DS, et al. Relapse-associated AURKB blunts the glucocorticoid sensitivity of B cell acute lymphoblastic leukemia. Proc Natl Acad Sci USA. 2019;116:3052–61.

102 Poulard C, Baulu E, Lee BH, Pufall MA, Stallcup MR. Increasing G9a automethylation sensitizes B acute lymphoblastic leukemia cells to glucocorticoid-induced death. Cell Death Dis. 2018;9:1–13.

103 Jang J, Eom J-I, Jeung H-K, Seol S-Y, Chung H, Kim YY, et al. Endoplasmic reticulum stress signaling comprises a G9a inhibitor tolerance pathway and PERK inhibition increases anti-leukemia activity of G9a inhibitor in leukemia cells and leukemia stem-like cells. Blood. 2018;132:1360.

104 Johnson C, Warmoes MO, Shen X, Locasale JW. Epigenetics and cancer metabolism. Cancer Lett. 2015;356:309–14.

105 Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. Cell Metab. 2016;23:27–47.

106 Dong C, Yuan T, Wu Y, Wang Y, Fan TW, Miriyala S, et al. Loss of FBP1 by Snail-mediated repression provides metabolic advantages in basal-like breast cancer. Cancer Cell. 2013;23:316–31.

107 Reina-Campos M, Diaz-Meco MT, Moscat J. The complexity of the serine glycine one-carbon pathway in cancer. J Cell Biol. 2020;219:e201907022.

108 Pan S, Fan M, Liu Z, Li X, Wang H. Serine, glycine and one-carbon metabolism in cancer. Int J Oncol. 2021;58:158–70.

109 Ding J, Li T, Wang X, Zhao E, Choi J-H, Yang L, et al. The histone H3 methyltransferase G9A epigenetically activates the serine-glycine synthesis pathway to sustain cancer cell survival and proliferation. Cell Metab. 2013;18:896–907.

110 Wang H, Cui L, Li D, Fan M, Liu Z, Liu C, et al. Overexpression of PSAT1 regulated by G9A sustains cell proliferation in colorectal cancer. Signal Transduct Target Ther. 2020;5:1–3.

111 Colyn L, Bárcena-Varela M, Álvarez-Sola G, Latasa MU, Uriarte I, Santamaría E, et al. Dual targeting of
G9a and DNMT1 for the treatment of experimental cholangiocarcinoma. *Hepatology*. 2020;73(6):2380–96.

115 Fan J-D, Lei P-J, Zheng J-Y, Wang X, Li S, Liu H, et al. The selective activation of p53 target genes regulated by SMYD2 in BIX-01294 induced autophagy-related cell death. *PLoS One*. 2015;10:e0116782.

116 Kim H, Park YJ. G9a, an epigenetic modifier, affects cell viability and serine-glycine synthesis pathway by altering ATF4 expression. *FASEB J*. 2017;31:lb331.

117 Liu S, Tao YG. Erratum to: chromatin remodeling factor LSH affects fumarate hydratase as a cancer driver. *Chin J Cancer*. 2016;35:99.

118 Song N, Wang J, Jiang H, Xie J. Ferroportin1 and hephaestin overexpression attenuate iron-induced oxidative stress in MES23.5 dopaminergic cells. *J Cell Biochem*. 2010;110:1063–72.

119 Torti SV, Torti FM. Iron and cancer: more ore to be mined. *Nat Rev Cancer*. 2013;13:342–55.

120 Wang Y-F, Zhang J, Su Y, Shen Y-Y, Jiang D-X, Hou Y-Y, et al. G9a regulates breast cancer growth by modulating iron homeostasis through the repression of ferroxidase hephaestin. *Nat Commun*. 2017;8:1–14.

121 Ahmad F, Dixit D, Joshi SD, Sen E. G9a inhibition induced PKM2 regulates autophagic responses. *Int J Biochem Cell Biol*. 2016;78:87–95.