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Noncanonical Functions of the Polycomb Group Protein EZH2 in Breast Cancer

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Enhancer of Zeste Homologue 2 (EZH2) is the catalytic subunit of the polycomb repressive complex 2 (PRC2) that is critical for determining cell identity. An epigenetic writer, EZH2 has a well-defined role in transcriptional repression by depositing trimethyl marks on lysine 27 of histone H3. However, there is mounting evidence that histone methyltransferases like EZH2 exert histone methyltransferase-independent functions. The relevance of these functions to breast cancer progression and their regulatory mechanisms are only beginning to become understood. Here, we review the current understanding of EZH2 H3K27me3-independent, noncanonical, functions and their regulation in breast cancer. (*Am J Pathol* 2021, 191: 774–783; <https://doi.org/10.1016/j.ajpath.2021.01.013>)

Polycomb Structure and Function

Epigenetic modifications, such as post-translational modifications that occur on histone tails, correlate with whether chromatin conforms to an open state (euchromatin) associated with active gene transcription, or a closed state that prevents gene transcription (heterochromatin). These modifications are broadly associated with three classes of proteins: those that place the modifications, those that read the modifications, and those that remove the modifications.¹

Polycomb group (PcG) and trithorax group (TrxG) are two of the most well-studied protein complexes responsible for post-translational modifications on histone tails, and consequently, epigenetic regulation. Genome-wide chromatin immunoprecipitation studies have revealed that PRC2 represses transcription of hundreds of genes to maintain pluripotency of embryonic stem cells.² The canonical catalytic subunit of PRC2 is EZH2, a 746–amino acid protein containing a C-terminal SET domain common to many lysine methyltransferases³ catalyzes the exchange of one to three methyl groups from donor S-adenosylmethionine (SAM) onto H3K27. The H3K27me3 form of H3K27 is associated with transcriptional repressive functions and, in

most contexts, is associated with genome-wide distribution of PRC2. Additional domains of EZH2 include its WD-40 binding domain (WDB) responsible for interaction with EED, and domains 1 and 2 thought to mediate protein–protein interactions.⁴ Two SANT (Swi3, Ada2, N-CoR, TFIIB) domains are less well characterized but may be important for interaction with histone tails.⁵ Interestingly, structural studies have found that SANT1 is a histone binding domain with specificity for the histone H4 N-terminal tail⁶ and sits adjoined to a stimulation response motif (SRM) that stabilizes SANT1. Additionally, a cysteine-rich domain (CXC) sits immediately N-terminal to the SET

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domain, which is important for methyltransferase activity.⁵ More recently, a transactivating domain (TAD) was observed comprising the SANT1 and SRM (stimulation response motif) regions.⁷

In addition to the well-described transcriptional and post-transcriptional mechanisms involving c-MYC,⁸ miRNAs, and other regulators,^{9,10} the function of EZH2 is regulated at a post-translational level in normal and neoplastic cells (Table 1).^{4,11–41}

EZH2 Function in Cancer

Deregulation of epigenetic proteins is commonly seen in cancer. Alterations in EZH2 have a complex role, exerting both tumor-suppressive and tumor-promoting functions depending on the context. Deregulation of EZH2 typically results in its overexpression, which often correlates to poor prognosis. Indeed, overexpression of EZH2 has been observed in multiple tumors, including prostate,^{42–44} breast,^{44–49} and others. Beyond its role as a biomarker, EZH2 has a well-established role as an oncogene; ectopic expression of EZH2 in nontumorigenic cells promotes their proliferation,^{42,45} and genetic knockdown of EZH2 reduces the proliferation, migration, invasion, and stem properties in multiple cancer cell lines and xenograft models.^{23,48–51}

How overexpression of EZH2 promotes cancer in individual tumor types is not well understood. Several putative tumor suppressors repressed by EZH2 have been described in prostate cancer and include repression of *CDH1*,⁴³ *RUNX3*,⁵² and others (reviewed by Kim and Roberts⁵³). However, for other tumor types, fewer targets have been identified, and their regulation by EZH2 may be context-specific, as in the case of tobacco-mediated induction of PRC2 repressing the tumor suppressor *DKK1* in lung cancer.⁵⁴

Activating somatic heterozygous mutations of EZH2 have been observed in up to 30% of non-Hodgkin lymphomas of follicular and germinal center diffuse large B-cell (GCB-DLBCL) subtypes.^{55,56} These predominantly occur within the SET domain (hotspot mutations at Y641, A677G, and A687) and alter EZH2 substrate specificity toward di- and trimethylated H3K27 over the monomethylated state.⁵⁷ The resulting altered specificity, such as in the case of Y641, affects both a global increase in H3K27me3 and a total redistribution of the repressive mark at PRC2-regulated loci that are sufficient for tumorigenesis⁵⁸ and is a rationale for clinical use of EZH2 inhibitors. Indeed, to date, three SAM-competitive EZH2 methyltransferase inhibitors (tazemetostat/EPZ-6348, GSK2816126, and CPI-1205) have reached phase I/phase II clinical trials for patients with follicular lymphomas, DLBCL harboring these gain-of-function mutations.⁵⁹ These inhibitors are also being examined in a genetically defined set of solid tumors harboring inactivating mutations in members of the ATP-dependent chromatin remodeling SWI/SNF complex that results in oncogenic

dependency on EZH2.^{60,61} A fourth compound, MAK683, is the only EZH2 inhibitor currently in clinical trials that does not target the methyltransferase activity directly, but rather disrupts EZH2–EED interaction, the proof of principle for which is described in Kim et al.⁶²

In breast cancer, EZH2 was first observed to be overexpressed in approximately 55% of invasive breast carcinomas. Its expression is inversely correlated with poor breast cancer survival,⁴⁵ suggesting its role as a potential biomarker. Multiple studies have corroborated this finding.^{44,47,63–66} High expression of EZH2 is significantly associated with estrogen receptor- (ER) and progesterone receptor- (PR) negative status, higher histologic grade, and triple-negative breast cancer (TNBC) status^{45,64} of breast tumors. Additionally, EZH2 may have a role that precedes frank tumor dissemination, because higher levels of EZH2 expression in normal breast epithelium correlate with increased risk of breast cancer.⁶⁷ Together, the data have demonstrated a role of EZH2 as a potential biomarker of breast cancer development and aggressive breast cancers.

Gain- and loss-of-function studies have demonstrated that EZH2 exerts oncogenic functions in breast cells. Overexpression of EZH2 in benign mammary epithelial cells is sufficient to increase anchorage-independent growth and invasion, and this phenotype is dependent on an intact SET domain.⁴⁵ Conversely, genetic inhibition of EZH2 using shRNA is sufficient to reduce the proliferation of ER-negative breast cancer cells *in vitro* and *in vivo* by delaying G2/M cell cycle transition.⁵⁰ Independent of proliferation, genetic inhibition with shRNA or pharmacologic depletion of EZH2 with 3-deazaneplanocin A (DZNep) is sufficient to reduce migration, invasion, and random motility of TNBC cells and is accompanied by the acquisition of a MET phenotype and restoration of E-cadherin.⁴⁸ EZH2 may also contribute to PARP inhibitor resistance, and pharmacologic inhibition of EZH2 with a specific inhibitor can restore PARP inhibitor sensitivity.⁴¹ EZH2 is capable of conferring tamoxifen resistance to ER-positive breast cancer cells by repressing the estrogen receptor alpha cofactor *GREB1*, which causes a redistribution of other ER α cofactors p300 and CBP. This may explain why tamoxifen can have a paradoxical growth-stimulating effect in tamoxifen-resistant ER-positive breast cancer cell lines.⁶⁶ These studies suggest that in breast cancer alone, EZH2 has a diverse set of oncogenic functions.

The role of EZH2 in breast cancer initiation is starting to be unraveled. Overexpression of EZH2 in the breast acinar epithelium is associated with an increased risk of invasive carcinoma.^{67,68} Transgenic mouse models of conditional EZH2 overexpression in mammary epithelial cells (MMTV LTR-driven EZH2) show disrupted terminal end bud development, a hyperbranching phenotype, and precocious intraductal epithelial hyperplasia, a precursor to carcinoma *in situ*.⁶⁹ Data suggest that EZH2 overexpression accelerates

Table 1 Post-Translational Modifications of EZH2

Modification	Site	Modifier	Function	Reference
Phosphorylation	S21	AKT	Suppresses EZH2 HMT activity by impeding affinity of EZH2 for nucleosome substrate Promotes the noncanonical methylation of STAT3 and STAT3 activity in GBM cells Appears to be required for androgen-independent growth of castration-resistant prostate cancer cells Appears to be required for the transcriptional activation activity of EZH2 in CRPC	4,11,12
Phosphorylation	Y244	JAK3	Promotes dissociation of PRC2 members; Increases noncanonical EZH2 interaction with RNA PolII, promotes proliferation of NK/T-cell lymphoma cells	13
Phosphorylation	T261	CDK5-related protein	Promotes degradation by F-box and WD repeat domain-containing 7	14
Phosphorylation	T311	AMP kinase	Disrupts interaction between EZH2 and SUZ12, resulting in reduced canonical H3K27 trimethylation Associated with better survival in breast and ovarian cancer patients	15
Phosphorylation	T345	CDK1, CDK2, PKC γ	Promotes degradation; cell-cycle dependent, increases binding to HOTAIR ncRNA and 5' end of Xist Promotes recruitment of EZH2 to target gene loci and maintenance of H3K27me3 levels at these target loci in prostate cells	16–18
Phosphorylation	S363	GSK3 β	Not yet known	19
Phosphorylation	T367	p38 α GSK3 β	Promotes satellite cell differentiation in response to TNF α through PJA1-mediated degradation of EZH2 Induces cytoplasmic localization of EZH2 and binding to vinculin and other cytoskeletal regulators of cell migration and invasion; critical for TNBC metastasis Contributes to histologic diversity and behavior of metaplastic breast tumors Reduces H3K27me3 activity and reduces migratory/invasive properties in MCF12A and MDA-MB-231 overexpressing cells	19–22
Phosphorylation	T416	CDK2	Enhances migration, invasion, stemness in TNBC cells Serves as a docking site for the forkhead-associated domain of NIPP1, which prevents dephosphorylation and is required for EZH2 association with proliferation loci	23,24
Phosphorylation	T487	CDK1 PKC γ	Promotes ubiquitination and degradation by the proteasome; negatively regulates proliferation in PC3 prostate cancer cells Disrupts binding with SUZ12 and EED, thereby suppressing H3K27 methyltransferase activity Promotes differentiation of mesenchymal stem cells	25–27
Phosphorylation	Y641	JAK2	Promotes B-TrCP (FBXW1)-mediated degradation	28
Phosphorylation	S652	ATM	May negatively regulate interaction with PRC2 members SUZ12 and EED and negatively regulate stability	29
Phosphorylation	S690	PKC γ	Not yet known	27
Phosphorylation	S734	ATM	May negatively regulate interaction with PRC2 members SUZ12 and EED and negatively regulate stability	29
O-GlcNacylation	S75	OGT	Likely positively regulates EZH2 protein stability	30
Acetylation	K348	PCAF and SIRT1 (deacetylation)	Decreases phosphorylation at T345 and T487, increases EZH2 stability, may affect H3K27me3-mediated repression of EZH2 target genes May enhance transcriptional silencing of HOXA10 in lung carcinoma cells; may enhance lung cancer cell migration and invasion	31

(table continues)

Table 1 (continued)

Modification	Site	Modifier	Function	Reference
Methylation	K307	SMYD2	Dimethylation at K307 critical for EZH2 protein stability, protecting from ubiquitination and degradation and thereby contributing to breast cancer cell invasion	32
Methylation	R432	PRMT1	Asymmetric demethylation which inhibits CDK-1 mediated phosphorylation of EZH2 at T345 and T487, thereby preventing ubiquitination by TRAF6	33
Methylation	K510	PRC2 (auto)	Contributes to breast cancer cell invasion and metastasis	34,35
Methylation	K514	PRC2 (auto)	Increases activity, promotes accessibility to H3 tail	
			Increases activity, promotes accessibility to H3 tail	34,35
			Significantly reduced in diffuse intrinsic pontine glioma cells carrying H3K27M mutation	
Methylation	K515	PRC2 (auto)	Not known	34,35
Ubiquitination	K421	SMURF2	Proteasomal degradation	36,37
Ubiquitination		Praja1, FBXW, FBXW7, TRAF6	Protein instability and degradation	14,20,33,38–40
PARYlation	D233, E239	PARP1	Loss of PARYlation results in PRC2 dissociation, EZH2 down-regulation, and decreased H3K27me3	41

Shown are the demonstrated post-translational modifications of EZH2 occurring across multiple systems. GBM, glioblastoma multiforme.

breast cancer initiation in MMTV-neu mice and increases the numbers of tumor initiating cells in this model through transcriptional activation of *Notch1* via EZH2 interaction with RelA and RelB NF- κ B components.⁵¹ Further investigations are necessary to define the role of EZH2 in breast cancer initiation.

Several putative mechanisms have been posited to explain how overexpression of EZH2 confers proliferative, migratory, and invasive potential to breast cancer cells. These include transcriptional repression of tumor suppressors such as RUNX3,⁵² RAD51-paralog proteins,⁷⁰ and RKIP.⁷¹ EZH2 has been shown to promote epithelial–mesenchymal transition by promoting signaling through protumorigenic kinases, such as by activating the p38 MAPK.⁴⁸ Several recent studies show that a subset of TNBCs display a decoupling of EZH2 and H3K27me3. TNBCs display higher levels of EZH2 and lower levels of H3K27me3, as indicated by immunohistochemistry.⁷² Stratification of patients by EZH2 and H3K27me3 shows that the combination of high EZH2/low H3K27me3 portends the worst overall survival in breast cancer patients, irrespective of ER status.⁷² These observations suggest the existence of H3K27me3-independent functions of EZH2, such as transactivating functions and nonhistone protein methylation, which are the focus of intense investigation and are summarized below.

Noncanonical Functions of EZH2 in Breast Cancer

The reported noncanonical mechanisms of EZH2 in breast tumorigenesis are summarized in Figure 1. Independent of its H3K27me3 activity, EZH2 can transcriptionally activate

genes. Underscoring the importance of cellular context are the different roles of EZH2 in studies of ER-positive versus ER-negative breast cancer. In ER-negative basal-like breast cancers, EZH2 activates transcription of NF- κ B targets independent of other PRC2 members.⁷³ In this setting, EZH2 forms a complex with RelA and RelB on NF- κ B target genes and is associated with activating H3K4 marks rather than H3K27me3 marks.⁷³ Interestingly, EZH2 may itself transcriptionally activate RelB in TNBC, adding an additional layer of complexity.⁷⁴ By contrast, in ER-positive breast cancer cell lines, EZH2 interacts with ER α and β -catenin upon E2 treatment. In response to estradiol treatment, ER α , β -catenin, and EZH2 form a complex on the *MYC* (alias *c-myc*) promoter and activate its transcription, independent of the histone methyltransferase (HMT) activity of EZH2.⁷⁵ Furthermore, EZH2 interacts with the transcription factor TRIM28 and positively regulates transcription of approximately 100 genes in ER-positive breast cancer cell lines. Through chromatin immunoprecipitation quantitative PCR, EZH2 and TRIM28 were found to co-occupy regions within 10 kb of the transcription start site.⁷⁶

EZH2 transcriptionally activates *Notch1* in TNBC to increase the number of tumor-initiating cells in cell lines and in animal models.⁵¹ In this instance, EZH2 binds to the proximal *Notch1* promoter with cofactors RelA and RelB to activate transcription independent of H3K27me3, and associated with H3K4 activating marks. Studies involving generation of myc-tagged EZH2 deletion mutants involving the amino-terminal homology domains I and II (Δ HI and Δ HII), the carboxyl-terminal SET domain (Δ SET), and the nuclear localization signal (Δ NLS), as well the EZH2-H689A mutant, which has reduced HMT activity, in MCF10A cells,⁵¹ indicate that EZH2 HMT activity is dispensable for *Notch1* transcriptional activation and stem

Noncanonical functions of EZH2 in breast cancer growth and metastasis

Figure 1 Noncanonical functions of EZH2 in breast cancer progression. The schematic summarizes current knowledge in estrogen receptor positive (asterisks) and in triple negative (double asterisks) breast cancer.

cell expansion in nontumorigenic breast cells. The translational relevance of these findings was validated in human tissue samples where high levels of EZH2 are significantly associated with activated NOTCH1 protein and increased tumor-initiating cells in triple-negative invasive carcinomas.⁵¹

A role for EZH2 in transcriptional activation was reported in other systems, including intestinal stem cells,⁷⁷ and in castration-resistant prostate cancer. In the latter, chromatin immunoprecipitation sequencing of EZH2 and H3K27me3 has shown sites where EZH2 does not colocalize with the repressive mark. These solo peaks are instead associated with H3K4me2 and -3 and RNA pol II, and EZH2 knock-down decreases levels of active mark at these sites. These data are interesting because they not only support a transactivating role of EZH2, but also suggest that EZH2 might function as both a transcriptional repressor and activator within the same type of cell.⁴ Taken together, these studies reveal the complexity of EZH2 functions in tumorigenesis where the protein is capable of exerting repressive and activating functions depending on cellular context.

How EZH2 switches from a transcriptional repression role to a transcriptional activation role is not fully understood. A disordered transactivation domain (TAD) contains the SRM and SANT1 regions and exists in a structurally locked state in an alpha-helix bundle structure bound by the SBD of EZH2. Phosphorylation at Y244 by JAK3 or at S21 by AKT reduces SBD binding, thereby allowing the transactivation domain to interact with transcriptional coactivators such as the histone acetyltransferase p300.⁷

In recent years, there has been growing evidence that EZH2 may undergo phosphorylation, which plays an important role in regulating EZH2 noncanonical functions. EZH2 phosphorylation events have been linked to decreased

EZH2 transcriptional repressor function through reduction of H3K27me3.

One of the first indications that phosphorylation modifies EZH2 functions in cancer progression was published in 2005, when Cha et al¹¹ characterized the phosphorylation of S21 by Akt in MDA-MB-453 breast cancer cells. Modification of this site results in decreased affinity of EZH2 for H3, and therefore less methyltransferase activity compared with the unphosphorylated enzyme.⁷⁸ The mechanism appears to rely on reduced affinity of EZH2 to chromatin but does not affect the composition of the PRC2. Interestingly, several novel functions of EZH2 in malignancies other than breast have been demonstrated as a result of S21 phosphorylation: in multiple myeloma, where S21 phosphorylation is implicated in cell-adhesion-mediated drug resistance⁷⁸; in glioblastoma, where S21 phosphorylation promotes cancer progression via nonhistone methylation of STAT3¹²; and in prostate cancer, where S21 phosphorylation switches EZH2 to a transcriptional coactivator of AR.⁴ However, these same mechanisms have not been definitively shown in breast cancers.

There are two phosphorylation sites that do not affect EZH2 methyltransferase activity directly, but instead lead to decreased activity due to the poor binding of phosphorylated forms to the scaffolding proteins of the polycomb repressive complex. Wan et al¹⁵ showed that AMPK, which is activated in conditions of energy stress, phosphorylates EZH2 at T311, leading to dissociation from the PRC2 and less H3K27 methylation at EZH2 target sites in breast cancer cells. Increased active AMPK and pT311-EZH2 are both found in breast and ovarian cancers, but only pT311-EZH2 correlates with higher survival.

Cyclin-dependent kinase 1 and 2 (CDK1 and CDK2) phosphorylation of EZH2 plays an important role in

Table 2 Nonhistone Targets of EZH2, Summarized

Substrate	Site	Peptide	Function and notes	Reference
β-Catenin	K49me3	LSGGNP	Promotes repression of Sox1 and Sox3 in ESCs, competes with acetylation at the same site	81
EloA	K754me	TVKIAP	Modulates expression of low-expression PRC2 target genes in mouse ESCs	82
Fra-2	K104me, K104me2	GVITIG	May prevent Fra-2 from transcriptionally regulating epidermal differentiation genes	83
GATA4	K299me	LYMLHG	Reduces its transcriptional activity by preventing p300 acetylation in HL-1 cardiac muscle cells	84
Histone H2B	K120me	AVTYTS	May compete with H2BK120-ub <i>in vitro</i> and in cancer cell lines	85
Jarid2	K116me3, K116me2	AQRFAQ	Mimics trimethylated histone tail and allosterically activates PRC2 in ESCs	86,87
p38α	K139me3, K165me3	RGLYIH CELILD	May regulate p38 activity	48 and unpublished data
PLZF	K430me	SGMTYG	Based on predictive software*	88
RORα	K38me	SARSEP	Promotes ubiquitination and degradation in HEK293 cells	89
Stat3	K49me2	AASESH	Promotes transcription of STAT3 target genes in response to IL-6 in colon cancer cells	90
Stat3	K180me3	KTLSQG	Promotes activation of STAT3 signaling in glioblastoma stem-like cells	12
Talin	K2454me3	EAMRLQ	Disrupts binding of Talin to F-actin in neutrophils and dendritic cells	91

Shown are the putative nonhistone targets of EZH2, along with the sequences of the three amino acids.
ESC, embryonic stem cell.

*Prediction of Methylation Sites Based on Enhanced Feature Encoding Scheme (PMeS).

regulating EZH2 in breast cancer, with CDK1 promoting noncanonical functions. CDK1 phosphorylation of EZH2 at T487, which lies within the SUZ12 binding region of EZH2, disrupts the interaction between EZH2 and SUZ12/EED, and regulates EZH2 protein stability.²⁵ Functionally, CDK1-dependent phosphorylation of EZH2 at T487 suppresses methylation of H3K27 and promotes osteogenic differentiation of human mesenchymal stem cells.⁸³ In breast cancer MCF7 cells, loss of phosphorylation at T487 does not affect cell proliferation but significantly increases cancer cell migration and invasion, suggesting that phosphorylation at this site is associated with EZH2 metastatic functions.^{17,26} In line with these findings, the methyltransferase PRMT1 promotes metastasis of MDA-MB-231 and MCF7 breast cancer cells by methylating EZH2 at R342, which prevents phosphorylation of nearby T487. Interestingly, phosphoproteomic analyses have demonstrated T487 phosphorylation is significantly elevated in basal-like breast cancers compared with other subtypes,⁷⁹ suggesting a context-specific role for this event.

p38 Mitogen activated kinase phosphorylates EZH2 at T367. First described in myoblasts and satellite cells, it was recently shown to play an important role in TNBC progression. In muscle cells, activation of the p38 signaling pathway via tumor necrosis factor α (TNFα), as in case of muscle injury, results in p38α-mediated phosphorylation of EZH2 at threonine 372 (which corresponds to T367; the five

amino acid difference resulting from using different EZH2 isoforms as reference), and recruitment of EZH2 to the Pax7 promoter.²¹ In skeletal muscle progenitors, MyoD induces an E3 ubiquitin ligase Praja1 to ubiquitinate and subsequently degrade EZH2 in response to p38-mediated phosphorylation at T372.^{20,21}

In breast cancer, p38 phosphorylation of EZH2 at T367 has a novel function. EZH2 and p38 are concordantly expressed in invasive breast carcinomas, and interact in breast cancer cells.⁴⁸ Recent studies further characterized this interaction by showing that p38 phosphorylates EZH2 at T367 and developed and validated a phosphorylation-specific antibody for this site.⁸⁰ Interestingly, in normal lobules, if present, pEZH2-T367 is exclusively nuclear, but in invasive breast cancer cells is expressed in the cytoplasm as well as in the nucleus. There is a significant increase in cytoplasmic pEZH2-T367 with cancer progression, and high cytoplasmic pEZH2-T367 in invasive carcinomas are significantly associated with higher histologic grade, ER-, PR-, and triple-negative status. Mechanistically, phosphorylation results in reduced global H3K27me3 levels by controlling EZH2 subcellular localization and promoting movement to the cytoplasm. In the cytoplasm, EZH2 complexes with cytoskeletal proteins such as vinculin to promote breast cancer cell adhesion, migration, and invasion. pEZH2-T367 retains the ability to bind to PRC2 members EED and SUZ12 in TNBC cells, suggesting the

intriguing hypothesis that phosphorylation of EZH2 at this specific site may promote methylation of cytoplasmic proteins, which warrants further investigation.⁸⁰ Supporting this hypothesis are preliminary studies suggesting that EZH2 catalyzes p38 α methylation, as well as data showing that EZH2 methylates nonhistone proteins in normal and in neoplastic cells (Table 2).^{12,48,81–91}

In vivo, using knockdown-rescue orthotopic xenograft mouse models of breast cancer, no difference was noted in primary tumor growth between tumors whose EZH2 had been replaced with EZH2 or with a phospho-deficient T367A mutant. However, luciferase analysis demonstrated that loss of T367 phosphorylation of EZH2 resulted in significantly less metastatic burden, supporting a role for T367 in mediating the migratory and invasive properties of EZH2 in breast cancer. Interestingly, the existence of cytoplasmic EZH2 has been described previously.⁴⁷ In metaplastic breast carcinomas, the most aggressive form of TNBC, there is differential expression and subcellular localization of pEZH2-T367 among different histologic subtypes, suggesting that pEZH2-T367 may play a role in the histologic diversity of these tumors.²²

Translational Implications of Noncanonical EZH2 Functions in Breast Cancer

There are still limited data on how inhibition of EZH2 functions affects tumorigenesis in preclinical models of breast cancer. Inhibition of canonical EZH2 function, assessed by global levels of H3K27me₃, using the SAM-competitive small molecule GSK126 at low micromolar concentrations appears to have no effect on cell proliferation of three TNBC cell lines, despite derepression of at least two canonical EZH2 targets, CDKN1A and CDKN1C, and may even promote invasion of breast cancer cells through derepression of MMPs.⁹² These data have been replicated recently in the MMTV-PyVmt model of breast cancer, in which mice develop tumors resembling luminal B breast cancer subtype. Interestingly, treatment of mice harboring luminal B tumors with the EZH2 inhibitor GSK126 has minimal effects on primary tumor size but significantly reduces lung metastases.⁹³ Recently, Yomtubian et al⁹⁴ found similar results using orthotopic LM2 as well as human TNBC patient-derived xenograft mouse models of breast cancer; GSK-126 treatment had no effect on primary tumor size, but significantly reduced breast cancer lung metastases, which was attributed to an effect on a small subpopulation of metastatic cells.

The oncogenic alterations in pathways directly involving EZH2 are frequent across multiple types of cancer, which make targeting EZH2 a tantalizing option for cancer treatment. However, the body of work of EZH2 function in breast cancer supports that it may have multiple, discrete oncogenic functions that are not fully explained by its histone methyltransferase activity and that have yet to be fully

delineated. This consideration is important, because several potent SAM-competitive EZH2 inhibitors have been developed over the past 5 years and have reached phase I to III clinical trials. The majority of these are with the inhibitor tazemetostat, which was approved by the Food and Drug Administration in 2020 for metastatic or locally advanced epithelioid sarcoma. However, given the increasing evidence of noncanonical functions of EZH2 in promoting cancer, the use of SAM-competitive inhibitors may not be as effective as other small molecules that disrupt EZH2 protein–protein interactions. These include MAK683, which binds to EED and disrupts the EZH2 interaction with histone tail, and is being actively investigated in clinical trials (reviewed by Eich et al⁹⁵). More recently, proteolysis-targeting chimeras (PROTACs), as well as EZH2 degraders, have offered novel mechanisms of degrading EZH2 and are exciting new modalities added to the armament of PRC2 inhibition.^{96–98} These data highlight the need for a greater understanding of the noncanonical functions of EZH2 in order to develop effective management options for breast cancers.

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