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Novel molecular targets in gastric adenocarcinoma

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ABSTRACT

Gastric adenocarcinoma (GAC) is the third leading cause of cancer-related death worldwide. A high mortality rate and resistance to treatment protocols due to a heterogeneous molecular pathogenesis has made discovering the key etiologic molecular alterations of the utmost importance. The remarkable role played by epigenetic modifications in repressing or activating many cancer-related genes and forming new epigenetic signatures can affect cancer initiation and progression. Hence, targeting the key epigenetic drivers could potentially attenuate cancer progression. MLLs, ARID1A and EZH2 are among the major epigenetic players that are frequently mutated in GACs. In this paper, we have proposed the existence of a network between these proteins that, together with PCAF and KDM6A, control the 3D chromatin structure and regulate the expression of tumor suppressor genes (TSGs) and oncogenes in GAC. Therefore, we suggest that manipulating the expression of *EZH2*, *PCAF*, and *KDM6A* or their downstream targets may reduce the cancerous phenotype in GAC.

Contents

Abbreviations: 5-FU, 5-Fluorouracil; ANR, Active, Not Recruiting; BET, Bromodomain and Extra-Terminal motif; ARID1A, AT-Rich Interaction Domain 1A; CBP, CREB-binding Protein; CDA, Cytidine Deaminase; CDH1, Cadherin-1; CNV, Copy Number Variation; DAC, Decitabine; DNMT, DNA Methyltransferase; EZH2, Enhancer of Zeste 2; GAC, Gastric Adenocarcinoma; H3K27, Histone H3 lysine 27; HER2, Human Epidermal growth factor Receptor 2; HDAC, Histone deacetylase; KDM6A, Lysine-specific demethylase 6A; MLL, Mixed Lineage Leukemianc; RNA, non-coding RNA; PCAF, P300/CBP-associated Factor; PRC2, Polycomb Repressive Complex 2; PIK3CA, Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha; T-ALL, T-cell Acute Lymphoblastic Leukemia; TCGA, The Cancer Genome Atlas; THU, Tetrahydrouridine; TP-53, Tumor Protein p53; TSG, Tumor Suppressor Gene; UTX, Ubiquitously Transcribed Tetratricopeptide repeat, X chromosome.

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1. Gastric adenocarcinoma; an epigenetic perspective

Gastric adenocarcinoma (GAC) constitutes approximately 95% of cancer cases originating in the stomach and it has a high mortality rate worldwide. The low median patient survival rates are due to the fact that many are only diagnosed at an advanced stage of the disease. However, other factors such as high levels of heterogeneity and a lack of effective therapeutic modalities leading to dramatically reduced survival rates means that GAC has imposed a substantial health burden globally. The high incidence and prevalence of GAC combined with high mortality rates has encouraged scientists to investigate the molecular mechanisms underlying the pathogenesis of this disease (Jaffer A Ajani et al., 2017; Padmanabhan, Ushijima, & Tan, 2017; Torre, Siegel, Ward, & Jemal, 2016).

Unfortunately, the high heterogeneity of GAC tumors with respect to phenotype and genotype is one of the main obstacles in terms of finding an efficient therapeutic approach (Gao, Xu, Liu, Yan, & Zhu, 2018). To overcome this issue, whole genome analysis has been exploited in order to investigate the wide range of genomic alterations in gastric tumors. As a result, The Cancer Genome Atlas (TCGA) and the Asian Cancer Research Group (ACRG) have identified four distinct subtypes of GAC based on the genetic, epigenetic, and gene expression characteristics of tumors. Each subtype represents a distinct pattern of genomic alterations, particularly mutations (Jaffer A Ajani et al., 2017; Padmanabhan et al., 2017; Sohn et al., 2017). Notably, such variations can be seen in genes encoding cell adhesion-related proteins such as CTNNB1 (Catenin Beta 1) and CDH1 (Cadherin-1). Although CTNNB1 mutations are common in both intestinal and diffuse GACs, they are more frequently observed in intestinal tumors (Ogasawara et al., 2006).

Overall, the main etiological factors for GAC can be summarized in three main areas consisting of environmental risk factors along with genetic and epigenetic drivers (Abdi, Latifi-Navid, Zahri, Yazdanbod, & Pourfarzi, 2019; Padmanabhan et al., 2017). With respect to environmental factors, *Helicobacter pylori* (*H. pylori*) and Epstein-Barr virus (EBV) infections constitute the leading contributors to the development of GAC especially in endemic areas (Teresa et al., 2019). In addition, many DNA alterations have been detected in GAC. Among the commonly mutated genes, a high rate of aberrations in DNA repair genes has attracted attention as loss of these genes makes the DNA sequences more unstable. The accumulation of various mutations leads to a weak and inefficient response by gastric tumors to drugs and combinational therapies.

Dysregulation of the gene expression profile, which is a feature of GAC, can be due to widespread changes in the epigenetic profile of the cancerous cell known as epimutation. Epimutations can promote the activation of oncogenes and silencing of tumor suppressor genes (TSGs) that can result in cancer progression, invasion and drug resistance. The range of epigenetic-related changes involve CpG island methylation, histone modifications, and various non-coding RNAs (ncRNAs), which all affect gene expression. It has already been shown that global DNA methylation and hydroxy methylation together with certain histone modifications of specific genes are altered in GAC cells (Yang et al., 2013; Zeng, Wang, & Chen, 2017). Furthermore, the expression of specific ncRNAs is modulated in gastric tumors (Yu & Rong, 2018). Changes in the 3D structure of chromatin, such as the conversion of euchromatin to heterochromatin or vice versa, can affect many TSGs or oncogenes. Hence, alterations in individual genes that are responsible for the epigenetic status of cells may be of great importance. Therefore, there is a possibility that targeting these factors that affect the epigenome could potentially alleviate the cancer phenotype or even lead to an increased apoptosis rate in GAC cells (Roberti, Valdes, Torrecillas, Fraga, & Fernandez, 2019).

2. Current standard treatments for GAC

After staging the cancer, appropriate therapy should be initiated using a multidisciplinary approach. The National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in the US, the European Society for Medical Oncology (ESMO) Clinical Practice Guidelines in Europe, and the Japanese Gastric Cancer Treatment Guidelines are examples of established practice guidelines which utilize treatment algorithms that should be applied for recommended therapies (J. A. Ajani et al., 2016; Japanese Gastric Cancer Association, 2020; Smyth et al., 2016). As an example, according to the ESMO Guidelines, the first treatment decision for GAC patients is made based on considering the three main stages of the disease: (i) operable cancers at stage T1N0 ("T" plus a number indicates the size and location of the tumor and "N" plus a number indicates the involved lymph nodes), (ii) operable cancers at stages >T1N0, and (iii) inoperable or metastatic cancers. The recommended therapy is then implemented for each type (Smyth et al., 2016). Below, we discuss the main clinical strategies for the management of GAC, including endoscopic therapy, surgical approaches, chemotherapy, and radiotherapy.

2.1. Endoscopic therapy

In the early phases of gastric cancers when invasion is limited to the mucosa or submucosa, the endoscopic resection of tumors is recommended. Endoscopic mucosal resection (EMR) for smaller lesions and endoscopic submucosal dissection (ESD) for larger tumors constitute the two main procedures for this treatment strategy (Ko, Song, Kim, Hong, & Cho, 2016). The main criteria that determine the necessity for endoscopic resection are the depth and diameter of the tumor along with the histologic grade and ulcerative component (Jaffer A Ajani et al., 2017).

2.2. Surgical approaches

Gastrectomy for complete resection of the tumor and lymphadenectomy are the two main surgery-based strategies. With a 5 cm proximal margin present between the tumor and the gastroesophageal junction, or 8 cm for diffuse cancers, a subtotal gastrectomy would be recommended; otherwise, a total gastrectomy may be performed (Smyth et al., 2016). The definition of a D0–D3 lymphadenectomy is mainly based on the 16 lymph node stations, each of which has a defined anatomical location surrounding the stomach. While D1 involves the dissection of perigastric lymph nodes, D2 implies the dissection of lymph nodes together with the left gastric, common hepatic and splenic arteries and the celiac axis, in addition to the perigastric ones (Garg, Jakhetiya, Sharma, Ray, & Pandey, 2016; Japanese Gastric Cancer Association, 2011; Smyth et al., 2016). However, a number of clinical GAC trials in western countries reported no clear survival benefit from extended lymphadenectomy or the combined removal of the spleen or bursa (Jaffer A Ajani et al., 2017). Laparoscopic gastrectomy is another interventional method recommended for early gastric cancers. This approach is safe and postoperative complications are lower, particularly when used in combination with enhanced recovery after surgery (ERAS) protocols (J. Wang et al., 2020).

2.3. Chemotherapy

Applying chemotherapy in a preoperative (neoadjuvant) and postoperative (adjuvant) manner for advanced and metastatic gastric cancer (GC) should result in improved survival and quality of life (J. A. Ajani et al., 2016). The main aims of neoadjuvant chemotherapy are to downstage the tumor's growth and disease progression, increase resection rate, eradicate remaining tumor cells, and reduce cancer-related symptoms (Orditura et al., 2014). The use of fluoropyrimidine-platinum (oxaliplatin in preference to cisplatin) doublet or triplet chemotherapy before surgery is considered reasonable (Smyth et al., 2016). While the results of the British Medical Research Council (MRC) MAGIC (MRC Adjuvant Gastric Infusional Chemotherapy) trial showed beneficial effects in the perioperative ECF arm (Epirubicin/Cisplatin/5-Fluorouracil

[5-FU]) for 5-year survival, it is suggested that 5-FU can be replaced with capecitabine (ECX regimen), which does not require an indwelling central venous access device (Cunningham et al., 2006; Cunningham et al., 2008). Moreover, in the Phase II/III FLOT4-Arbeitsgemeinschaft Internistische Onkologie (AIO) trial, patients with locally advanced, resectable gastric or gastroesophageal junction adenocarcinoma receiving the FLOT regimen (fluorouracil, leucovorin, oxaliplatin, and docetaxel) showed a higher overall survival in comparison with the ECF/X regimen (Al-Batran et al., 2019).

Conventionally, radiotherapy (RT) is considered as a palliative therapy and can be employed in a pre- or post-operative manner and in combination with chemotherapy agents. It has been shown that the administration of chemoradiotherapy in an adjuvant setting is an effective treatment regimen that improves overall survival in treated patients (Macdonald et al., 2001; Smalley et al., 2012). In a clinical trial of 559 patients with stages IB to IV GC, the treatment efficacy of post-surgical chemoradiotherapy compared to surgery alone was studied. The chemoradiotherapy group received 5-FU and leucovorin, followed by chemoradiotherapy 28 days after the start of the initial cycle of chemotherapy. Over a three-year time frame, the overall survival rate of patients increased to 50% compared with the surgical arm alone (Smalley et al., 2012).

On the other hand, targeted therapy with active biologicals such as those against human epidermal growth factor receptor 2 (HER2), vascular endothelial growth factor receptor 2 (VEGFR2), vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), fibroblast growth factor receptor 2 (FGFR2), FGFR2B, AKT, MET, mammalian target of rapamycin (mTOR), ATM, and Claudin18.2 are currently under investigation in various trials. The combination of chemotherapy and trastuzumab (an anti-HER2 antibody) for patients with HER2 protein overexpression, in comparison with chemotherapy alone, has already shown a survival advantage (Jaffer A Ajani et al., 2017; Bang et al., 2010).

The complex nature of GAC and the limitations associated with current standard treatments have inspired scientists to investigate novel therapeutic strategies. To this end, molecular targets may provide promising insights into drug development in cancer medicine, and particularly for GAC.

3. Translational perspective on epigenetic therapy in GAC

The capacity of epigenetic drivers to induce different genes to up- or down-regulate, makes them potential targets for cancer therapy. There is plenty of evidence showing that individual epigenetic effectors can regulate the expression of various oncogenes and TSGs, which are involved in numerous cancer-related biological processes. Furthermore, alteration of the epigenetic status of chromatin plays a role in the process through which cancer cells become resistant to cytotoxic agents. In this regard, some clinical data have already shown the therapeutic effects of epigenetic modifiers prevailing over resistance after chemotherapy and irradiation in GAC and other tumors (Emran et al., 2019; Strauss & Figg, 2016). Moreover, given the role of epigenetic modifications in silencing tumor-associated antigens, epigenetic therapies may demonstrate beneficial results in terms of unmasking tumor antigens that have been hidden from the immune system (Dunn & Rao, 2017).

The fact that epigenetic alterations, unlike genetic mutations, are reversible has made them ideal targets for cancer therapies. Two types of epigenetic drugs, inhibitors of DNA methyltransferases (iDNMTs) and histone deacetylase inhibitors (iHDACs), have been approved by the American Food and Drug Administration (FDA) (Roberti et al., 2019). In addition, multiple epigenetic modulating agents have shown promising results in several late-stage clinical trials (Table 1).

Cancers present a highly heterogeneous, dynamic and complicated microenvironment and the mechanisms recruited by each type of cancer may vary according to their origin and specific microenvironment conditions. Therefore, applying a single strategy, such as inhibiting the DNMT enzyme, may not have the same effect in different tumor types or even in similar tumors in different individuals. Moreover, as has been previously shown, this therapeutic approach still lacks specificity and it is possible that the methylated oncogenes may reactivate (Nishigaki et al., 2005) and even cause hypomethylation of the entire genome (Gius et al., 2004). Another issue in terms of epigenetic therapy is the variation in epigenetic status of various cancers such as hematological malignancies and solid tumors, which makes this type of therapy a context-dependent treatment strategy (Cheng et al., 2019).

Targeting distinct epigenetic drivers that are known to affect certain TSGs or oncogenes in specific tumor backgrounds may be of more value for therapeutic interventions. One example of such an intervention is the application of the H3K27 methyltransferase, anti-EZH2 (Enhancer of zeste homolog 2) antibody that is being investigated in early-stage clinical trials for hematological malignancies (Tremblay-LeMay, Rastgoo, Pourabdollah, & Chang, 2018). Similarly, Pinometostat, an epigenetic small molecule modulator can decrease mixed lineage leukemia (MLL) target gene expression by reducing the epigenetic modification, H3K79me2, through inhibition of the disruptor of telomeric silencing 1-like (DOT1L) enzyme, resulting in the elimination of leukemia cells (Table 1) (Stein et al., 2018). However, it is important to note that the epigenetic modifications that occur during tumorigenesis are complex and involve multiple steps. Therefore, combination therapies with the aim of targeting various key epigenetic drivers seem to have the ability to synergistically inhibit the expression of oncogenes and promote the reactivation of TSGs. A possible candidate for such a strategy is 4SC-202, a small molecule inhibitor with dual effects on HDAC1/2/3 and lysine-specific demethylase 1 (LSD1), which is currently under clinical investigation (Cheng et al., 2019).

In summary, given the well accepted fact that the epigenetic status of the genome is usually widely modified during cancer initiation, targeting certain epigenetic determinants with recognized effects on the expression of tumorigenesis-related genes may be a promising strategy in cancer therapy and more specifically in precision cancer medicine.

4. Potential drug targets with possible different roles in diverse backgrounds

Re-evaluating the importance of the distinctive role played by epigenetics status in GAC in combination with investigating the major players can be of great importance in discovering novel treatments. This research topic is gaining international attention among clinical and basic research groups (Gan et al., 2018; Guo, Yang, Liang, Guo, & Wang, 2014; Lim et al., 2018; Mathur, 2018; Strauss & Figg, 2016; C. Wang et al., 2018). With this in mind, it is worth mentioning the role of mutations in MLLs and AT-rich interaction domain 1A (ARID1A) proteins, which are relatively common in GAC, and their interactions with other key regulators of epigenetic status, which have been frequently reported (Jaffer A Ajani et al., 2017). It has been shown that *ARID1A* is recurrently mutated in GACs and a broad range of other tumor types. Three main tumor promoting functions: enhanced proliferation, disrupted differentiation, and apoptosis elimination, have proven associations with mutations in this gene involving probable alterations in the role of ARID1A within the SWI/SNF chromatin remodeling complex (Wu & Roberts, 2013). It is also noted that in GACs, as in several other tumors, there is an association between *ARID1A* and *phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha* (*PIK3CA*) mutations. In fact, *PIK3CA* gene mutations have been proven to have an association with the EBV subtype according to the TCGA classification, and are associated with microsatellite-stable (MSS)/TP53 and microsatellite instability (MSI)-high subtypes in the ACRG classification. The rate of ARID1A loss-of-function mutations is also higher in these subtypes (Jaffer A Ajani et al., 2017). Moreover, in one study which sequenced the exons of 15 GAC samples, it was revealed that the most frequently mutated genes were *TP53*, *PIK3CA*, and *ARID1A*, which underlines the importance of these genes in tumorigenesis (Zang et al., 2012).

Table 1
Epigenetic modification-related clinical trials.

No.	Drug	Mechanism of action	Participants	Status	Phase	Country	Trial identifier
Non-small cell lung cancer							
1	Azacytidine Entinostat	DNMT inhibitor HDAC inhibitor	120	R	2	USA	NCT01928576
2	CC-486 (oral azacytidine)	DNMT inhibitor	240	ANR	2	USA	NCT02250326
3	Oral DAC THU	DNMT inhibitor CDA inhibitor	13	C	2	USA	NCT02664181
4	Vidaza (azacytidine)	DNMT inhibitor	6	C	–	USA	NCT01209520
5	DAC THU	DNMT inhibitor CDA inhibitor	75	R	1,2	USA	NCT03233724
Lung cancer							
6	Guadecitabine Mocetinostat	DNMT inhibitor HDAC inhibitor	40	R	1	USA	NCT03220477
Pancreatic cancer							
7	THU DAC	CDA inhibitor DNMT inhibitor	15	C	Early 1	USA	NCT02847000
8	Azacytidine	DNMT inhibitor	80	R	2	USA	NCT01845805
Solid Tumors							
9	Hydralazine Magnesium valproate	DNMT inhibitor HDAC inhibitor	15	C	2	Mexico	NCT00404508
10	CHR-3996	HDAC inhibitor	40	C	1	Netherlands	NCT00697879
11	Azacytidine	DNMT inhibitor	70	ANR	1,2	USA Spain UK	NCT02959437
12	aza-TdCyd	DNMT inhibitor	46	R	1	USA	NCT03366116
13	5-Azacytidine	DNMT inhibitor	54	R	1	USA Australia Canada	NCT03206021
14	TdCyd	DNMT inhibitor	24	S	1	USA	NCT02423057
15	CHR-3996	HDAC inhibitor	40	C	1	Netherlands UK	NCT00697879
16	Azacytidine	DNMT inhibitor	70	ANR	1,2	USA	NCT02959437
17	TdCyd	DNMT inhibitor	24	S	1	USA	NCT02423057
18	MG98	DNMT inhibitor	20	C	1	USA Canada	NCT00003890
Cervical cancer							
19	Hydralazine Magnesium valproate	DNMT inhibitor HDAC inhibitor	143	Un-known	3	Mexico	NCT00532818
20	Hydralazine Magnesium valproate	DNMT inhibitor HDAC inhibitor	230	Un-known	3	Mexico	NCT02446652
Ovarian cancer							
21	Hydralazine Magnesium valproate	DNMT inhibitor HDAC inhibitor	211	Un-known	3	Mexico	NCT00533299
22	DAC	DNMT inhibitor	500	R	2	China	NCT02159820
23	CC-486	DNMT inhibitor	32	ANR	2	USA	NCT02900560
Breast cancer							
24	Vorinostat	HDAC inhibitor	65	NYR	2	USA	NCT04190056
25	ZEN003694	BET inhibitor	49	R	2	USA Belgium Spain	NCT03901469
Hematological malignancy							
26	THU DAC	CDA inhibitor DNMT inhibitor	7	C	Early 1	USA	NCT02846935
27	Azacytidine DAC	DNMT inhibitor	20	NYR	Early 1	USA	NCT04187703
28	Valporic acid	HDAC inhibitor	52	Unknown	2	Puerto Rico	NCT01016990
29	DAC	DNMT inhibitor	156	C		Korea	NCT01400633
30	DAC Vorinostat	DNMT inhibitor HDAC inhibitor	24	R	1	USA	NCT03263936
31	Azacytidine	DNMT inhibitor	15	C	1	USA	NCT01861002
32	Vorinostat Azacytidine	HDAC inhibitor DNMT inhibitor	15	R	1	USA	NCT03843528
33	5-Azacytidine	DNMT inhibitor	41	ANR	2	USA	NCT02497404
34	Azacytidine	DNMT inhibitor	120	C	2	France	NCT01301820
35	Azacytidine	DNMT inhibitor	27	NYR	2	Korea	NCT03719989
36	Vorinostat Azacytidine	HDAC inhibitor DNMT inhibitor	15	R	1	USA	NCT03843528
37	DAC	DNMT inhibitor	9	C	1	USA	NCT01834248
38	Azacytidine	DNMT inhibitor	120	C	2	France	NCT01301820
39	Azacytidine	DNMT inhibitor	30	C	2	Denmark	NCT01048034
40	Azacytidine DAC	DNMT inhibitor	200	R	2	USA	NCT03164057
41	Azacytidine	DNMT inhibitor	34	C	1	USA	NCT00005639
42	5-Azacytidine	DNMT inhibitor	41	ANR	2	USA	NCT02497404
43	DAC	DNMT inhibitor	44	C	2	USA	NCT01829503
44	Azacytidine Vorinostat	DNMT inhibitor HDAC inhibitor	17	C	1,2	USA	NCT01120834
45	Vorinstat	HDAC inhibitor	52	C	1,2	France	NCT00776503
46	DAC	DNMT inhibitor	30	C	1	USA	NCT00538876
47	Azacytidine Vorinostat	DNMT inhibitor HDAC inhibitor	260	ANR	2	UK	NCT01617226
48	DAC	DNMT inhibitor	204	C	2	Germany	NCT00867672

Table 1 (*continued*)

No.	Drug	Mechanism of action	Participants	Status	Phase	Country	Trial identifier
	Valporic acid	HDAC inhibitor					
49	Vorinstat	HDAC inhibitor	754	C	3	USA	NCT01802333
50	Panobinostat	HDAC inhibitor	62	ANR	1,2	Germany	NCT01451268
51	OTX015/MK-8628	Bromodomain inhibitor	141	C	1		NCT01713582
52	RO6870810	BET inhibitor	26	C	1	USA	NCT02308761
53	GSK525762	BET inhibitor	110	ANR	2	USA Australia Spain	NCT01943851
54	EPZ-5676	HMT inhibitor	51	C	1	USA Germany	NCT01684150
55	Romidepsin	HDAC inhibitor	102	C	2	USA	NCT00106431
Urothelial Carcinoma							
56	Guadecitabine	DNMT inhibitor	53	R	2	USA	NCT03179943
Gastrointestinal cancer							
57	Domatinostat	HDAC inhibitor	75	R	2	UK	NCT03812796
Prostate cancer							
58	ZEN003694	BET inhibitor	44	C	1	USA	NCT02705469
59	ZEN003694	BET inhibitor	58	ANR	1,2	USA	NCT02711956

Abbreviations: ANR: Active, not recruiting; BET: Bromodomain and Extra-Terminal motif; C: completed; CDA: Cytidine Deaminase; DAC: Decitabine; DNMT: DNA methyltransferase; HDAC: Histone deacetylase; HMT: Histone methyltransferase; NYR: Not yet recruiting; R: Recruiting; S: Suspended; THU: Tetrahydrouridine; UK: United Kingdom, USA: United States.

The MLLs family (MLL1, MLL2, MLL3 and MLL4) are tumor suppressors and have a considerable impact on gene activation through histone H3 lysine 4 (H3K4) methylation. According to the TCGA database, the total genetic alterations in MLL1–MLL4 occurred in 38% (184/478) of GACs (Jaffer A Ajani et al., 2017).

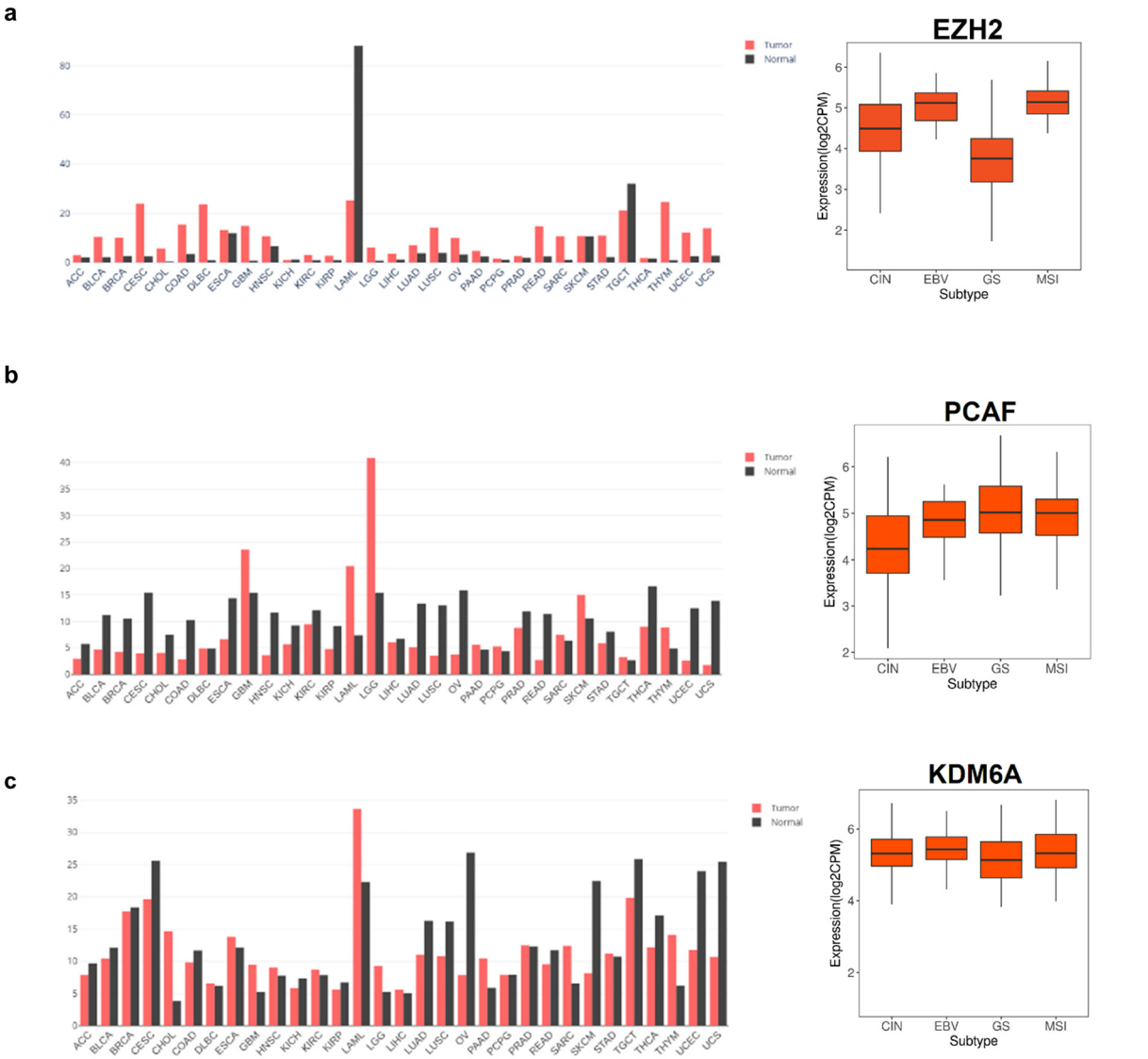
Along with the specified functions of ARID1A and MLLs in GAC molecular pathways, their association with the protein, EZH2 (an H3K27 methyltransferase and a component of PRC2 [polycomb repressive complex 2]) has also been confirmed. Interestingly, EZH2 inhibition was shown to be useful in the suppression of certain tumors with *ARID1A* mutations. In addition to MLLs, EZH2 is also involved in bivalent histone marking, which can regulate the expression of a variety of genes and there is evidence to show the loss of bivalent chromatin status in GACs (Bernhart et al., 2016; Bitler et al., 2015). EZH2 function and expression level are regulated through several molecular mechanisms including those involving ncRNAs as certain microRNAs (miRNAs) reduce the invasion and migration rate of cancerous cells by inhibiting *EZH2* expression (Benetatos, Voulgaris, Vartholomatos, & Hatzimichael, 2013; H.-J. Wang et al., 2010). Many studies have demonstrated an oncogenic role for EZH2 in tumor progression, malignancy, and a poor prognosis, and have revealed its high expression level in GAC and other cancers (Figs. 1a and d, 2) (Gan et al., 2018; Guo et al., 2014; Tang et al., 2017; C. Wang et al., 2018; Wassef et al., 2019; Yan et al., 2017). Moreover, it has been shown that EZH2 is capable of promoting angiogenesis mechanisms and is associated with drug resistance in certain types of malignancies (Göllner et al., 2017; Nakagawa et al., 2018; Tsou et al., 2019; Yamagishi & Uchimaru, 2017).

However, in certain cancers, EZH2 inhibition can result in cancer progression, which would indicate a tumor suppressor role for this protein. A reduction in the activity of the PRC2 complex and the H3K27me3 mark caused by oncogenic activation of NOTCH1 signaling in T-cell acute lymphoblastic leukemia (T-ALL) cells results in T-ALL progression (Ntziachristos et al., 2012). Schäfer et al. indicated that the EZH2 promoter is highly methylated and therefore inactivated in T-ALL patients compared to healthy children. Furthermore, loss-of-function mutations in *EZH2* were also found in myeloproliferative neoplasms (Ernst et al., 2010; Schäfer et al., 2016) This contrasts with the oncogenic role of this gene in a number of other cancers such as GAC and lymphoid malignancies, making it a potential therapeutic target in certain types of cancers (Morin et al., 2010).

Taken together, the data suggests two possible contradictory roles for EZH2 in different cancers. Kim et al. have suggested that the local chromatin environment determines the role of EZH2, which can function either as a tumor activator or repressor. In fact, it has been demonstrated that EZH2 can occupy promoters marked by either H3K27ac or H3K27me3 resulting in gene activation or repression, respectively (Kim et al., 2018). On the other hand, it is well-known that the genes repressed in malignancies are usually TSGs while those that are over activated are listed as oncogenes. Lavarone et al. have implied that the global loss of H3K27 methylation results in diffuse chromatin invasion by acetyltransferases leading to an aberrant accumulation of H3K27 acetylation (Lavarone, Barbieri, & Pasini, 2019). Furthermore, it was demonstrated that there is an antagonistic switch between H3K27 methylation and acetylation associated with the transcriptional regulation of polycomb group (PcG) target genes (Pasini et al., 2010). Altogether, these data indicate that, for certain types of cancers, the chromatin environment of different genes and their interactions with PRC2 and acetyltransferase complexes can play a crucial role in defining the transcriptional state of the genome.

In other words, we propose that the "local" chromatin state of specific genes, i.e. whether they are H3K27 tri-methylated or acetylated, justifies the discrete outcome of *EZH2* loss-of-function mutations in two particular types of cancer: specific hematological-related malignancies and gastric tumors. Therefore, in GAC, it is possible that due to the aberrant methylation of H3K27, not only has the local chromatin state been disrupted but H3K27 acetylation of specific genes (presumably TSGs) is also reduced. It was reported by Pasini et al. that the knockdown of *Histone acetyltransferase 1* (*Hat1*), *Lysine Acetyltransferase 2B* (*Kat2b*), *CREB-binding protein* (*CBP*) and p300 genes led to a significant reduction in H3K27Ac (Pasini et al., 2010). Although it is widely accepted that CBP and p300 along with Hat1 inhibition exerts antitumor effects (Gu et al., 2016), in 2019, Liu et al. suggested that downregulation of *Kat2b* (also known as *P300/CBP-associated factor* [*PCAF*]) results in an increased resistance to 5-FU in colorectal cancer. Additionally, the in silico data analysis of a TCGA GAC cohort shows the downregulation of *PCAF* across tumors in comparison with normal tissues (Figs. 1b and e, Fig. 2) (Tang et al., 2017). However, it has been suggested that the ability of PCAF to acetylate p53 is the probable mechanism for this event (Liu et al., 2019). In addition, Brasacchio et al. have shown that the loss of *PCAF* expression is associated with a poor clinical outcome in GAC patients. Although the mechanisms underlying the association between the loss of PCAF and initiation of GAC are not fully understood it has been suggested that PCAF and adaptor protein 3 (ADA3) are in charge of modulating the intrinsic apoptotic pathway through the "epigenetic regulation" of phosphofurin acidic cluster sorting proteins 1 and 2 (PACS1, PACS2) (Brasacchio et al., 2018).

Lysine-specific demethylase 6A (KDM6A), also known as Ubiquitously transcribed tetratricopeptide repeat, X chromosome (UTX) protein, plays a significant role in the regulation of gene expression


a
EZH2
Tumor
Normal
Expression(log2CPM)
CIN
EBV
GS
MSI
Subtype
b
PCAF
c
KDM6A
d
EZH2
e
PCAF
f
KDM6A
Copy Number
Percentage (%)
Loss Neutral Gain
Methylation (beta value)
Expression (log2CPM)
Tumor
Sample

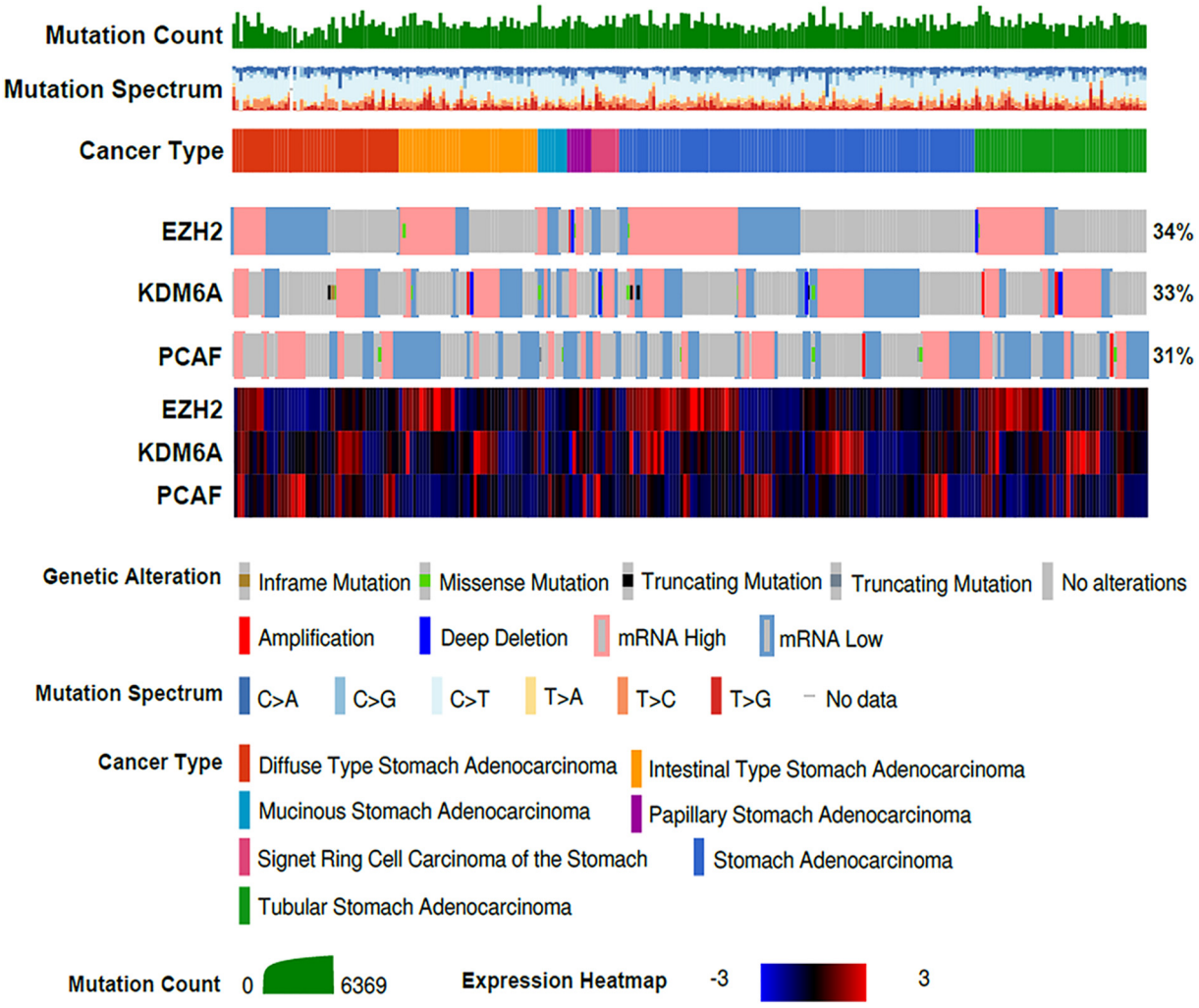


Fig. 2. OncoPrint illustrating the genomic alteration frequency (expression, mutation and CNV) of EZH2, KDM6A, and PCAF across a cohort of 440 stomach cancer samples with various subtypes registered in The Cancer Genome Atlas database (TCGA). Cluster analysis reveals high expression of EZH2 among patients diagnosed with an adenocarcinoma. Additionally, expression of EZH2 is also enriched in intestinal and diffuse type gastric cancer compared with other types. In contrast, PCAF was mostly enriched in patients with diffuse type tumors, while the majority of samples reflected low PCAF expression. All data were analyzed using TCGAbiolinks, R/Bioconductor software package.

through H3K27 demethylation (Fig. 1c and f) (Tang et al., 2017). KDM6A/UTX acts together with COMPASS (complex of proteins associated with Set1), which controls H3K4 methylation and consists of a core complex named WRAD together with MLL3 or MLL2/4 proteins (Lang et al., 2019). Mutations in *KDM6A* are associated with a wide range of human cancers, in particular urothelial carcinoma and certain T-cell leukemias (Schulz, Lang, Koch, & Greife, 2019; Van Haaften et al., 2009). However, it does not function as a tumor suppressor gene in all cancers as it is associated with oncogenic reprogramming in particular T-ALL subtypes. Moreover, knocking out *EZH2*, which we believe has a related function with KDM6A, promotes T-cell leukemia in mice (Schulz et al., 2019). In line with our hypothesis and given the fact that EZH2 is essential for cancer stem cell self-renewal and can repress TSGs such as *CDH1*, we raise the possibility that H3K27 demethylases may also have a role in reversing the epigenetic changes that have occurred. For example, in colon cancer, KDM6A has the ability to demethylate H3K27me3 at the *CDH1* promoter while also recruiting CBP, which leads to increased H3K27 acetylation (Fig. 3) (Zha et al., 2016).

In humans, *KDM6A* has two paralogs, *Jumonji Domain-Containing Protein 3* (JMJD3(and *Ubiquitously Transcribed Tetratricopeptide Repeat*

Fig. 1. *EZH2, PCAF*, and *KDM6A* expression levels compared to their normal counterparts in a range of cancers including STAD (Stomach adenocarcinoma) are shown in *a*, *b*, and *c*, respectively (left). The y axis represents the median expression in each tumor type or in normal tissue. The plots implies a higher level of *EZH2* expression in STAD samples in which PCAF is downregulated in comparison with normal tissues. Data was acquired from the GEPIA (Gene Expression Profiling Interactive Analysis) database. The box plots show the expression of the aforementioned genes in different cancer subtypes (right). Methylation vs expression vs CNV of *EZH2*, *PCAF*, and *KDM6A* are demonstrated in *d*, *e*, and *f*. The data were analyzed using the TCGABiolinks, R/Bioconductor software package (Mounir et al., 2019). ACC: Adrenocortical carcinoma, BLCA: Bladder urothelial carcinoma, BRCA: Breast invasive carcinoma, CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma, CHOL: Cholangiocarcinoma, CNV: Copy number variation, COAD: Colon adenocarcinoma, DLBC: Diffuse large B-cell lymphoma (lymphoid neoplasm), ESCA: Esophageal carcinoma, GBM: Glioblastoma multiforme, HNSC: Head and neck squamous cell carcinoma, KICH: Kidney chromophobe, KIRC: Kidney renal clear cell carcinoma, KIRP: Kidney renal papillary cell carcinoma, LAML: Acute myeloid leukemia, LGG: Low grade glioma (brain), LIHC: Liver hepatocellular carcinoma, LUAD: Lung adenocarcinoma, LUSC: Lung squamous cell carcinoma, MESO: Mesothelioma, OV: Ovarian serous cystadenocarcinoma, PAAD: Pancreatic adenocarcinoma, PCPG: Pheochromocytoma/paraganglioma, PRAD: Prostate adenocarcinoma, READ: Rectum adenocarcinoma, SARC: Sarcoma, SKCM: Skin cutaneous melanoma, STAD: Stomach adenocarcinoma, TGCT: Testicular germ cell tumors, THCA: Thyroid carcinoma, THYM: Thymoma, UCEC: Uterine corpus endometrial carcinoma, UCS: Uterine carcinosarcoma, UVM: Uveal melanoma.

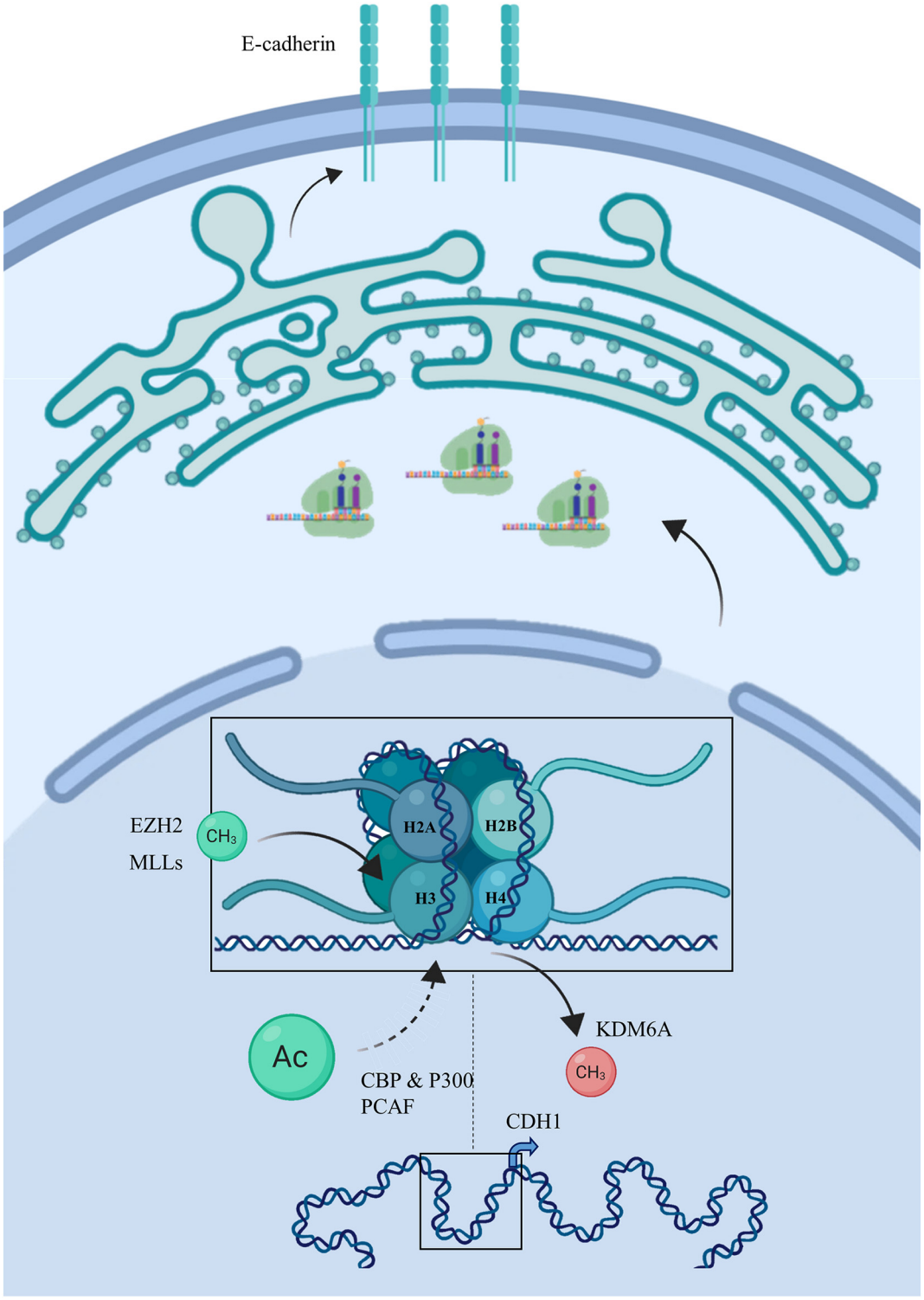


Fig. 3. Roles of EZH2, PCAF, and KDM6A in regulating the epigenetic status of specific gene targets such as CDH1. The confirmed negative correlation between E-cadherin and EZH2/PCAF is illustrated. The positive correlation between E-cadherin and KDM6A in certain types of cancers is also demonstrated. EZH2 and PCAF are in charge of modulating expression of TSGs, either directly or indirectly, by changing the 3D structure of the related chromatin ((Fujii & Ochiai, 2008; Zhou et al., 2019). KDM6A, on the other hand, due to its histone demethylation activity may increase the expression of certain TSGs and reverse the effect of EZH2 in a cancer type-dependent manner (Created by Biorender.com).

Protein, Y-Linked (*UTY*). It is worth noting that *UTY*, located on the Y chromosome, has weaker activity than KDM6A/UTX (Schulz et al., 2019) and also that *KDM6A* is one of the X chromosomal genes that largely escapes X inactivation (Lederer et al., 2012). These observations may explain the biased ratio of GAC incidence between the two sexes, as the incidence is twice as high in males as it is in females (Jaffer A Ajani et al., 2017), which agrees with the fact that females possess an overall higher activity of H3K27me2/3 demethylase.

Given the local chromatin status in different cancerous cells, the function of EZH2 as a key modulator of 3D chromatin structure can vary. This situation is particularly apparent in hematological malignancies compared to solid tumors such as GACs, in which H3K27

trimethylation or H3K27 acetylation play important roles in defining the function of EZH2 as an oncogene or TSG (Ernst et al., 2010; Morin et al., 2010; Ntziachristos et al., 2012; Schäfer et al., 2016). Therefore, it is possible that the disruption of a precise balance between these two epigenetic modifications may be one of the main events occurring in cancer initiation. Such disruption can alter the expression of many cancer-related genes without leaving traces in their genetic code. This phenomenon raises the possibility that modifications of both EZH2 and H3K27 acetylation modulators may have the potential for attenuating distinct cancer phenotypes in regards with their origins. PCAF is a potential candidate for one of the suggested acetylation factors as it was recently demonstrated that its loss is associated with GAC initiation (Brasacchio et al., 2018). This may suggest a mechanism through which increased H3K27 trimethylation and a reduction in H3K27 acetylation of specific genes could be among the main events in cancer initiation.

On the other hand, targeting certain histone demethylases that have the ability to alter the chromatin architecture seems an interesting strategy for mitigating the GAC phenotype. With this in mind, we propose KDM6A, which is involved in H3K27 demethylation and may have the potential to reverse the epigenetic modifications that occur in specific cancer phenotypes such as GAC.

5. Conclusion

Epigenetic regulators are often mutated in GAC and many other cancers and consequently the expression of numerous downstream cancer-related genes could also be affected. Therefore, targeting these epigenetic regulators may have clinical value. Based on a large number of studies looking at variations in the epigenome during cancer initiation, epigenetic-related therapies can be considered as a promising strategy for anticancer treatments. Several epigenetic-related therapies have already been developed and are successfully used in different types of cancer. However, targeting those enzymes that play roles in universal DNA modifications may reduce the need to target particular oncogenes or TSGs. Thus, understanding the specific mechanisms underlying critical alterations in epigenetic drivers is necessary. Due to the complexity of the mechanisms involved in cancer initiation and progression (particularly in GACs), the discovery of key players in cancer pathogenesis is of great importance. Here, we propose that identifying the specific targets of EZH2, PCAF, and KDM6A in GAC would most probably offer new gene targets for cancer treatment including key genetic regulators. Identifying the common target genes of the above mentioned epigenetic modulators in combination with the possibility of modifying one of these three molecules and/or their targets would enhance the treatments currently available and help to alleviate the related cancer. Further studies are required to investigate the applicability of the desired epigenetic alterations as the basis for targeted molecular therapy.

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Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Availability of data and material

The data used to support the findings of this study are included within the article.

Declaration of Competing Interest

The authors have nothing to declare.

References

Ajani, J. A., D'Amico, T. A., Almhanna, K., Bentrem, D. J., Chao, J., Das, P., ... Sundar, H. (2016). Gastric cancer, Version 3.2016, NCCN clinical practice guidelines in oncology. *Journal of the National Comprehensive Cancer Network 14*, 1286–1312.

Abdi, E., Latifi-Navid, S., Zahri, S., Yazdanbod, A., & Pourfarzi, F. (2019). Risk factors predisposing to cardia gastric adenocarcinoma: Insights and new perspectives. *Cancer Medicine 8*, 6114–6126.

Ajani, J. A., Lee, J., Sano, T., Janjigian, Y. Y., Fan, D., & Song, S. (2017). Gastric adenocarcinoma. *Nature Reviews. Disease Primers 3*, 1–19.

Al-Batran, S. -E., Homann, N., Pauligk, C., Goetze, T. O., Meiler, J., Kasper, S., ... Hofheinz, R. D. (2019). Perioperative chemotherapy with fluorouracil plus leucovorin, oxaliplatin, and docetaxel versus fluorouracil or capecitabine plus cisplatin and epirubicin for locally advanced, resectable gastric or gastro-oesophageal junction adenocarcinoma (FLOT4): A randomised, phase 2/3 trial. *The Lancet 393*, 1948–1957.

Bang, Y. J., Van Cutsem, E., Feyereislova, A., Chung, H. C., Shen, L., Sawaki, A., ... Kang, Y. K. (2010). Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): A phase 3, open-label, randomised controlled trial. *Lancet 376*, 687–697.

Benetatos, L., Voulgaris, E., Vartholomatos, G., & Hatzimichael, E. (2013). Non-coding RNAs and EZH2 interactions in cancer: Long and short tales from the transcriptome. *International Journal of Cancer 133*, 267–274.

Bernhart, S. H., Kretzmer, H., Holdt, L. M., Jühling, F., Ammerpohl, O., Bergmann, A. K., ... Stadler, P. F. (2016). Changes of bivalent chromatin coincide with increased expression of developmental genes in cancer. *Scientific Reports 6*, 37393.

Bitler, B. G., Aird, K. M., Garipov, A., Li, H., Amatangelo, M., Kossenkov, A. V., ... Conejo-Garcia, J. R. (2015). Synthetic lethality by targeting EZH2 methyltransferase activity in ARID1A-mutated cancers. *Nature Medicine 21*, 231.

Brasacchio, D., Busuttil, R. A., Noori, T., Johnstone, R. W., Boussioutas, A., & Trapani, J. A. (2018). Down-regulation of a pro-apoptotic pathway regulated by PCAF/ADA3 in early stage gastric cancer. *Cell Death & Disease 9*, 1–10.

Cheng, Y., He, C., Wang, M., Ma, X., Mo, F., Yang, S., ... Wei, X. (2019). Targeting epigenetic regulators for cancer therapy: Mechanisms and advances in clinical trials. *Signal Transduction and Targeted Therapy 4*, 62.

Cunningham, D., Allum, W. H., Stenning, S. P., Thompson, J. N., Van de Velde, C. J., Nicolson, M., ... Participants, M. T. (2006). Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *The New England Journal of Medicine 355*, 11–20.

Cunningham, D., Starling, N., Rao, S., Iveson, T., Nicolson, M., Coxon, F., ... Norman, A. R. (2008). Capecitabine and oxaliplatin for advanced esophagogastric cancer. *The New England Journal of Medicine 358*, 36–46.

Dunn, J., & Rao, S. (2017). Epigenetics and immunotherapy: The current state of play. *Molecular Immunology 87*, 227–239.

Emran, A. A., Marzese, D. M., Menon, D. R., Hammerlindl, H., Ahmed, F., Richtig, E., ... Schaider, H. (2019). Commonly integrated epigenetic modifications of differentially expressed genes lead to adaptive resistance in cancer. *Epigenomics 11*, 732–737.

Ernst, T., Chase, A. J., Score, J., Hidalgo-Curtis, C. E., Bryant, C., Jones, A. V., ... Reiter, A. (2010). Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nature Genetics 42*, 722.

Fujii, S., & Ochiai, A. (2008). Enhancer of zeste homolog 2 downregulates E-cadherin by mediating histone H3 methylation in gastric cancer cells. *Cancer Science 99*, 738–746.

Gan, L., Xu, M., Hua, R., Tan, C., Zhang, J., Gong, Y., ... Guo, W. (2018). The polycomb group protein EZH2 induces epithelial–mesenchymal transition and pluripotent phenotype of gastric cancer cells by binding to PTEN promoter. *Journal of Hematology & Oncology 11*, 9.

Gao, J. -P., Xu, W., Liu, W. -T., Yan, M., & Zhu, Z. -G. (2018). Tumor heterogeneity of gastric cancer: From the perspective of tumor-initiating cell. *World Journal of Gastroenterology 24*, 2567.

Garg, P. K., Jakhetiya, A., Sharma, J., Ray, M. D., & Pandey, D. (2016). Lymphadenectomy in gastric cancer: Contentious issues. *World Journal of Gastrointestinal Surgery 8*, 294–300.

Gius, D., Cui, H., Bradbury, C. M., Cook, J., Smart, D. K., Zhao, S., ... Feinberg, A. P. (2004). Distinct effects on gene expression of chemical and genetic manipulation of the cancer epigenome revealed by a multimodality approach. *Cancer Cell 6*, 361–371.

Göllner, S., Oellerich, T., Agrawal-Singh, S., Schenk, T., Klein, H. -U., Rohde, C., ... Tavor, S. (2017). Loss of the histone methyltransferase EZH2 induces resistance to multiple drugs in acute myeloid leukemia. *Nature Medicine 23*, 69.

Gu, M. -L., Wang, Y. -M., Zhou, X. -X., Yao, H. -P., Zheng, S., Xiang, Z., & Ji, F. (2016). An inhibitor of the acetyltransferases CBP/p300 exerts antineoplastic effects on gastrointestinal stromal tumor cells. *Oncology Reports 36*, 2763–2770.

Guo, L., Yang, T. -F., Liang, S. -C., Guo, J. -X., & Wang, Q. (2014). Role of EZH2 protein expression in gastric carcinogenesis among Asians: A meta-analysis. *Tumor Biology 35*, 6649–6656.

Japanese Gastric Cancer Association (2011). Japanese classification of gastric carcinoma: 3rd English edition. *Gastric Cancer: Official Journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association 14*, 101–112.
Japanese Gastric Cancer Association (2020). Japanese gastric cancer treatment guidelines 2018. *Gastric cancer: official journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association* (5th ed.).
Kim, J., Lee, Y., Lu, X., Song, B., Fong, K. -W., Cao, Q., ... Yu, J. (2018). Polycomb- and methylation-independent roles of EZH2 as a transcription activator. *Cell Reports 25* 2808-2820.e2804.
Ko, W. J., Song, G. W., Kim, W. H., Hong, S. P., & Cho, J. Y. (2016). Endoscopic resection of early gastric cancer: Current status and new approaches. *Translational Gastroenterology and Hepatology 1*, 24.
Lang, A., Yilmaz, M., Hader, C., Murday, S., Kunz, X., Wagner, N., ... Koch, J. (2019). Contingencies of UTX/KDM6A action in urothelial carcinoma. *Cancers 11*, 481.
Lavarone, E., Barbieri, C. M., & Pasini, D. (2019). Dissecting the role of H3K27 acetylation and methylation in PRC2 mediated control of cellular identity. *Nature Communications 10*, 1–16.
Lederer, D., Grisart, B., Digilio, M. C., Benoit, V., Crespin, M., Ghariani, S. C., ... Verellen-Dumoulin, C. (2012). Deletion of KDM6A, a histone demethylase interacting with MLL2, in three patients with kabuki syndrome. *The American Journal of Human Genetics 90*, 119–124.
Lim, B., Kim, H. J., Heo, H., Huh, N., Baek, S. J., Kim, J. H., ... Song, K. S. (2018). Epigenetic silencing of miR-1271 enhances MEK1 and TEAD4 expression in gastric cancer. *Cancer Medicine 7*, 3411–3424.
Liu, T., Wang, X., Hu, W., Fang, Z., Jin, Y., Fang, X., & Miao, Q. R. (2019). Epigenetically down-regulated acetyltransferase PCAF increases the resistance of colorectal cancer to 5-fluorouracil. *Neoplasia 21*, 557–570.
Macdonald, J. S., Smalley, S. R., Benedetti, J., Hundahl, S. A., Estes, N. C., Stemmermann, G. N., ... Martenson, J. A. (2001). Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. *The New England Journal of Medicine 345*, 725–730.
Mathur, R. (2018). ARID1A loss in cancer: Towards a mechanistic understanding. *Pharmacology & Therapeutics 190*, 15–23.
Morin, R. D., Johnson, N. A., Severson, T. M., Mungall, A. J., An, J., Goya, R., ... Kuchenbauer, F. (2010). Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nature Genetics 42*, 181.
Mounir, M., Lucchetta, M., Silva, T. C., Olsen, C., Bontempi, G., Chen, X., ... Papaleo, E. (2019). New functionalities in the TCGAbiolinks package for the study and integration of cancer data from GDC and GTEx. *PLoS Computational Biology 15*, e1006701.
Nakagawa, S., Okabe, H., Ouchi, M., Tokunaga, R., Umezaki, N., Higashi, T., ... Kuroki, H. (2018). Enhancer of zeste homolog 2 (EZH2) regulates tumor angiogenesis and predicts recurrence and prognosis of intrahepatic cholangiocarcinoma. *HPB 20*, 939–948.
Nishigaki, M., Aoyagi, K., Danjoh, I., Fukaya, M., Yanagihara, K., Sakamoto, H., ... Sasaki, H. (2005). Discovery of aberrant expression of R-RAS by cancer-linked DNA hypomethylation in gastric cancer using microarrays. *Cancer Research 65*, 2115.
Ntziachristos, P., Tsirigos, A., Van Vlierberghe, P., Nedjic, J., Trimarchi, T., Flaherty, M. S., ... Siegle, J. (2012). Genetic inactivation of the polycomb repressive complex 2 in T cell acute lymphoblastic leukemia. *Nature Medicine 18*, 298.
Ogasawara, N., Tsukamoto, T., Mizoshita, T., Inada, K., Cao, X., Takenaka, Y., ... Tatematsu, M. (2006). Mutations and nuclear accumulation of β-catenin correlate with intestinal phenotypic expression in human gastric cancer. *Histopathology 49*, 612–621.
Orditura, M., Galizia, G., Sforza, V., Gambardella, V., Fabozzi, A., Laterza, M. M., ... De Vita, F. (2014). Treatment of gastric cancer. *World Journal of Gastroenterology 20*, 1635–1649.
Padmanabhan, N., Ushijima, T., & Tan, P. (2017). How to stomach an epigenetic insult: The gastric cancer epigenome. *Nature Reviews Gastroenterology & Hepatology 14*, 467.
Pasini, D., Malatesta, M., Jung, H. R., Walfridsson, J., Willer, A., Olsson, L., ... Jensen, O. N. (2010). Characterization of an antagonistic switch between histone H3 lysine 27 methylation and acetylation in the transcriptional regulation of Polycomb group target genes. *Nucleic Acids Research 38*, 4958–4969.
Roberti, A., Valdes, A. F., Torrecillas, R., Fraga, M. F., & Fernandez, A. F. (2019). Epigenetics in cancer therapy and nanomedicine. *Clinical Epigenetics 11*, 81.
Schäfer, V., Ernst, J., Rinke, J., Winkelmann, N., Beck, J. F., Hochhaus, A., ... Ernst, T. (2016). EZH2 mutations and promoter hypermethylation in childhood acute lymphoblastic leukemia. *Journal of Cancer Research and Clinical Oncology 142*, 1641–1650.
Schulz, W. A., Lang, A., Koch, J., & Greife, A. (2019). The histone demethylase UTX/KDM6A in cancer: Progress and puzzles. *International Journal of Cancer 145*, 614–620.
Smalley, S. R., Benedetti, J. K., Haller, D. G., Hundahl, S. A., Estes, N. C., Ajani, J. A., ... Macdonald, J. S. (2012). Updated analysis of SWOG-directed intergroup study 0116: A phase III trial of adjuvant radiochemotherapy versus observation after curative gastric cancer resection. *Journal of Clinical Oncology 30*, 2327–2333.
Smyth, E. C., Verheij, M., Allum, W., Cunningham, D., Cervantes, A., & Arnold, D. (2016). Gastric cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up†. *Annals of Oncology 27*, v38–v49.
Sohn, B. H., Hwang, J. -E., Jang, H. -J., Lee, H. -S., Oh, S. C., Shim, J. -J., ... Lee, S. H. (2017). Clinical significance of four molecular subtypes of gastric cancer identified by the cancer genome atlas project. *Clinical Cancer Research 23*, 4441–4449.
Stein, E. M., Garcia-Manero, G., Rizzieri, D. A., Tibes, R., Berdeja, J. G., Savona, M. R., ... Tallman, M. S. (2018). The DOT1L inhibitor pinometostat reduces H3K79 methylation and has modest clinical activity in adult acute leukemia. *Blood 131*, 2661–2669.
Strauss, J., & Figg, W. D. (2016). Using epigenetic therapy to overcome chemotherapy resistance. *Anticancer Research 36*, 1–4.
Tang, Z., Li, C., Kang, B., Gao, G., Li, C., & Zhang, Z. (2017). GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Research 45*, W98–W102.
Teresa, F., Serra, N., Capra, G., Mascarella, C., Gagliardi, C., Di Carlo, P., ... Sciortino, M. (2019). Helicobacter pylori and Epstein–Barr virus infection in gastric diseases: Correlation with IL-10 and IL1RN polymorphism. *Journal of Oncology 2019*.
Torre, L. A., Siegel, R. L., Ward, E. M., & Jemal, A. (2016). Global cancer incidence and mortality rates and trends—An update. *Cancer Epidemiology and Prevention Biomarkers 25*, 16–27.
Tremblay-LeMay, R., Rastgoo, N., Pourabdollah, M., & Chang, H. (2018). EZH2 as a therapeutic target for multiple myeloma and other haematological malignancies. *Biomarker Research 6*.
Tsou, P. -S., Campbell, P., Amin, M. A., Coit, P., Miller, S., Fox, D. A., ... Sawalha, A. H. (2019). Inhibition of EZH2 prevents fibrosis and restores normal angiogenesis in scleroderma. *Proceedings of the National Academy of Sciences 116*, 3695–3702.
Van Haaften, G., Dalgliesh, G. L., Davies, H., Chen, L., Bignell, G., Greenman, C., ... Teague, J. (2009). Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer. *Nature Genetics 41*, 521–523.
Wang, C., Li, X., Zhang, J., Ge, Z., Chen, H., & Hu, J. (2018). Ezh2 contributes to 5-fu resistance in gastric cancer by epigenetically suppressing fbxo32 expression. *Oncotargets and Therapy 11*, 7853.
Wang, J., Luo, Y., Wang, Q., Bai, J., Liao, Q., Feng, X., ... Li, Y. (2020). Evaluation of the application of laparoscopy in enhanced recovery after surgery (ERAS) for gastric cancer: A Chinese multicenter analysis. *Annals of Translational Medicine 8*, 543.
Wang, H. -J., Ruan, H. -J., He, X. -J., Ma, Y. -Y., Jiang, X. -T., Xia, Y. -J., ... Tao, H. -Q. (2010). MicroRNA-101 is down-regulated in gastric cancer and involved in cell migration and invasion. *European Journal of Cancer 46*, 2295–2303.
Wassef, M., Luscan, A., Aflaki, S., Zielinski, D., Jansen, P. W., Baymaz, H. I., ... Wallace, M. R. (2019). EZH1/2 function mostly within canonical PRC2 and exhibit proliferation-dependent redundancy that shapes mutational signatures in cancer. *Proceedings of the National Academy of Sciences 116*, 6075–6080.
Wu, J. N., & Roberts, C. W. (2013). ARID1A mutations in cancer: Another epigenetic tumor suppressor? *Cancer Discovery 3*, 35–43.
Yamagishi, M., & Uchimaru, K. (2017). Targeting EZH2 in cancer therapy. *Current Opinion in Oncology 29*, 375–381.
Yan, K. -S., Lin, C. -Y., Liao, T. -W., Peng, C. -M., Lee, S. -C., Liu, Y. -J., ... Chou, R. -H. (2017). EZH2 in cancer progression and potential application in cancer therapy: A friend or foe? *International Journal of Molecular Sciences 18*, 1172.
Yang, Q., Wu, K., Ji, M., Jin, W., He, N., Shi, B., & Hou, P. (2013). Decreased 5-hydroxymethylcytosine (5-hmC) is an independent poor prognostic factor in gastric cancer patients. *Journal of Biomedical Nanotechnology 9*, 1607–1616.
Yu, H., & Rong, L. (2018). Emerging role of long non-coding RNA in the development of gastric cancer. *World Journal of Gastrointestinal Oncology 10*, 260.
Zang, Z. J., Cutcutache, I., Poon, S. L., Zhang, S. L., McPherson, J. R., Tao, J., ... Tan, P. (2012). Exome sequencing of gastric adenocarcinoma identifies recurrent somatic mutations in cell adhesion and chromatin remodeling genes. *Nature Genetics 44*, 570–574.
Zeng, X. -Q., Wang, J., & Chen, S. -Y. (2017). Methylation modification in gastric cancer and approaches to targeted epigenetic therapy. *International Journal of Oncology 50*, 1921–1933.
Zha, L., Cao, Q., Cui, X., Li, F., Liang, H., Xue, B., & Shi, H. (2016). Epigenetic regulation of E-cadherin expression by the histone demethylase UTX in colon cancer cells. *Medical Oncology 33*, 21.
Zhou, R., Yang, Y., Park, S. -Y., Seo, Y. -W., Jung, S. -C., Kim, K. K., ... Kim, H. (2019). p300/CBP-associated factor promotes autophagic degradation of δ-catenin through acetylation and decreases prostate cancer tumorigenicity. *Scientific Reports 9*, 1–14.