Elevated expression of a pharmacologic Polycomb signature predicts poor prognosis in gastric and breast cancer.

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ABSTRACT

Aims: Polycomb Group (PcG) complexes are epigenetic repressors that silence tumor suppressive genes. Studies demonstrated that pharmacologic inhibition of PcG complexes with 3-deazaneplanocin A (DZNeP) induces cancer cell death by re-expressing silenced genes. Here, we evaluate the prognostic significance of DZNeP target genes in gastric and breast cancer.

Patients & Methods/Materials: The prognostic impact of a DZNeP-regulated gene signature was investigated using the KM Plotter and cBio Portal resources containing microarray data from tumor tissue.

Results: We report that elevated expression of DZNeP targets is associated with poor clinical outcome in gastric and breast cancer. In gastric cancer, elevated expression of DZNeP signature is inversely correlated with decreased overall survival. In breast cancer, DZNeP signature predicted poor prognosis in HER2+ tumors but not in HER2- neoplasms.

Conclusions: These findings demonstrate that DZNeP target genes are not predictive of better but rather of poor clinical outcome in gastric and breast cancer.

KEYWORDS

Cancer, Epigenetics, Polycomb, EZH2, DZNeP, Prognostic, Signature

SUMMARY POINTS

- EZH2 is an epigenetic repressor that is frequently overexpressed in advanced malignancies.
- 3-deazaneplanocin A (DZNeP) is an EZH2 inhibitor that has shown some anti-tumor activity in pre-clinical models.
- Tan et al. have previously identified a 44-gene expression signature that was upregulated following treatment with DZNeP in cancer cells.
- In gastric cancer, high expression of DZNeP signature is associated with lower overall survival in patients independently of other clinicopathologic factors.
- In breast cancer, elevated expression of DZNeP signature is correlated with poor clinical outcome.
- More specifically, expression of DZNeP target genes is associated with patient prognosis in HER2+ but not in HER2- breast cancer.
- The results presented in this article suggest that analyzing the expression of genes modulated by an epigenetic drug may not be a perfect surrogate to determine the efficacy of that drug.
- There are multiple proposed mechanisms by which higher expression of DZNeP targets may be associated with poor clinical outcome in different tumor types.

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INTRODUCTION

For many years, cancer has been described as a genetic disease resulting from a series of DNA mutations [1]. However, it has become widely accepted that epigenetic alterations that do not alter DNA sequence can also drive cancer initiation and progression in concert with genetic alterations [2, 3]. This paradigm shift has generated considerable interest in exploring the cancer epigenome in the search for clinical tools to diagnose, classify, and treat human tumors [4]. Notably, extensive evidence has demonstrated that a group of epigenetic regulators called the Polycomb Group (PcG) proteins play critical roles in human cancer and may be exploited clinically [5, 6]. PcG proteins assemble in two main Polycomb Repressive Complexes (PRC1 and PRC2) known to silence key tumor suppressor genes, thereby contributing to tumorigenesis and metastasic dissemination [7].

Among PcG proteins, EZH2 has been the most extensively studied protein given its recurrent implication in aggressive neoplasms [8, 9]. EZH2 functions as the catalytic component of PRC2 by trimethylating histone 3 at lysine 27 (H3K27me3), an epigenetic mark associated with transcriptional repression [10]. H3K27me3 serves as a docking site for PRC1, which further promotes heterochromatin formation at target loci [11]. In a number of tumor types, EZH2 is overexpressed and its elevated expression correlates with metastatic spreading and poor patient prognosis [12-14]. Moreover, single nucleotide polymorphisms (SNPs) present in the EZH2 gene have been associated with survival and drug response in different cancer types [15, 16]. In line with these findings, pharmacologic inhibition of EZH2 decreased proliferation, tumorigenicity, and invasion while inducing apoptosis *in vitro* and *in vivo* [8, 17]. Accordingly, the efficacy of EZH2 inhibitors has been well documented in animal models and come of them are currently undergoing highly anticipated clinical testing [18, 19].

Given the transcriptional effects of EZH2, altered gene expression induced by EZH2 may be used as an important tool to prognosticate human tumors [20, 21]. Tan et al. have previously analyzed gene expression following pharmacologic EZH2 inhibition with the EZH2 inhibitor 3-deazaneplanocin A (DZNeP) in a panel of human cancer cell lines [22]. They identified 44 EZH2 target genes that were consistently upregulated following DZNeP treatment. We have previously shown that this gene signature is down-regulated in metastatic compared to primary neoplasms [9]. However, the prognostic role of this signature in primary tumors remains unexplored. In this article, we analyze the prognostic significance of this signature and demonstrate that its elevated expression associates with poor clinical outcome in breast and gastric cancer.

FINDINGS AND DISCUSSION

To assess the clinical significance of EZH2 target genes, we employed a list of genes, referred to as the DZNeP signature, comprised of 44 genes that were significantly overexpressed following DZNeP treatment in a number of human cancer cell lines (Table S1), as reported by Tan et al [22]. Briefly, RNA was extracted from cancer cell lines after treatment with 5μM of DZNeP and subsequently profiled using Illumina microarray technology. The mean expression of these 44 genes was used and correlated to different clinical endpoints using the KM Plotter platform (http://kmplot.com/analysis/) [23, 24]. The optimal microarray probes to represent a gene were selected in an unbiased fashion according to the previously published Jetset method [25]. KM

Plotter analyses were conducted in gastric cancer [23] and breast cancer [24, 26] cancer with the available endpoints.

As the first step, we investigated the association between the expression of DZNeP signature with overall survival (OS) in gastric cancer. In an unsorted cohort of gastric cancer patients, there was a significant correlation between silencing of DZNeP signature and shorter OS, (Figure 1, Logrank test, $p<10^{-7}$, hazard ratio (HR)=1.86). Next, we analyzed whether this relationship was maintained in specific clinical subgroups. We observed that downregulation of the signature was also significantly associated with lower OS in gastric tumors of different differentiation status, metastatasis, Lauren class, and HER2 status (Figure 1). Taken together, these results indicate that repression of DZNeP target genes is observed in more lethal gastric cancers, independently of other clinical features.

To further evaluate the prognostic impact of the DZNeP signature, we investigated the correlation between poor prognosis and the expression of individual genes from the signature. We conducted a Kaplan-Meier analysis using the cBio Portal in a large cohort of gastric cancer made up of 265 patients (TCGA, Nature 2014 gastric adenocarcinoma [27]). We report a strong association between lower disease-free survival and elevated expression (fold change >2) of the genes KLF6 (Figure S1, p=3.8e-4) and SLPI (Figure S1, p=6.7e-3). In line with these findings, SLPI has already been shown to promote gastric cancer growth and metastasis [28]. Thus, these data indicate that KLF6 likely to represent the most relevant genes within the signature in gastric cancer and may be independent prognostic markers, therefore warranting further functional evaluation.

To determine whether this association was restricted to gastric cancer, we also conducted survival analysis in a breast cancer cohort. Expression data was correlated with three available clinical endpoints: overall survival (OS), recurrence-free survival (RFS), and distant-metastasis-free survival (DMFS). As observed in gastric cancer, upregulation of DZNeP signature was significantly associated with poorer outcome with regards to OS (Figure 2A, Logrank test, p<0.05, HR=1.43), RFS (Figure 2B, Logrank test, p<0.002, HR=1.29), and DMFS (Figure 2C, Logrank test, p<0.03, HR=1.46). Thus, upregulation of DZNeP signature occurred preferentially in aggressive breast tumors, as reported in gastric cancer.

Next, we assessed whether the signature was selectively upregulated in a particular breast cancer subtype. In HER2+ breast cancer, we found that elevated expression of the signature was significantly associated with lower RFS (Figure 3A, Logrank test, p<0.006, HR=2.19) and DMFS (Figure 3B, Logrank test, p<0.006, HR=4.24). However, this was not found in HER2- breast tumors, where RFS (Figure 3A, Logrank test, p=0.15, HR=1.24) and DMFS (Figure 3B, Logrank test, p=0.98, HR=1.02) did not significantly correlate with expression of DZNeP. This suggests that upregulation of DZNeP targets can occur preferentially in distinct clinical subgroups in breast cancer but appears to be a widespread event in gastric cancer.

To further validate the prognostic value of the signature, we sought to determine whether individual genes within the signature may be independently associated with clinical outcome in breast cancer patients. We queried the cBio Portal to analyze whether elevated expression (fold change >2) of each gene was significantly associated with poor prognosis using a Kaplan-Meier analysis. We selected the Breast Cancer (METABRIC, Nature 2012 & Nat Commun 2016 [29]) cohort, which contained microarray data, derived from 2509 patients. In accordance with our results, we found that elevated expression of four genes was significantly linked with lower overall survival: KCNC4 (p=0.03), SQSTM1 (p=0.005), TGFB1 (p=0.02), and ZNF286A (p=0.04, Figure S2). Interestingly,

KCNC4, SQSTM1, and TGFB1 have all been linked to promoting increased cell migration and stem-like phenotypes of breast cancer cells in the literature [30-32]. Therefore, the elevated expression of these pro-metastatic genes may explain the reduced overall survival.

In accordance with numerous other articles, we have previously demonstrated that silencing of PcG targets can predict poor prognosis in some aggressive tumor types [5, 20]. Here, we report the opposite in gastric and breast cancer, wherein an upregulation of DZNeP target genes is observed in more aggressive tumors. In this report, since EZH2 overexpression but not silencing of EZH2 targets correlates with poor prognosis, we conclude that EZH2 likely promotes tumor progression through PcG-independent mechanisms and not solely through silencing of a few specific genes such as the DZNeP signature [33].

Although these results argue against the classical model of PcG-mediated oncogenesis [34], a number of factors can explain why the upregulation of DZNeP targets can be linked to poorer prognosis. First, since the effect of EZH2's silencing activity extends to a large amount of genes and therefore affects global gene expression [35]. Thus, repression of a few genes such as the signature may not be sufficient to promote tumor aggressiveness. In addition, EZH2 acts not solely through gene silencing, but also through ncRNAs or other macromolecules [36, 37]. Since our analysis contained exclusively protein-coding genes, it is possible that a DZNeP signature including ncRNAs would yield different results. DZNeP may have off-target effects and interact with proteins other than EZH2 that regulate gene expression. Another possibility is that DZNeP treatment preferentially affects a subset of genes (the signature) but not all EZH2 target genes, as a microarray analysis revealed that EZH2 siRNA and DZNeP affect a partially overlapping set of genes [22]. Furthermore, the expression and the effect of DZNeP targets may differ in a cancer-specific manner. For example, it is possible that post-translational modifications (PTMs) of EZH2 in distinct cell contexts may direct EZH2 towards gene silencing while other PTMs may prime EZH2 for cellular roles that are independent of transcriptional repression [37, 38].

While our findings open up novel avenues regarding the mechanisms through which EZH2 promotes tumor growth, our study also brings up key implications for clinical management. Importantly, our findings raise the issue that it may be inaccurate to use the expression of gene targets to quantify the activity of a drug targeting an epigenetic regulator. This is because epigenetic regulators such as EZH2 can promote tumor progression through mechanisms other than solely transcriptional repression [39, 40]. Further supporting this idea, our study identified different genes that were significantly associated with poor clinical outcome in breast and gastric cancer. Moreover, as chemotherapy represents the backbone of systemic therapy in most solid malignancies (such as gastric and breast cancers), insights into the molecular interactions of cytotoxic agents and drugs acting on the epigenetic machinery are needed to design a personalized therapeutic approach. Indeed, evidence exists that EZH2 is involved in sensitivity to chemotherapy of gastric cancer cell lines through ncRNAs regulation [41]. Finally, we believe that it should become imperative to integrate genetic and epigenetic data from individual tumors to provide a more comprehensive overview of the molecular alterations driving cancer progression in each patient. This will provide additional tools to guide clinicians in selecting treatment sequence and in providing a more accurate prognosis.

TABLE AND FIGURE LEGEND

Table S1: Composition of DZNeP gene signature.

Figure 1: Analysis of DZNeP signature expression and correlation with overall survival in different clinical subtypes of gastric cancer.

Figure 2: DZNeP signature analysis in full breast cancer clinical cohort: A) Overall survival (OS): Logrank test, $p<0.05$, HR=1.43 B) Recurrence-free survival (RFS): Logrank test, $p<0.002$, HR=1.29 C) Distant metastasis-free survival (DMFS): Logrank test, $p<0.03$, HR=1.46.

Figure 3: Prognostic value of DZNeP signature in HER2 subtypes. A) Kaplan Meier analysis of DZNeP signature expression and RFS in HER2+ (Logrank test, $p<0.006$, HR=2.19) and HER2- (Logrank test, $p=0.15$, HR=1.24) breast cancer B) Kaplan Meier analysis of DZNeP signature expression and DMFS in HER2+ (Logrank test, $p<0.006$, HR=4.24) and HER2- (Logrank test, $p=0.98$, HR=1.02) breast cancer.

Figure S1: Prognostic analysis of individual DZNeP signature genes in gastric cancer using the cBio Portal. Assessment of individual prognostic genes in the TCGA, Nature 2014 gastric adenocarcinoma cohort demonstrates that high expression (FC>2) of KLF6 ($p=3.8e-4$) and SLPI ($p=6.7e-3$) is associated with lower disease-free survival.

Figure S2: Analysis of individual DZNeP signature genes in breast cancer using the cBio Portal. Data from the breast cancer METABRIC trial was analyzed and demonstrated that lower overall survival was correlated with high expression of KCNC4 ($p=0.03$), SQSTM1 ($p=0.005$), TGFB1 ($p=0.02$), and ZNF286A ($p=0.04$).

TABLES

Table S1: Composition of DZNeP gene signature

#	Accession #	Gene	Affy gene ID
1	NM_000358	TGFBI	201506_at
2	NM_000228	LAMB3	209270_at
3	NM_000598	IGFBP3	210095_s_at
4	NM_005556	KRT7	209016_s_at
5	NM_022748	TNS3	217853_at
6	NM_001394	DUSP4	226034_at
7	NM_148177	FBXO32	225328_at
8	NM_001630	ANXA8	203074_at
9	NM_002658	PLAU	205479_s_at
10	NM_003900	SQSTM1	213112_s_at
11	NM_003064	SLPI	203021_at
12	NM_012385	NUPR1	209230_s_at
13	NM_000362	TIMP3	201150_s_at
14	NM_058229	FBXO32	225345_s_at
15	NM_014330	PPP1R15A	202014_at
16	NM_001300	KLF6	1555832_s_at
17	NM_004405	DLX2	207147_at
18	XM_166376	PPP1R18	224927_at
19	NM_004089	TSC22D3	208763_s_at
20	NM_000422	KRT17	205157_s_at
21	NM_005115	MVP	202180_s_at
22	NM_033452	TRIM47	225868_at
23	NM_007207	DUSP10	221563_at
24	NM_002673	PLXNB1	215807_s_at
25	NM_003954	MAP3K14	205192_at
26	NM_153271	SNX30	226249_at
27	NM_002510	GPNMB	201141_at
28	XM_290536	PHRF1	234952_s_at
29	NM_033219	TRIM14	210846_x_at
30	NM_000413	HSD17B1	205829_at
31	NM_014387	LAT	211005_at
32	NM_016263	FZR1	209414_at
33	NM_020652	ZNF286	239898_x_at
34	NM_152449	LYSMD4	228954_at
35	NM_014830	ZBTB39	205256_at

36	NM_173217	ST6GAL1	201998_at
37	NM_138768	MYEOV	227342_s_at
38	NM_005065	SEL1L	202061_s_at
39	NM_145102	ZFP95	203731_s_at
40	NM_153763	KCNC4	228436_at
41	NM_024408	NOTCH2	212377_s_at
42	NM_006943	SOX12	228358_at
43	NM_018986	SH3TC1	219256_s_at
44	NM_030767	AKNA	225701_at

DECLARATIONS

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The datasets generated during and/or analysed during the current study are all publically available in the KM Plotter resource (http://kmplot.com/analysis/) and the cBio Portal (http://www.cbioportal.org/).

Competing interests

The authors declare no conflict of interest

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REFERENCES

1. Nowell PC. The clonal evolution of tumor cell populations. *Science* 194(4260), 23-28 (1976).
2. Baylin SB, Ohm JE. Epigenetic gene silencing in cancer - a mechanism for early oncogenic pathway addiction? *Nature reviews. Cancer* 6(2), 107-116 (2006).
3. Esteller M. Epigenetics in cancer. *The New England journal of medicine* 358(11), 1148-1159 (2008).
4. Dawson MA, Kouzarides T. Cancer epigenetics: from mechanism to therapy. *Cell* 150(1), 12-27 (2012).*

*This paper describes the important role that epigenetics can have in the management of cancer patients.

5. Clermont PL, Crea F, Chiang YT *et al*. Identification of the epigenetic reader CBX2 as a potential drug target in advanced prostate cancer. *Clinical epigenetics* 8 16 (2016).
6. Crea F, Di Paolo A, Liu HH *et al*. Polycomb genes are associated with response to imatinib in chronic myeloid leukemia. *Epigenomics* 7(5), 757-765 (2015).
7. Richly H, Aloia L, Di Croce L. Roles of the Polycomb group proteins in stem cells and cancer. *Cell death & disease* 2 e204 (2011).
8. Crea F, Hurt EM, Mathews LA *et al*. Pharmacologic disruption of Polycomb Repressive Complex 2 inhibits tumorigenicity and tumor progression in prostate cancer. *Molecular cancer* 10 40 (2011).
9. Crea F, Fornaro L, Bocci G *et al*. EZH2 inhibition: targeting the crossroad of tumor invasion and angiogenesis. *Cancer metastasis reviews* 31(3-4), 753-761 (2012).
10. Simon JA, Kingston RE. Mechanisms of polycomb gene silencing: knowns and unknowns. *Nat Rev Mol Cell Biol* 10(10), 697-708 (2009).
11. Simon JA, Kingston RE. Occupying chromatin: Polycomb mechanisms for getting to genomic targets, stopping transcriptional traffic, and staying put. *Molecular cell* 49(5), 808-824 (2013).*

*This article highlights the diverse mechanisms through which Polycomb proteins can modulate gene expression.

12. Varambally S, Dhanasekaran SM, Zhou M *et al*. The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* 419(6907), 624-629 (2002).
13. Crea F, Hurt EM, Farrar WL. Clinical significance of Polycomb gene expression in brain tumors. *Molecular cancer* 9 265 (2010).
14. Crea F, Fornaro L, Paolicchi E *et al*. An EZH2 polymorphism is associated with clinical outcome in metastatic colorectal cancer patients. *Annals of oncology : official journal of the European Society for Medical Oncology* 23(5), 1207-1213 (2012).
15. Paolicchi E, Pacetti P, Giovannetti E *et al*. A single nucleotide polymorphism in EZH2 predicts overall survival rate in patients with cholangiocarcinoma. *Oncology letters* 6(5), 1487-1491 (2013).
16. Fornaro L, Faviana P, De Gregorio V *et al*. Molecular and pathological characterization of the EZH2 rs3757441 single nucleotide polymorphism in colorectal cancer. *BMC cancer* 15 874 (2015).
17. Avan A, Crea F, Paolicchi E *et al*. Molecular mechanisms involved in the synergistic interaction of the EZH2 inhibitor 3-deazaneplanocin A with gemcitabine in pancreatic cancer cells. *Molecular cancer therapeutics* 11(8), 1735-1746 (2012).
18. Kim KH, Roberts CW. Targeting EZH2 in cancer. *Nature medicine* 22(2), 128-134 (2016).

19. Morera L, Lubbert M, Jung M. Targeting histone methyltransferases and demethylases in clinical trials for cancer therapy. *Clinical epigenetics* 8 57 (2016).
20. Clermont PL, Lin D, Crea F *et al*. Polycomb-mediated silencing in neuroendocrine prostate cancer. *Clinical epigenetics* 7 40 (2015).
21. Sato T, Kaneda A, Tsuji S *et al*. PRC2 overexpression and PRC2-target gene repression relating to poorer prognosis in small cell lung cancer. *Scientific reports* 3 1911 (2013).
22. Tan J, Yang X, Zhuang L *et al*. Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. *Genes & development* 21(9), 1050-1063 (2007).**

**This article describes the generation of the 44 gene list that was derived from microarray data following DZNeP treatment and that was used in this paper.

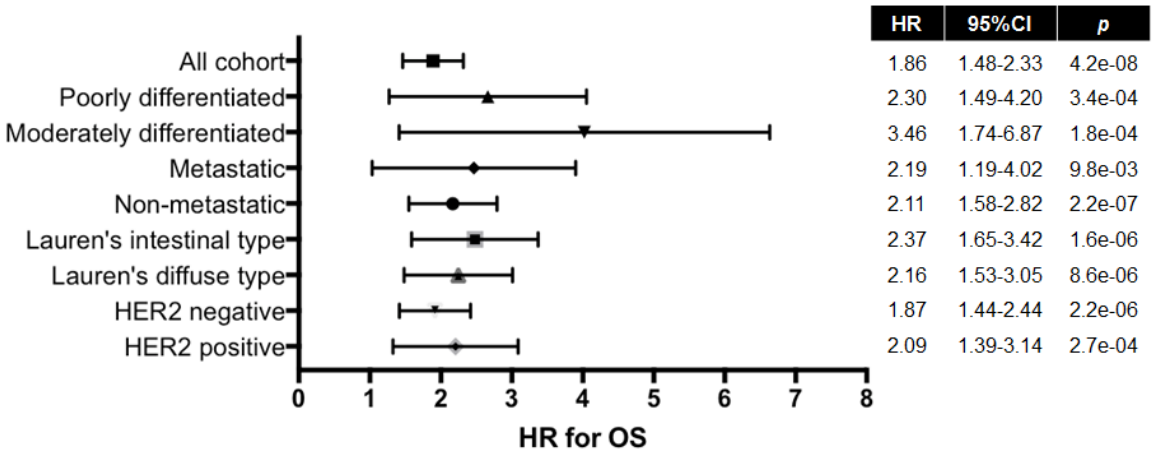
23. Szasz AM, Lanczky A, Nagy A *et al*. Cross-validation of survival associated biomarkers in gastric cancer using transcriptomic data of 1,065 patients. *Oncotarget* 7(31), 49322-49333 (2016).**

**This article outlines the development of the KMplotter resource that was used to create Kaplain-Meier plots used in this manuscript.

24. Gyorffy B, Lanczky A, Eklund AC *et al*. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast cancer research and treatment* 123(3), 725-731 (2010).
25. Li Q, Birkbak NJ, Gyorffy B, Szallasi Z, Eklund AC. Jetset: selecting the optimal microarray probe set to represent a gene. *BMC Bioinformatics* 12 474 (2011).
26. Mihaly Z, Kormos M, Lanczky A *et al*. A meta-analysis of gene expression-based biomarkers predicting outcome after tamoxifen treatment in breast cancer. *Breast cancer research and treatment* 140(2), 219-232 (2013).
27. Cancer Genome Atlas Research N. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 513(7517), 202-209 (2014).
28. Du XY, Liu X, Wang ZJ, Wang YY. SLPI promotes the gastric cancer growth and metastasis by regulating the expression of P53, Bcl-2 and Caspase-8. *Eur Rev Med Pharmacol Sci* 21(7), 1495-1501 (2017).
29. Pereira B, Chin SF, Rueda OM *et al*. The somatic mutation profiles of 2,433 breast cancers refines their genomic and transcriptomic landscapes. *Nat Commun* 7 11479 (2016).
30. Guo DQ, Zhang H, Tan SJ, Gu YC. Nifedipine promotes the proliferation and migration of breast cancer cells. *PloS one* 9(12), e113649 (2014).
31. Xu LZ, Li SS, Zhou W *et al*. p62/SQSTM1 enhances breast cancer stem-like properties by stabilizing MYC mRNA. *Oncogene* 36(3), 304-317 (2017).
32. Moses H, Barcellos-Hoff MH. TGF-beta biology in mammary development and breast cancer. *Cold Spring Harb Perspect Biol* 3(1), a003277 (2011).
33. Kerppola TK. Polycomb group complexes--many combinations, many functions. *Trends in cell biology* 19(12), 692-704 (2009).
34. Morey L, Helin K. Polycomb group protein-mediated repression of transcription. *Trends in biochemical sciences* 35(6), 323-332 (2010).
35. Miranda TB, Cortez CC, Yoo CB *et al*. DZNep is a global histone methylation inhibitor that reactivates developmental genes not silenced by DNA methylation. *Molecular cancer therapeutics* 8(6), 1579-1588 (2009).
36. Kaneko S, Bonasio R, Saldana-Meyer R *et al*. Interactions between JARID2 and noncoding RNAs regulate PRC2 recruitment to chromatin. *Molecular cell* 53(2), 290-300 (2014).

37. Kaneko S, Li G, Son J *et al*. Phosphorylation of the PRC2 component Ezh2 is cell cycle-regulated and up-regulates its binding to ncRNA. *Genes & development* 24(23), 2615-2620 (2010).
38. Wan J, Zhan J, Li S *et al*. PCAF-primed EZH2 acetylation regulates its stability and promotes lung adenocarcinoma progression. *Nucleic acids research* 43(7), 3591-3604 (2015).
39. Yan J, Li B, Lin B *et al*. EZH2 phosphorylation by JAK3 mediates a switch to noncanonical function in natural killer/T-cell lymphoma. *Blood* 128(7), 948-958 (2016).
40. Gao SB, Zheng QF, Xu B *et al*. EZH2 represses target genes through H3K27-dependent and H3K27-independent mechanisms in hepatocellular carcinoma. *Molecular cancer research : MCR* 12(10), 1388-1397 (2014).
41. Wang P, Li Z, Liu H, Zhou D, Fu A, Zhang E. MicroRNA-126 increases chemosensitivity in drug-resistant gastric cancer cells by targeting EZH2. *Biochem Biophys Res Commun* 479(1), 91-96 (2016).

Figure 1



	HR	95%CI	*p*
All cohort	1.86	1.48-2.33	4.2e-08
Poorly differentiated	2.30	1.49-4.20	3.4e-04
Moderately differentiated	3.46	1.74-6.87	1.8e-04
Metastatic	2.19	1.19-4.02	9.8e-03
Non-metastatic	2.11	1.58-2.82	2.2e-07
Lauren's intestinal type	2.37	1.65-3.42	1.6e-06
Lauren's diffuse type	2.16	1.53-3.05	8.6e-06
HER2 negative	1.87	1.44-2.44	2.2e-06
HER2 positive	2.09	1.39-3.14	2.7e-04

Figure 2

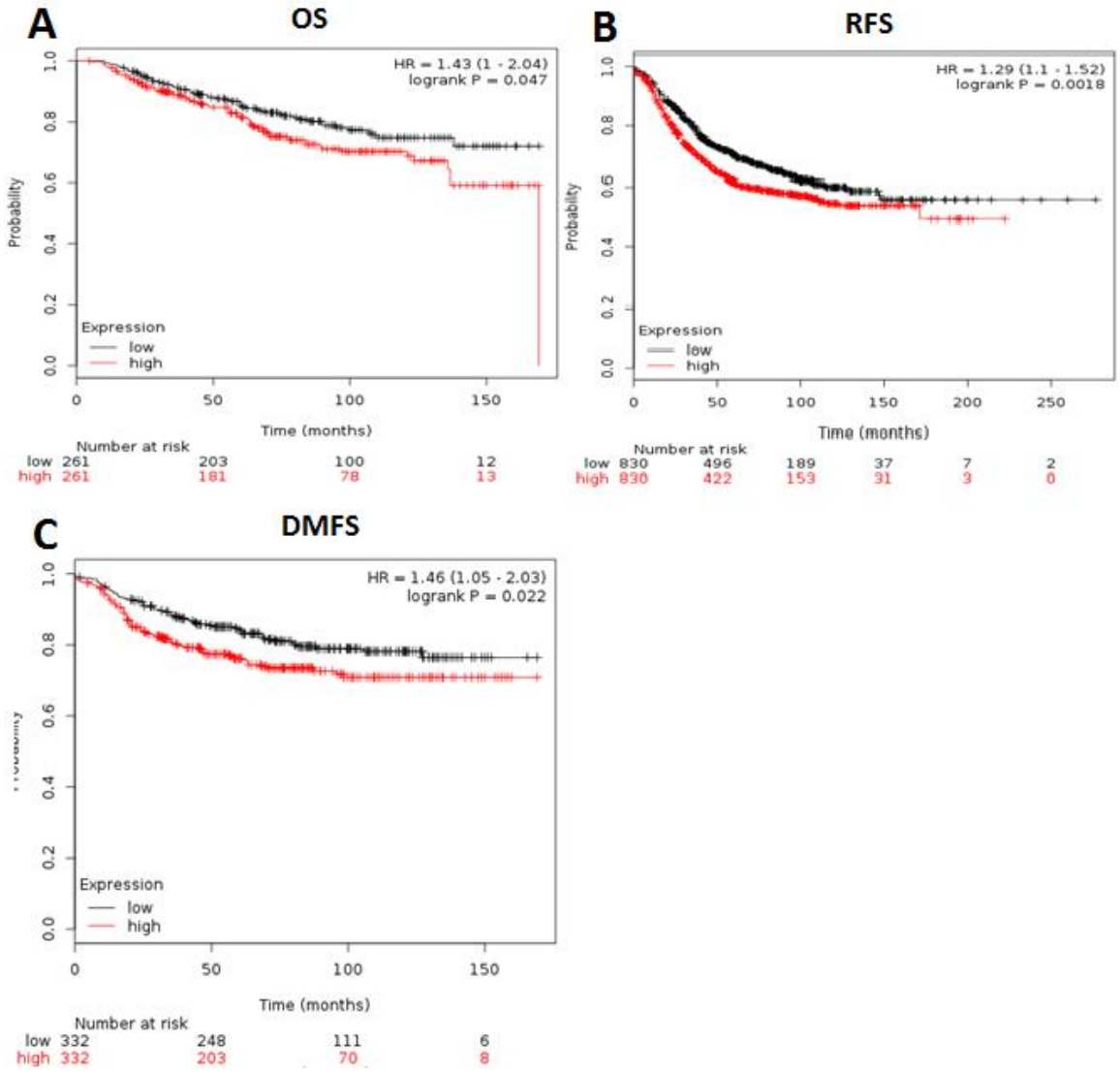
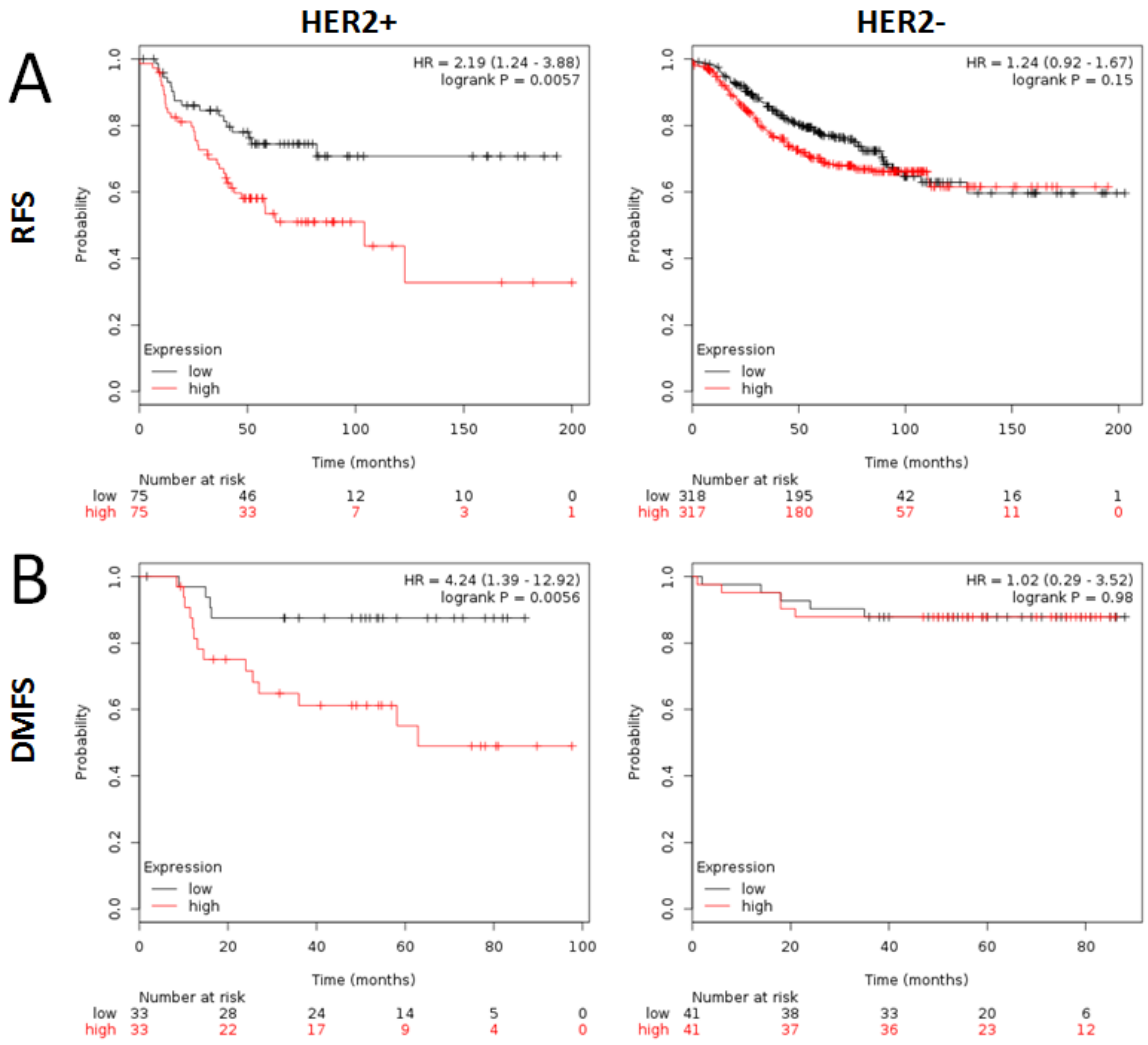
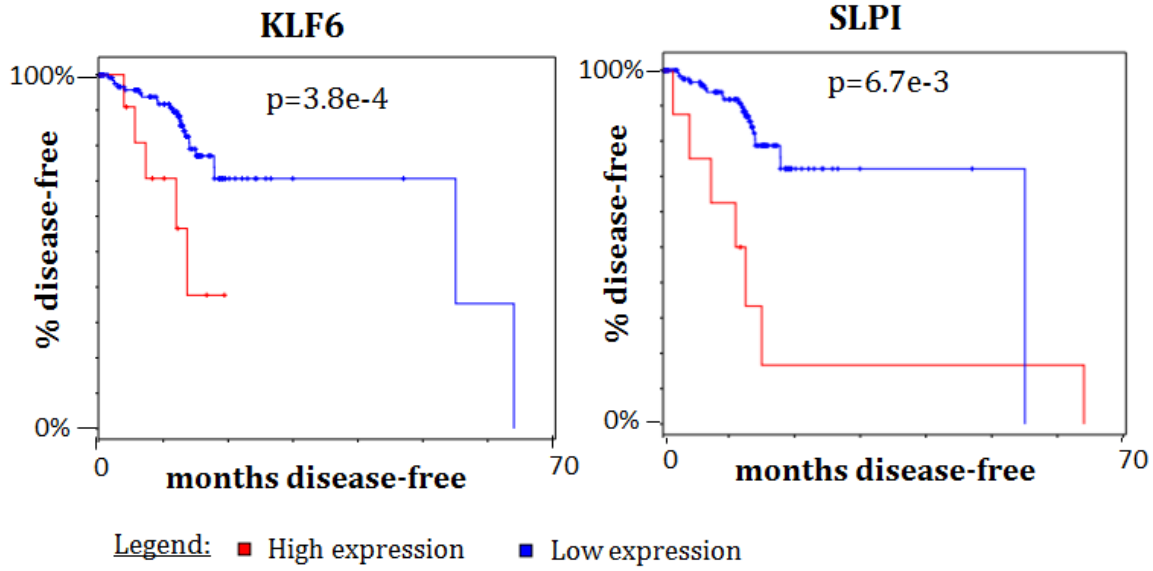

A
OS
HR = 1.43 (1 - 2.04)
logrank P = 0.047
Probability
Expression
low
high
Time (months)
Number at risk
low 261 203 100 12
high 261 181 78 13
B
RFS
HR = 1.29 (1.1 - 1.52)
logrank P = 0.0018
Probability
Expression
low
high
Time (months)
Number at risk
low 830 496 189 37 7 2
high 830 422 153 31 3 0
C
DMFS
HR = 1.46 (1.05 - 2.03)
logrank P = 0.022
Expression
low
high
Time (months)
Number at risk
low 332 248 111 6
high 332 203 70 8

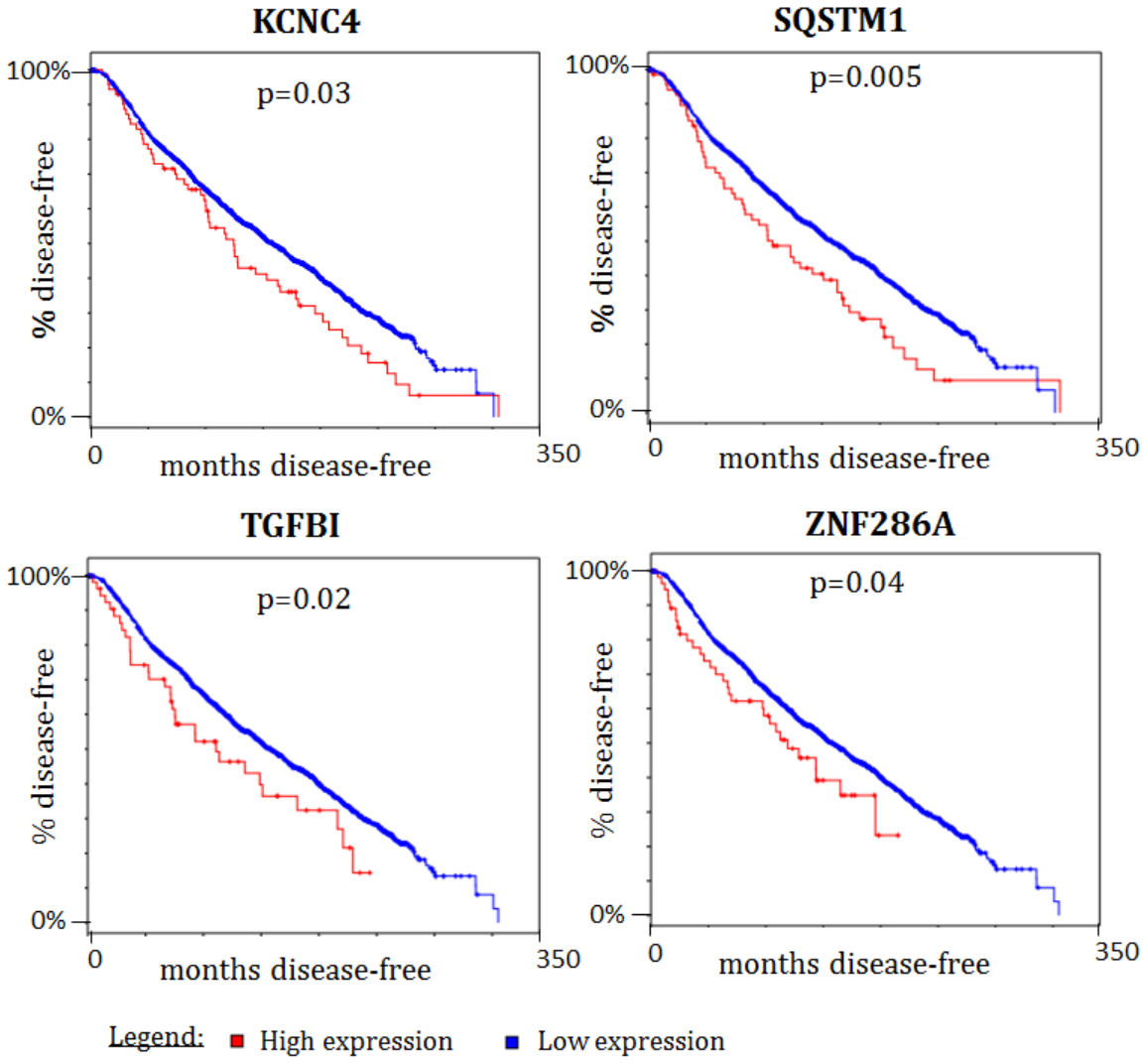
Figure 3


A
RFS
HER2+
HR = 2.19 (1.24 - 3.88)
logrank P = 0.0057
Probability
Expression
low
high
Time (months)
Number at risk
low 75 46 12 10 0
high 75 33 7 3 1
HER2-
HR = 1.24 (0.92 - 1.67)
logrank P = 0.15
Probability
Expression
low
high
Time (months)
Number at risk
low 318 195 42 16 1
high 317 180 57 11 0
B
DMFS
HR = 4.24 (1.39 - 12.92)
logrank P = 0.0056
Probability
Expression
low
high
Time (months)
Number at risk
low 33 28 24 14 5 0
high 33 22 17 9 4 0
HR = 1.02 (0.29 - 3.52)
logrank P = 0.98
Probability
Expression
low
high
Time (months)
Number at risk
low 41 38 33 20 6
high 41 37 36 23 12

S1


KLF6
100%
p=3.8e-4
% disease-free
0%
0
70
months disease-free
SLPI
100%
p=6.7e-3
% disease-free
0%
0
70
months disease-free
Legend:
High expression
Low expression

S2


KCNC4
p=0.03
100%
0%
% disease-free
0
months disease-free
350
SQSTM1
p=0.005
100%
0%
% disease-free
0
months disease-free
350
TGFBI
p=0.02
100%
0%
% disease-free
0
months disease-free
350
ZNF286A
p=0.04
100%
0%
% disease-free
0
months disease-free
350
Legend:
High expression
Low expression