Published in final edited form as:

Cancer Res. 2016 January 15; 76(2): 275–282. doi:10.1158/0008-5472.CAN-15-1938.

PRC2 epigenetically silences Th1-type chemokines to suppress effector T cell trafficking in colon cancer

Nisha Nagarsheth^{1,2}, Dongjun Peng¹, Ilona Kryczek^{1,2}, Ke Wu³, Wei Li^{1,3}, Ende Zhao^{1,3}, Lili Zhao⁴, Shuang Wei¹, Timothy Frankel¹, Linda Vatan¹, Wojciech Szeliga¹, Yali Dou⁵, Scott Owens⁵, Victor Marquez⁶, Kaixiong Tao³, Emina Huang⁷, Guobin Wang³, and Weiping Zou^{1,2,8}

¹Department of Surgery, University of Michigan School of Medicine, Ann Arbor, MI ²Department of Graduate Programs in Immunology and Cancer Biology, University of Michigan School of Medicine, Ann Arbor, MI ³Department of Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430022; China ⁴Department of Biostatistics, University of Michigan School of Medicine, Ann Arbor, MI ⁵Department of Pathology, University of Michigan School of Medicine, Ann Arbor, MI ⁶Chemical Biology Laboratory, Center for Cancer Research, NCI-Frederick, Frederick, 21702; Maryland ⁷Departments of Colorectal Surgery and Stem Cell Biology and Regenerative Medicine, Case Western Reserve University, Cleveland, 44106; Ohio ⁸Department of University of Michigan Comprehensive Cancer Center, University of Michigan School of Medicine, Ann Arbor, MI

Abstract

Infiltration of tumors with effector T cells is positively associated with therapeutic efficacy and patient survival. However, the mechanisms underlying effector T cell trafficking to the tumor microenvironment remain poorly understood in patients with colon cancer. The polycomb repressive complex 2 (PRC2) is involved in cancer progression, but the regulation of tumor immunity by epigenetic mechanisms has yet to be investigated. In this study, we examined the relationship between the repressive PRC2 machinery and effector T cell trafficking. We found that PRC2 components and demethylase JMJD3-mediated histone H3 lysine 27 trimethylation (H3K27me3) repress the expression and subsequent production of Th1-type chemokines CXCL9 and CXCL10, mediators of effector T cell trafficking. Moreover, the expression levels of PRC2 components, including EZH2, SUZ12, and EED, were inversely associated with those of CD4, CD8, and Th1-type chemokines in human colon cancer tissue, and this expression pattern was significantly associated with patient survival. Collectively, our findings reveal that PRC2-mediated epigenetic silencing is not only a crucial oncogenic mechanism, but also a key circuit controlling tumor immunosuppression. Therefore, targeting epigenetic programs may have significant implications for improving the efficacy of current cancer immunotherapies relying on effective T cell-mediated immunity at the tumor site.

Contact Information: Weiping Zou, M.D., Ph.D. at the Department of Surgery, University of Michigan School of Medicine, 109 Zina Pitcher Place Ann Arbor, MI, 48109 or at wzou@med.umich.edu.

Conflict of Interest: The authors declare no competing financial interests.

Keywords

CXCL9 and CXCL10; effector T cell trafficking; EZH2; epigenetics; cancer immunotherapy

INTRODUCTION

Effector T cells are indispensable for protective tumor immunity and therapeutic efficacy of cancer treatment (1–3). In colon cancer, the presence of CD8⁺ T cells and its cytotoxic antitumor molecules is a parameter for improved patient survival and tumors without signs of metastasis (4). Th1-type chemokines CXCL9 and CXCL10 mediate the trafficking of the main antitumor immune cells, including Th1 and CD8⁺ T cells, into the tumor microenvironment. However, it is not well understood how Th1-type chemokine expression is controlled in the tumor and subsequently how it affects effector T cell trafficking into the human cancer microenvironment.

Epigenetic regulation, including histone modifications, can mediate gene repression (5). One epigenetic repressive machinery involves the polycomb repressive complex 2 (PRC2), which trimethylates histone 3 lysine 27 (H3K27me3) (6,7). H3K27me3 is a repressive histone mark associated with facultative heterochromatin that functions to recruit regulatory proteins to repress gene transcription (7). PRC2 component, enhancer of zeste homolog 2 (EZH2) is highly expressed in multiple cancers (8,9). Its role in cancer cell proliferation, invasiveness, and differentiation has been widely studied (8,9). However, it is not known if EZH2 and the other PRC2 proteins are involved in the regulation of human T cell tumor trafficking, and, in turn, tumor immunity. Cancer epigenetic studies suggest that an abnormal evolution of repressive epigenetic marks including histone modification may directly contribute to cancer development and progression (10,11). However, the nature of cancer epigenetic repression is involved in the control of cancer immunity remains unanswered. Given the relevance of effector T cells and their tumor homing in anti-tumor immunity (1,2,4), we hypothesized that the cancer epigenetic repressive PRC2 machinery represses Th1-type chemokines in colon cancer, and in turn, alters effector T cell tumor migration and effective anti-tumor immunity. The validation of this hypothesis will lead to a notion that cancer epigenetic repressive machinery-mediated Th1-type chemokine repression is a novel immune evasive mechanism and the repressive machinery may be a target for novel cancer immunotherapy. Thus, in the current work, we tested this hypothesis in the context of human colon cancer at the molecular and clinical level.

MATERIALS AND METHODS**Human subjects and colon cancer tissues**

Patients diagnosed with colon carcinomas were recruited in the study. All usage of human subjects in this study was approved by local Institutional Review Boards. 6–9 fresh colon tissues were collected from patients with colon cancer and ulcerative colitis. Primary colon cancer cells, colon epithelial cells, and all the *in vitro* functional assays were performed with single cells from fresh colon cancer and colitis tissues as previously described (12). In addition, patients with colorectal carcinoma were evaluated from datasets in [OncoPrint.org](https://www.oncoPrint.org/).

Cell culture

Primary colon cancer cell lines (C1) were established from fresh colon cancer tissue. Routine short tandem repeat analysis is done to confirm the uniqueness of the primary line (12). DLD-1 and SW480 cell lines (ATCC) were used in the experiments. Single colon epithelial cells were made from fresh colon tissues from patients with colon cancer and ulcerative colitis. Colon cancer cells were treated with recombinant IFN γ (R&D systems), DZNep (Sigma), GSK-126 (GlaxoSmithKline), and GSK-J4 (GlaxoSmithKline), for different time points and concentrations.

Real-Time reverse-transcriptase polymerase chain reaction (RT-PCR)

RNA was isolated from the cells by Trizol (Ambion) and converted to cDNA using reverse transcriptase PCR (cloned AMV reverse transcriptase, Invitrogen). The mRNA was then quantified by real-time RT-PCR using StepOnePlus (Applied Biosystems). Specific primers are included in the supplementary information (Supplementary Table I). Fast SYBR Green Master Mix (Applied Biosystems) was used to detect fluorescence. Relative quantification was calculated according to the comparative Ct method with normalization to *GAPDH*. Unless otherwise noted, fold change with normalization to control is shown in the figures.

Lentiviral transduction and transfection

The lentiviral vectors, pGIPz or pGreen, encoding gene specific shRNAs were used to transduce colon cancer cells to establish stable cell knockdowns. Lentiviral shRNAs (Supplementary Table II) were from the Vector Core at the University of Michigan or provided by Arul Chinnaiyan (8). The lentiviral transduction efficiency was confirmed by GFP which was co-expressed by the lentiviral vector. The knockdown efficiency was assessed by Western blotting and real-time PCR. For transfections, Fugene HD (Promega) was used to transfect colon cancer cells with PKH3 (empty vector) and pCMV HA JMJD3 (Addgene (#24167)) according to the manufacturer's protocol. The overexpression was confirmed by Western blotting.

Enzyme-Linked Immunosorbent Assay (ELISA)

The protein levels of CXCL9 and CXCL10 were detected by ELISA (R&D) from the supernatants of treated colon cancer cells or single cells from fresh colon cancer and colitis tissue.

Western Blot

Western blotting was performed with specific antibodies against Histone H3 (9715, Cell Signaling), β -Actin (A5441, Sigma), EZH2 (612667, BD), SUZ12 (46264, Santa Cruz), EED (28701, Santa Cruz), and H3K27me3 (07449, Millipore). Signals were detected by ECL reagents (GE Healthcare, Buckinghamshire, UK).

T cell migration assays

In vitro migration assay was performed in a Transwell system with a polycarbonate membrane of 6.5-mm diameter with a 3- μ m pore size as described (13,14). Activated T cells were treated with anti-human CXCR3 or isotype, and added to the upper chamber.

Supernatant from the cultured colon cancer cells was added to the lower chamber. After incubation at 37°C for 12 hours, the phenotype and number of T cells in the upper and lower chambers were determined by FACS (LSRII, BD).

Chromatin immunoprecipitation (ChIP)

ChIP was performed and as previously described (12,15). Crosslinking was performed with 1% formaldehyde or 1% paraformaldehyde for 10 minutes. Sonication was performed with the Misonix 4000 water bath sonication unit at 15% amplitude for 10 minutes. Protein/DNA complex was precipitated with specific antibodies against H3K27me3 (6002, Abcam) and IgG control (Millipore). ChIP-enriched chromatin was used for RT-PCR with SYBR Green Master Mix, normalizing to input. Specific primers are listed in supplementary information (16) (Supplementary Table I).

Statistical analysis

Dependent on data distribution and experimental design, paired or unpaired Student's t-test and Mann-Whitney U-tests were used. Correlation coefficients (Spearman correlation) denoted by r , together with a p-value, were computed to measure correlation between different genes. Survival functions were estimated by Kaplan-Meier methods using genes classified as high or low based on mean or median expression values. Data was censored at the last follow-up for patients who were disease-free or alive at the time of analysis. All analyses were done using SAS 9.3 software. $P < 0.05$ was considered significant.

RESULTS

Inverse correlation exists between Th1-type chemokines and PRC2 in colon cancer

Th1-type chemokines CXCL9 and CXCL10 mediate effector CD8⁺ T cell tumor trafficking. CD8⁺ T cell tumor infiltration is associated with improved cancer patient survival. Th1-type chemokines are correlated with effector T cell density in some human tumors, including colon cancer, and positively associated with cancer patient survival (17,18). However, it is poorly understood how Th1-type chemokine expression is controlled in human colon cancer. We found that the levels of CXCL9 and CXCL10 mRNA (Fig. 1A) and protein (Fig. 1B) were higher in colitis colon compared to colon cancer tissue. The chemokines CXCL9 and CXCL10 can be stimulated by interferon gamma (IFN γ). When we treated single epithelial cells from the microenvironments of colon cancer, adjacent colon tissue, and colitis with IFN γ , the levels of CXCL9 and CXCL10 were higher in adjacent tissues (Fig. 1C) and colitis tissues (Fig. 1D) than colon cancer tissues. Patients with ulcerative colitis are at increased risk for developing colorectal cancer. The data suggest that Th1-type chemokine expression in colon cancer may evolve and become repressed when going from inflammatory tissue to cancer.

The PRC2 complex (including EZH2, embryonic ectoderm development (EED), and suppressor of zeste 12 homolog (SUZ12)) represses gene transcription through methylation of H3K27 (6,19). We hypothesized that the PRC2 complex repressed Th1-type chemokines in colon cancer. To test this hypothesis, we analyzed a colon cancer tissue microarray from [Oncomine.org](https://www.oncomine.org) (20) for potential correlations between PRC2 and Th1-type chemokines,

CXCL9 and CXCL10. In support of our hypothesis, we found significant negative correlations between the PRC2 complex components and CXCL9 and CXCL10 (Fig. 1E–I). The data suggests that the high levels of PRC2 may control and repress Th1-type chemokine expression in colon cancer.

PRC2 represses Th1-type chemokine expression in colon cancer

Given the inverse relationship between PRC2 and Th1-type chemokines (Fig. 1), we investigated if PRC2 machinery represses Th1-type chemokine expression in colon cancer. We initially examined the potential effect of 3-Deazaneplanocin A (DZNep), a pharmacological PRC2 inhibitor (19), on Th1-type chemokine expression in human colon cancer cells. In response to IFN γ treatment, we observed that treatment with DZNep led to higher levels of CXCL9 and CXCL10 expression in a primary colon cancer cell line (C1) (Fig. 2A, B), DLD-1 (Fig. 2C, D) and SW480 (Fig. 2E, F) colon cancer cells. As expected, DZNep reduced the expression of EZH2, SUZ12, and EED (Fig. S1A). However, the effect of DZNep was specific to chemokines as other IFN γ associated genes, including IFN γ receptor (*IFNGR2*) (Fig. S1B) and HLA-B (Fig. S1C) was not affected.

We next genetically knocked down EZH2 expression with lentivirus-based short hairpin RNA for EZH2 (shEZH2) in primary colon cancer C1 cells. shEZH2 specifically reduced the expression of EZH2 and SUZ12 (Fig. S1D), and resulted in elevated Th1 type chemokine mRNA (Fig. 2G,H) release in response to IFN γ stimulation (Fig. 2G, H). In addition, specific knockdown of EZH2 with shEZH2 and SUZ12 with shSUZ12 in DLD-1 cells (Fig. S1E, F) also increased Th1-type chemokine protein expression (Fig. S1F). Similar results were observed in shEED expressing DLD-1 cells (Fig. S1G). Thus, the PRC2 complex mediates Th1-type chemokine repression in colon cancer cells.

Th1-type chemokine repression is mediated via H3K27 methylation

The PRC2 complex mediates H3K27 methylation (6,7). We next examined whether Th1-type chemokine repression mediated by PRC2 depends on H3K27me3 changes at the promoter level of the chemokine genes. To test this possibility, we performed specific chromatin immunoprecipitation (ChIP) assay to detect H3K27me3 marks, based on the ENCODE database for H3K27me3 in GM12878 cells (16) (Fig. S2A). We observed high levels of H3K27me3 marks on the promoter of CXCL9 (Fig. 3A), the intergenic regions in between the CXCL9 and CXCL10 genes (Fig. 3B) and the promoter of CXCL10 (Fig. 3C). Knockdown of SUZ12 with shSUZ12, importantly, removed H3K27me3 on these areas (Fig. 3A–C) and increased Th1-type chemokine expression (Fig. S1F). *GAPDH* and *HoxB1* were used as a negative and positive control, respectively (Fig. 3D). Thus, H3K27me3 may be involved in the Th1-type chemokine gene silencing in colon cancer.

GSK126 is a highly selective, potent small molecule inhibitor of EZH2 methyltransferase activity (21). 5 μ M GSK126 treatment abolished the global level of H3K27me3 without inhibiting EZH2 (Fig. S2B). GSK126 treatment led to higher levels of IFN γ -induced CXCL10 (Fig. 3E) expression in primary colon cancer C1 cells. Similar results were observed in DLD-1 cells (Fig. S2C, D).

Jumonji C (JmjC)-domain-containing protein, JMJD3, is an H3K27 specific demethylase (22). As an activator of gene expression, ectopic expression of JMJD3 increased Th1-type chemokine expression (Fig. 3F). GSK-J4, a small molecular catalytic site inhibitor, selectively targets the H3K27-specific JmjC demethylase subfamily (23). GSK-J4 treatment led to reduced *CXCL9* and *CXCL10* expression in primary colon cancer C1 cells (Fig. 3G, H). Thus, H3K27me3 specific methyltransferase and demethylase regulate Th1-type chemokine repression in colon cancer cells.

PRC2 affects T cell migration toward colon cancer

Next, we examined whether the PRC2 complex can affect T cell migration via controlling *CXCL9* and *CXCL10* expression. We collected supernatants from colon cancer cells transfected with shEZH2 and control lentiviral vectors. We showed that CD4⁺ (Fig. 4A) and CD8⁺ (Fig. 4B) T cells more efficiently migrated towards shEZH2 supernatant than control. The migration was blocked by monoclonal antibody (mAb) against CXCR3, the receptor for *CXCL9* and *CXCL10* on T cells (Fig. 4A, B). Thus, the PRC2 component, EZH2, controls T cell trafficking toward colon cancer-derived *CXCL9* and *CXCL10*.

Finally, we assessed the clinical relevance of this phenomenon by examining the association of chemokines and the PRC2 complex with patient survival. We first performed survival analysis on chemokines by combining multiple datasets of colorectal carcinoma patients from oncomine.org. The patients were stratified in groups based on the mean expression level of each gene. When we divided patients with high or low expression of both *CXCL9* and *CXCL10*, high *CXCL9/CXCL10* associated with better patient overall survival (Fig. S3A). As a confirmation, we also observed positive correlation with *CD8* and *CXCL9* and *CXCL10* in different cohorts. It suggests that higher chemokines may lead to higher CD8⁺ T cell tumor infiltration (Fig. S3B). To examine the association between the 3 immune gene signature (*CD8*, *CXCL9*, and *CXCL10*) and the 3 PRC2 gene components (*EZH2*, *EED*, and *SUZ12*) with patient survival, we divided patients with high or low expression of these genes. We found that high *CD8/CXCL9/CXCL10* (Fig. 4C) and low *EZH2/SUZ12/EED* (Fig. 4D) associated with better patient overall survival. We also analyzed the relationship between PRC2 complex component transcripts and *CD4* and *CD8* mRNA in patients with colorectal cancer. We found that *CD4* and *CD8* mRNA expression negatively correlated with the PRC2 complex components, *EZH2*, *SUZ12*, and *EED* mRNA (Fig. 4E–G). Altogether, the data suggests that PRC2 controls T cell trafficking via repressing Th1-type chemokines and impacts colon cancer pathology.

DISCUSSION

Epigenetic changes are biologically vital and often linked to cancer proliferation, progression and metastasis (8,9,24,25). Our studies have shown that the PRC2 components in colon cancer cells silence the tumor production of Th1-type chemokines, potentially restrain effector T cell tumor infiltration, and in turn lessen anti-cancer immunity. Thus, PRC2 and H3K27me3-mediated Th1-type chemokine silencing is a novel immune evasion mechanism in human colon cancer. We propose a unifying mechanistic model of cancer in

which epigenetic silencing plays both biological and immunological roles in supporting tumor progression.

Previous reports show that the expression of CXCR3 and CXCL10 correlates with metastasis in certain cancer including colon cancer (26–28). However, whether the expression of CXCR3 in the tumor cells leads to CXCL10 dependent metastasis is not fully elucidated. Nonetheless, our data shows that there is a positive correlation between *CD8* and *CXCL9* and *CXCL10*, and the expression of CD8, CXCL9 and CXCL10 is positively associated with colon cancer patient survival. In support of this observation, it has been shown that CD8⁺ T cells and Th1-type chemokines positively predict colon cancer patient outcome (4,29–31).

A critical question is what drives the epigenetic changes in the first place. Several reports suggest microRNA involvement of EZH2 regulation (particularly loss of microRNA101) in prostate cancer (8). Further studies will be needed to demonstrate the drivers of this repression in cancer cells versus normal epithelium and if epigenetic changes are one of the earliest events in cancers, leading to immune evasion. We assume that this mechanism progressively evolves in cancer cells as colon inflammatory tissues express high levels of Th1-type chemokines. In contrast to irreversible genetic mutations, epigenetic alterations can be manipulated, making them a valuable target for therapy (32). To this end, it is crucial to understand the immune associated epigenetic mechanisms in the human cancer and to dissect how different epigenetic modifiers differentially affect cancer immune signature gene expression. In this regard, we have noticed that GSK126, a compound acting as a direct histone methyltransferase (HMT) inhibitor and DZNep, a compound acting as an indirect HMT inhibitor have shown certain differences in potencies in promoting cancer Th1-type chemokine expression. Perhaps additional mechanisms of DZNep, such as proteasomal degradation of PCR2 subunits, inhibition of other methylations, and reactivation of thioredoxin-binding protein 2 (TXNIP) which causes disruption of PCR2 could explain this difference (33).

Current cancer immunotherapies (34–36) and classic therapies (1,3) largely rely on efficient T cell tumor trafficking and T cell-mediated tumor immunity. Epigenetic reprogramming may unlock Th1-type chemokine repression, transform the tumor from poor T cell infiltration to rich T cell infiltration, and, ultimately, improve the effectiveness of any given cancer therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We thank Bruce Richardson and Venkat Keshamouni for valuable discussions, and Deborah Postiff, Michelle Vinco and Jackline Barikdar in the Tissue Procurement Core for their technical assistance. We are grateful for Dr. Weisheng Wu in the Bioinformatics Core for his help. The authors declare no competing financial interests.

Financial support: This work is supported (in part) by the National Institutes of Health grants (CA123088, CA099985, CA156685 and CA171306 for WZ; CA142808 and CA157663 for EH; F31CA189440 for NN), the Chinese Ministry of Science and Technology (973 program, 2015CB554000), the Wuhan Union Hospital Research

Fund, Herman and Dorothy Miller Award for Innovative Immunology Research, the Intramural Research Program of the NIH, and the National Cancer Institute.

REFERENCES

1. Zitvogel L, Apetoh L, Ghiringhelli F, Andre F, Tesniere A, Kroemer G. The anticancer immune response: indispensable for therapeutic success? *The Journal of clinical investigation*. 2008; 118(6): 1991–2001. [PubMed: 18523649]
2. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. *Nat Med*. 2004; 10(9):909–915. [PubMed: 15340416]
3. Park S, Jiang Z, Mortenson ED, Deng L, Radkevich-Brown O, Yang X, et al. The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity. *Cancer Cell*. 2010; 18(2):160–170. [PubMed: 20708157]
4. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nature reviews Cancer*. 2012; 12(4):298–306.
5. Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. *Nature reviews Genetics*. 2012; 13(5):343–357.
6. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, et al. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science*. 2002; 298(5595):1039–1043. [PubMed: 12351676]
7. Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. *Nature*. 2011; 469(7330):343–349. [PubMed: 21248841]
8. Varambally S, Cao Q, Mani RS, Shankar S, Wang X, Ateeq B, et al. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. *Science*. 2008; 322(5908):1695–1699. [PubMed: 19008416]
9. Mimori K, Ogawa K, Okamoto M, Sudo T, Inoue H, Mori M. Clinical significance of enhancer of zeste homolog 2 expression in colorectal cancer cases. *European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology*. 2005; 31(4):376–380.
10. Schlesinger Y, Straussman R, Keshet I, Farkash S, Hecht M, Zimmerman J, et al. Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for de novo methylation in cancer. *Nature genetics*. 2007; 39(2):232–236. [PubMed: 17200670]
11. Feinberg AP, Ohlsson R, Henikoff S. The epigenetic progenitor origin of human cancer. *Nature reviews Genetics*. 2006; 7(1):21–33.
12. Kryczek I, Lin Y, Nagarsheth N, Peng D, Zhao L, Zhao E, et al. IL-22CD4 T Cells Promote Colorectal Cancer Stemness via STAT3 Transcription Factor Activation and Induction of the Methyltransferase DOT1L. *Immunity*. 2014
13. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med*. 2004; 10(9):942–949. [PubMed: 15322536]
14. Curiel TJ, Wei S, Dong H, Alvarez X, Cheng P, Mottram P, et al. Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. *Nat Med*. 2003; 9(5):562–567. [PubMed: 12704383]
15. Cui TX, Kryczek I, Zhao L, Zhao E, Kuick R, Roh MH, et al. Myeloid-derived suppressor cells enhance stemness of cancer cells by inducing microRNA101 and suppressing the corepressor CtBP2. *Immunity*. 2013; 39(3):611–621. [PubMed: 24012420]
16. Harth-Hertle ML, Scholz BA, Erhard F, Glaser LV, Dolken L, Zimmer R, et al. Inactivation of intergenic enhancers by EBNA3A initiates and maintains polycomb signatures across a chromatin domain encoding CXCL10 and CXCL9. *PLoS pathogens*. 2013; 9(9):e1003638. [PubMed: 24068939]
17. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *The New England journal of medicine*. 2003; 348(3):203–213. [PubMed: 12529460]

18. Kryczek I, Banerjee M, Cheng P, Vatan L, Szeliga W, Wei S, et al. Phenotype, distribution, generation, and functional and clinical relevance of Th17 cells in the human tumor environments. *Blood*. 2009; 114(6):1141–1149. [PubMed: 19470694]
19. Tan J, Yang X, Zhuang L, Jiang X, Chen W, Lee PL, et al. Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. *Genes Dev*. 2007; 21(9):1050–1063. [PubMed: 17437993]
20. Khambata-Ford S, Garrett CR, Meropol NJ, Basik M, Harbison CT, Wu S, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2007; 25(22):3230–3237. [PubMed: 17664471]
21. McCabe MT, Ott HM, Ganji G, Korenchuk S, Thompson C, Van Aller GS, et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature*. 2012; 492(7427):108–112. [PubMed: 23051747]
22. Agger K, Cloos PA, Christensen J, Pasini D, Rose S, Rappsilber J, et al. UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. *Nature*. 2007; 449(7163):731–734. [PubMed: 17713478]
23. Kruidenier L, Chung CW, Cheng Z, Liddle J, Che K, Joberty G, et al. A selective jumonji H3K27 demethylase inhibitor modulates the proinflammatory macrophage response. *Nature*. 2012; 488(7411):404–408. [PubMed: 22842901]
24. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. *Nature reviews Genetics*. 2002; 3(6):415–428.
25. Fussbroich B, Wagener N, Macher-Goeppinger S, Benner A, Falth M, Sultmann H, et al. EZH2 depletion blocks the proliferation of colon cancer cells. *PLoS One*. 2011; 6(7):e21651. [PubMed: 21765901]
26. Wightman SC, Uppal A, Pitroda SP, Ganai S, Burnette B, Stack M, et al. Oncogenic CXCL10 signalling drives metastasis development and poor clinical outcome. *British journal of cancer*. 2015; 113(2):327–335. [PubMed: 26042934]
27. Murakami T, Kawada K, Iwamoto M, Akagami M, Hida K, Nakanishi Y, et al. The role of CXCR3 and CXCR4 in colorectal cancer metastasis. *International journal of cancer Journal international du cancer*. 2013; 132(2):276–287. [PubMed: 22689289]
28. Zipin-Roitman A, Meshel T, Sagi-Assif O, Shalmon B, Avivi C, Pfeffer RM, et al. CXCL10 promotes invasion-related properties in human colorectal carcinoma cells. *Cancer Res*. 2007; 67(7):3396–3405. [PubMed: 17409450]
29. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science*. 2006; 313(5795):1960–1964. [PubMed: 17008531]
30. Mlecnik B, Tosolini M, Kirilovsky A, Berger A, Bindea G, Meatchi T, et al. Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2011; 29(6):610–618. [PubMed: 21245428]
31. Pages F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molitor R, et al. Effector memory T cells, early metastasis, and survival in colorectal cancer. *The New England journal of medicine*. 2005; 353(25):2654–2666. [PubMed: 16371631]
32. Yoo CB, Jones PA. Epigenetic therapy of cancer: past, present and future. *Nature reviews Drug discovery*. 2006; 5(1):37–50. [PubMed: 16485345]
33. Zhou J, Bi C, Cheong L-L, Mahara S, Liu S-C, Tay K-G, et al. The histone methyltransferase inhibitor, DZNep, up-regulates TXNIP, increases ROS production, and targets leukemia cells in AML. *Blood*. 2011; 118(10):2830–2809. [PubMed: 21734239]
34. Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. *Nature reviews Cancer*. 2008; 8(4):299–308. [PubMed: 18354418]
35. Scholler J, Brady TL, Binder-Scholl G, Hwang WT, Plesa G, Hege KM, et al. Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells. *Sci Transl Med*. 2012; 4(132):132ra53.

36. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nature reviews Cancer*. 2012; 12(4):252–264. [PubMed: 22437870]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1. Inverse correlation exists between Th1-type chemokines and the PRC2 complex in colon cancer

A: CXCL9 and CXCL10 mRNA in colitis tissue and colon cancer tissue. CXCL9 and CXCL10 mRNA levels were detected by real-time PCR in human colon cancer and colitis tissue (n=6 tissues, *p<0.05).

B: CXCL9 and CXCL10 protein in colitis tissue and colon cancer tissue. CXCL9 and CXCL10 protein was detected by ELISA in 1 gram of human colon cancer and colitis tissue (n=6 tissues, *p<0.05).

C, D: CXCL9 and CXCL10 mRNA in IFN γ -treated colitis tissue, colon cancer tissue and tissue adjacent to colon cancer. Single epithelial cells were prepared from colon cancer tissue (C, D), tissue adjacent to colon cancer (C) and colitis tissue (D), and treated with IFN γ (5ng/ml). CXCL9 and CXCL10 mRNA levels were detected by real-time PCR. One representative of 3 independent experiments is shown.

E–I: Correlation between Th1-type chemokines and PRC2 components in patients with colon cancer. Numbers represent r-values with significance ($p < 0.05$). Spearman analysis for correlation were conducted from Khambata-Ford Colon database from Oncomine (oncomine.org, n=80).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2. PRC2 represses Th1-type chemokine expression in colon cancer

A–F: Effects of DZNep on IFN γ -stimulated Th1-type chemokine production. CXCL9 and CXCL10 mRNA levels were detected by real-time PCR in C1 cells (A, B), DLD-1 (C, D), and SW480 (E, F) cells treated with IFN γ (0.5 or 10 ng/ml) and DZNep (0.5, 2, or 5 μ M) (3 independent experiments shown, * p <0.05).

G, H: Effects of shEZHZ2 on IFN γ -mediated chemokine production. C1 cells expressing shEZHZ2 or scr vector were cultured with IFN γ (0.5 ng/ml), and CXCL9 (G) and CXCL10 (H) mRNA was detected by real-time PCR (3 independent experiments shown, * p <0.05); fold change shown relative to scr with IFN γ .

Figure 3. Th1-type chemokine repression is mediated via H3K27 methylation

A–C: H3K27me3 occupancy in the promoter area of *CXCL9* and *CXCL10* in colon cancer cells. H3K27me3-ChIP assay was performed in DLD-1 colon cancer cells for the promoter areas of *CXCL9* (A), the intergenic region of *CXCL9* and *CXCL10* (B), and the promoter areas of *CXCL10* (C) (3 independent experiments shown, * $p < 0.01$). kb: kilobase; TSS: transcription start site.

D: ChIP positive and negative control. H3K27me3-ChIP assay was performed in DLD-1 colon cancer cells for *HoxB1* and *GAPDH* (3 independent experiments shown).

E: Effects of GSK126 on the IFN γ -mediated chemokine production. C1 cells were treated with IFN γ (5 ng/ml) and 0.5 μ M GSK126. CXCL10 mRNA was detected by real-time PCR (3 independent experiments shown, * $p < 0.05$).

F: Effects of JMJD3 overexpression on IFN γ -mediated chemokine production. DLD-1 cells were transfected with PKH3 (empty vector) and pCMV HA JMJD3 (JMJD3 OE) before IFN γ (0.5 ng/ml) was added and RT-PCR was performed for CXCL10 (4 independent experiments shown, * $p < 0.05$).

G,H: Effects of GSK-J4 on IFN γ -mediated chemokine production. C1 cells were treated with IFN γ (0.5 ng/ml) and 5 μ M or 10 μ M GSK-J4 and CXCL9 and CXCL10 mRNA was detected by real-time PCR (3 independent experiments shown, * $p < 0.05$).

Figure 4. PRC2 affects CD8⁺ T cell migration toward colon cancer

A, B: Effect of shEZH2 on migration of CD4⁺ (A) and CD8⁺ (B) T cells. shEZH2 and control vector expressing DLD-1 cells were treated with 5 ng/ml IFN γ and the supernatants were collected for T cell migration assay. Activated T cells were subject to the migration toward the supernatants in the presence of anti-human CXCR3 or control. The migrated T cells were assessed by flow cytometry analysis. One representative of 3 independent experiments is shown.

C: The association between *CD8/CXCL9/CXCL10* transcripts and survival in patients with colorectal carcinoma (Smith colorectal, Smith colorectal 2, Jorissen Colorectal 3, and Staub colorectal). The analyses were conducted by combining multiple databases from Oncomine (oncomine.org) (n= 185, p=0.0089).

D: The association between *EZH2/SUZ12/EED* transcripts and survival in patients with colorectal carcinoma (Smith colorectal, Smith colorectal 2, Jorissen Colorectal 3, and Staub colorectal). The analyses were conducted by combining multiple databases from Oncomine (oncomine.org) (n=105, p=0.037).

E–G: Correlation between PRC2 components and CD4 and CD8A mRNA in patients with colorectal carcinoma. Numbers represent r-values with significance ($p < 0.05$). Spearman analysis for correlation was conducted from multiple databases (Khambata-Ford Colon, Staub Colorectal, Smith Colorectal 2, TCGA, and Jorissen Colorectal 3) from Oncomine (oncomine.org). NS: not significant.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript