Epigenetics

**Major finding:** Tumor-intrinsic MLL1 drives transcriptional reprogramming in response to EZH2 inhibition.

**Mechanism:** MLL1 interacts with the p300/CBP complex to drive reciprocal H3K27ac gain at sites of H3K27me loss.

**Impact:** MLL1 status may inform precision therapy for patients with EZH2-overexpressing tumors.

---

**MLL1-INDUCED ONCOGENIC REPROGRAMMING DRIVES EZH2 INHIBITOR RESISTANCE**

Overexpression or gain-of-function mutations in EZH2, the enzymatic subunit of the histone methyltransferase polycomb repressive complex 2 (PRC2), promote oncogenesis in solid tumors and hematologic malignancies, respectively. Given that mutant EZH2 has been shown to drive H3K27 methylation (H3K27me)-dependent growth, Huang, Yan, Zhang, Wang, and colleagues epigenetically profiled a panel of EZH2-overexpressing blood and solid cancer cell lines with varying sensitivities to EZH2 inhibition (EZH2i). EZH2i reduced H3K27me in both hematologic and solid-cancer cell lines, inhibited growth of hematologic cancer cell lines, and induced H3K27 acetylation (H3K27ac) in EZH2i-resistant cell lines. Knockdown of p300 or CBP, both of which comprise the complex that catalyzes H3K27ac, sensitized EZH2i-resistant cell lines to express MLL1, which facilitates binding of p300/CBP to target sites, to EZH2 inhibition. Consistent with these findings, MLL1 expression was correlated with increased H3K27ac in EZH2i-resistant cell lines, and ablation of MLL1 resulted in decreased formation of p300/CBP complexes and H3K27ac. Integrated chromatin immunoprecipitation sequencing, RNA sequencing, and proteomic analysis showed that EZH2i treatment induced transcriptional reprogramming via a H3K27me-to-H3K27ac switch in EZH2i resistant cell lines. Treatment with a BRD4 inhibitor, which targets H3K27ac, sensitized EZH2i-resistant cells to EZH2 inhibition *in vitro*, and combined inhibition of BRD4 and EZH2 reduced growth of a subset of patient-derived xenografts (PDX) *in vivo*. Proteomic analyses revealed that PDXs that did not respond to combined inhibition of BRD4 and EZH2 exhibited increased MAPK signaling due to BRD4i-mediated inactivation of ERK1 and subsequent activation of ERK2. Combined targeting of EZH2, BRD4, and ERK1/2 suppressed the growth of liver and pancreatic cancer xenografts without significant toxicity *in vivo*. These results show that resistance to EZH2 inhibition is driven by MLL1-mediated transcriptional reprogramming in patients with EZH2-overexpressing tumors, who potentially could be stratified by MLL1 status for EZH2i either as a monotherapy or in combination with therapies targeting H3K27ac and/or MAPK signaling.


---

**Tumor Microenvironment**

**Major finding:** The hepatic microenvironment epigenetically directs lineage commitment to promote ICC or HCC liver tumors.

**Concept:** A necroptotic microenvironment promotes the development of ICC whereas apoptosis promotes HCC.

**Impact:** The microenvironment determines if hepatocytes with identical oncogenic mutations develop into ICC or HCC.

---

**THE TUMOR MICROENVIRONMENT DRIVES LIVER CANCER LINEAGE COMMITMENT**

Chronic liver inflammation and liver cirrhosis increase the risk of developing two distinct types of liver cancer, hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC). HCC and ICC have distinct morphologies and metastatic potentials, but both have been suggested to arise from hepatocytes, which have a high degree of cellular plasticity. However, the mechanism by which transformed hepatocytes commit to an HCC or ICC lineage has not been determined. To investigate liver tumorigenesis, Seehawer and colleagues used mosaic mouse models of liver cancer. Hydrodynamic tail-vein (HDTV) injection of transposable elements to promote hepatic delivery of the oncogenes Myc and NRAS<sup>G12V</sup> or Myc and Akt1 induced HCC. In contrast, *in vivo* electroporation of the same oncogenes induced ICC or combined ICC–HCC. *In vivo* lineage tracing revealed that both HCC and ICC arise from differentiated hepatocytes, and the mutational profiles of HCC and ICC were similar. Mechanistically, HDTV primarily induced apoptosis, whereas electroporation predominantly induced necroptosis. The necroptotic-associated cytokine microenvironment induced by electroporation resulted in the development of ICC. In the absence of the necroptotic microenvironment, the same oncogenes induced HCC development instead of ICC development. Chromatin accessibility profiling via assay for transposase-accessible chromatin using sequencing (ATAC-seq) revealed that *Tbx3* locus, which encodes a transcription factor involved in carcinogenesis, was accessible in HCC but inaccessible in ICC, resulting in increased expression of *Tbx3* in HCC. Conversely, the transcription factor *Prdm5* was accessible and highly expressed in ICC compared with HCC, indicating that Tbx3 and Prdm5 may be essential regulators of lineage commitment in liver cancer. Collectively, these findings reveal that the microenvironment can regulate lineage commitment in liver cancer, with hepatocytes giving rise to ICC in a necroptotic microenvironment and HCC in an apoptotic microenvironment, even when the driver mutations are identical.

The Tumor Microenvironment Drives Liver Cancer Lineage Commitment

Cancer Discov 2018;8:1344. Published OnlineFirst September 21, 2018.

Updated version
Access the most recent version of this article at:

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerdiscovery.aacrjournals.org/content/8/11/1344.2. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.