IN THIS ISSUE

Androgen Signaling Can Be Monitored in Circulating Tumor Cells

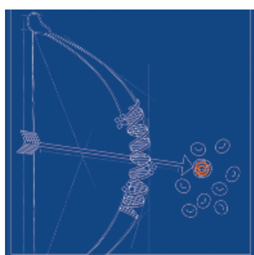
- Single circulating tumor cells (CTC) were stained for markers of androgen receptor signaling.
- CTC AR signaling is active in untreated prostate cancer but heterogenous in patients with CRPC.
- An "AR-mixed" CTC signature is associated with poor response to secondary hormonal therapy.

Castration-resistant prostate cancer (CRPC) is thought to arise due to androgen receptor (AR) reactivation following androgen deprivation therapy. Secondary inhibitors of AR signaling, such as abiraterone acetate, are in clinical development, but responses to these inhibitors vary, and no predictive biomarkers exist. In an exploratory study, Miyamoto and colleagues quantitatively measured AR signaling in circulating tumor cells (CTC) to noninvasively assay AR signaling in tumor cells of patients with prostate cancer. An automated immunofluorescence imaging platform detected levels of prostate-specific antigen (PSA), a marker of AR activation, and prostate-specific membrane antigen (PSMA), a marker of AR suppression. The vast majority of CTCs from newly diagnosed patients showed an "AR-on" ($PSA^+/PSMA^-$) signature, consistent with an androgen-dependent phenotype. After the initiation of androgen deprivation therapy, most CTCs switched to an "AR-off" ($PSA^-/PSMA^+$) signature and ultimately disappeared. In contrast, "AR-on," "AR-off," and "AR-mixed" ($PSA^+/PSMA^+$) CTC signatures were all observed in patients with CRPC, and abiraterone acetate treatment had variable effects on each CTC population. Notably, the presence of "AR-mixed" CTCs was significantly associated with decreased overall survival, suggesting that aberrant AR signaling in patients with CRPC may affect sensitivity to hormonal therapy. Although these findings require validation in larger prospective studies, they suggest that AR signaling is incompletely reactivated in CRPC and that monitoring of AR signaling in CTCs has the potential to identify patients with CRPC that are likely to respond to secondary hormonal therapy. ■

See article, p. 995.

B-ALL Fusion Proteins Induce Aberrant DNA Methylation Profiles

- DNA methylation and gene expression were profiled in *BCR–ABL1*, *E2A–PBX1*, and *MLL*-rearranged B-ALL.
- E2A–PBX1 and MLL fusion protein binding is directly linked to changes in DNA methylation.
- CD25 and BCL6 may be therapeutic targets in *BCR–ABL1* and *MLL*-rearranged B-ALL, respectively.



The aggressiveness of adult B-cell precursor acute lymphoblastic leukemia (B-ALL), compared with pediatric B-ALL, is thought to be associated with an increased frequency of high-risk genetic lesions, but the molecular basis for this link is not understood. Given the importance of DNA methylation in normal hematopoiesis and reports of abnormal DNA methylation in pediatric B-ALL, Geng and colleagues performed an integrated analysis of DNA methylation and gene expression patterns in a large cohort of adult patients with B-ALL that were enrolled in the same clinical trial. Interestingly, *BCR–ABL1*, *E2A–PBX1*, and *MLL*-rearranged B-ALLs each had distinct DNA methylation patterns that were associated with the deregulation of specific genes. *Interleukin receptor 2α chain*, encoding CD25, was preferentially hypomethylated and overexpressed in *BCR–ABL1*-positive B-ALL, and CD25 expression was associated with a worse clinical outcome. Although the biochemical mechanism by which the *BCR–ABL1* gene product mediates changes in DNA methylation remains unclear, the unique DNA methylation signature and gene expression patterns of *E2A–PBX1* and *MLL*-rearranged B-ALLs were directly linked to the genome-wide binding patterns of the fusion proteins. MLL fusions were specifically associated with hypomethylation and upregulation of *BCL6*, and genetic or pharmacologic inhibition of BCL6 selectively inhibited the growth of *MLL*-rearranged cells. These findings thus establish a link between leukemic fusion protein expression and distinct DNA methylation signatures and implicate CD25 and BCL6 as potential biomarkers and therapeutic targets for specific adult B-ALL subtypes. ■

See article, p. 1004.

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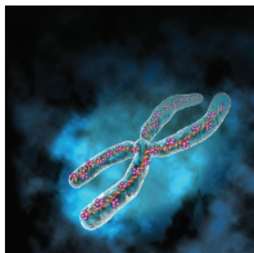
Altered DNA Methylation Distinguishes Fusion-Negative Prostate Cancers

- Genome-wide DNA methylation patterns in prostate cancer differ based on *TMPRSS2–ERG* status.
- *miR-26a* hypermethylation leads to EZH2 overexpression in the absence of *TMPRSS2–ERG*.
- DNA methylation inhibitors may be effective in *TMPRSS2–ERG* fusion-negative tumors.



Approximately half of all prostate cancers harbor a genomic rearrangement that places the oncogenic transcription factor v-ets erythroblastosis virus E26 oncogene homolog (ERG) under the control of the androgen-responsive *transmembrane protease serine 2* (*TMPRSS2*) promoter. Androgen-dependent *ERG* expression in the prostate drives cancer progression, but mechanisms underlying the development of *TMPRSS2–ERG* fusion-negative (FUS^-) tumors are less well understood. Börno and colleagues analyzed genome-wide DNA methylation patterns in prostate cancer and normal prostate tissue samples using methylated DNA immunoprecipitation followed by high-throughput sequencing. Interestingly, both tumor and normal tissues and *TMPRSS2–ERG* fusion-positive (FUS^+) and FUS^- tumors could be clearly differentiated by their DNA methylation patterns, with FUS^- tumors having a significantly higher number of differential methylation events than FUS^+ tumors and normal tissue. Differential methylated regions in FUS^- tumors were significantly enriched for regions encompassing cancer-related, homeobox, and microRNA (miRNA) genes. One hypermethylated miRNA gene, *miR-26a*, was significantly downregulated in FUS^- tumors, and its expression was negatively correlated with that of *enhancer of zeste homolog 2* (*EZH2*), a prostate cancer oncogene. Treatment with an *miR-26a* mimic or the DNA methylation inhibitor 5-aza-dC selectively decreased *EZH2* expression in FUS^- prostate cancer cells. Given that *EZH2* is a known target of ERG in FUS^+ tumors, these findings implicate *miR-26a* hypermethylation as an alternative mechanism of *EZH2* activation in FUS^- tumors. Because a deregulated epigenome may substitute for fusion genes in FUS^- prostate cancers, DNA methylation inhibitors may be preferentially effective in FUS^- tumors. ■

See article, p. 1024.

PI3K Blockade Sensitizes *BRCA*–Wild-type Tumors to PARP Inhibitors

- PI3K blockade decreases BRCA1/2 expression and impairs homologous recombination.
- PI3K suppression enhances the sensitivity of *BRCA*–wild-type breast tumors to PARP inhibitors.
- ERK-mediated activation of ETS1 upon PI3K inhibitor treatment represses BRCA1/2 expression.

PARP inhibitors provide therapeutic benefit to patients with *BRCA*-mutant triple-negative breast cancer (TNBC), in which homologous recombination-mediated DNA repair is defective. Ibrahim and colleagues investigated whether inhibition of phosphoinositide 3-kinase (PI3K), which is activated in TNBC, also impaired homologous recombination in *BRCA*–wild-type tumors. PI3K suppression in *BRCA*-proficient breast cancer cells resulted in increased phosphorylation of histone H2AX and reduced expression of BRCA1/2, indicating that loss of PI3K activity prevents efficient DNA repair. Moreover, PI3K blockade stimulated PARP activity and thus enhanced the sensitivity of *BRCA*–wild-type TNBC cell lines to PARP inhibition. Combined treatment with PI3K and PARP inhibitors more efficiently suppressed soft agar colony formation compared with single-agent treatment and significantly reduced the growth of patient-derived TNBC tumor xenografts that exhibited decreased BRCA1/2 expression and elevated PARP activity. Downregulation of BRCA1/2 in these tumors was dependent on elevated extracellular signal-regulated kinase (ERK) signaling; overexpression of active mitogen-activated protein/ERK kinase (MEK) diminished *BRCA1* transcription, whereas MEK inhibition enhanced BRCA1/2 levels. In addition, ERK-mediated phosphorylation of the v-ets erythroblastosis virus E26 oncogene homolog 1 (ETS1) transcription factor was required for BRCA downregulation, as ETS1 depletion was sufficient to increase BRCA1/2 expression, inhibit PARP activity, and diminish the sensitivity of TNBC cell lines to dual PI3K and PARP blockade. These findings suggest that this combinatorial strategy may improve the clinical efficacy of PARP inhibitors in TNBC. ■

See article, p. 1036.

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Combined PI3K and PARP Inhibition Reduces *BRCA1*-Mutant Tumor Growth

- PI3K inhibition slows *BRCA1*-deficient tumor growth, but MAPK activation confers resistance.
- Inhibition of PI3Kα impairs RAD51 foci formation and leads to accumulation of DNA damage.
- PI3K and PARP inhibitors synergize *in vivo* to attenuate *BRCA1*-mutant tumor growth.

BRCA1 mutation results in defective DNA repair by homologous recombination, thereby sensitizing breast cancer cells to inhibition of other DNA damage response proteins such as PARP. However, the clinical success of PARP inhibitors has been limited, indicating the need to identify additional therapeutic targets. Juvekar and colleagues observed activation of the phosphoinositide 3-kinase (PI3K) pathway in a mouse model of *BRCA1*-deficient breast cancer, suggesting that PI3K inhibitors might be therapeutically effective. Treatment with a PI3K inhibitor, NVP-BKM120, resulted in decreased AKT phosphorylation and delayed tumor growth, in part through reduction of glucose uptake and suppression of tumor angiogenesis; however, compensatory activation of mitogen-activated protein kinase (MAPK) signaling enabled outgrowth of resistant tumors, particularly at the proliferative tumor rim. PI3Kα inhibition also prevented RAD51 focus formation in response to irradiation and led to accumulation of phosphorylated histone H2AX and increased PARP activity, suggesting that loss of PI3K activity compromises the DNA damage response and might improve the efficacy of PARP inhibitors. In support of this idea, treatment with either NVP-BKM120 or the PARP inhibitor olaparib alone modestly reduced tumor growth, but the combination of both drugs synergized *in vivo* to significantly diminish the growth of mouse and human xenograft tumors derived from patients with *BRCA1*-mutant breast cancer. These results support the initiation of clinical trials to test this therapeutic combination in patients with *BRCA1*-mutant breast cancer. ■

See article, p. 1048.

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