Germ Cells

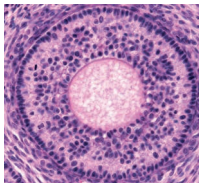
Major Finding: Checkpoint inhibitor immunotherapy can harm ovarian follicles and decrease oocyte quality in mice.

Concept: Immune checkpoint inhibitors activate immune cell–mediated extrinsic apoptosis within the ovary.

Impact: This study suggests strategies to combat immunotherapy-induced ovarian damage may be needed.

IMMUNE CHECKPOINT INHIBITORS INDUCE APOPTOSIS IN OVARIAN FOLLICLES

Immunotherapy has revolutionized the standard of care for many types of cancer, but organs that rely on immune cells, such as the ovary, may be especially susceptible to adverse events that stem from disruption of immune function. Within the ovary, T cells play critical roles in mediating follicle growth, supporting ovulation, and regulating the hormone-secreting corpus luteum. However, although immunotherapy is often administered to reproductive-age women, the effects of immunotherapy on ovarian function and fertility are not well understood. To address this, Winship, Alesi, Sant, Loi, Hutt, and colleagues investigated the off-target effects of immune checkpoint blockade on the ovary in tumor-bearing mice, demonstrating that anti–PD-L1 treatment increased the proportion of effector memory and tissue resident memory T cells while hindering ovarian follicle development and ovulation as soon as 4 days following final treatment. Similarly, analysis of tumor-free mice following 21 days of anti–PD-L1 or anti–CTLA-4 treatment indicated dysregulation of the estrous cycle and a significant decrease in the number of primordial follicles, revealing the long-term effects on fertility, as primordial follicles cannot be replaced. Furthermore, the quality



of oocytes diminished with immune checkpoint blockade, as evidenced by the increased number of fragmented and dead oocytes following stimulation of ovulation in treated mice. Supporting the role of immune cells in these immunotherapy-mediated defects, anti–PD-L1 treatment had no significant effect on follicle development or ovulation in mice lacking mature B, T, and natural killer cells. Immune checkpoint blockade increased intraovarian levels of inflammatory cytokines such as TNFα, as well as cleaved caspase-8 and caspase-3, suggesting that extrinsic apoptosis, amplified by intrinsic apoptosis, contributed to the observed phenotypes of ovarian damage and follicle loss. Indeed, genetic knockout of *Bid*, which prevented apoptosis downstream of cleaved caspase-8, abrogated follicle loss induced by anti–PD-L1. In summary, this work illustrates the effects of immune checkpoint blockade on ovarian function, highlighting this as an important area for future therapeutic focus. ■

Winship AL, Alesi LR, Sant S, Stringer JM, Cantavenera A, Hegarty T, et al. Checkpoint inhibitor immunotherapy diminishes oocyte number and quality in mice. Nat Cancer 2022;3:1–13.

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Biotechnology

Major Finding: Single-molecule profiling of circulating chromatin and biomarkers detects cancer and tissue of origin.

Concept: Plasma contains tumor-derived DNA and proteins that can reflect a tumor's molecular profile.

Impact: This method integrates layers of epigenetic information and informs cancer diagnosis and monitoring.

EPINUC ENABLES MULTIPLEXED EPIGENETIC PROFILING OF CIRCULATING TUMOR DNA

Cell-free DNA (cfDNA) is released by dying cells and circulates within the blood in the form of nucleosomes that often retain their epigenetic state. In patients with cancer, circulating tumor-derived cfDNA can be collected from liquid biopsies, enabling noninvasive molecular profiling, but current methods have been limited in their ability to integrate multiple parameters of epigenetic information with low input. To address this, Fedyuk, Erez, and colleagues developed a method called epigenetics of plasma-isolated nucleosomes (EPINUC), which utilizes total internal reflection fluorescence (TIRF) microscopy for single-molecule assessment of histone modifications from liquid biopsies of less than one milliliter. First, plasma-isolated nucleosomes are fluorescently labeled and polyadenylated prior to hybridization to a surface, followed by binding of fluorescently tagged antibodies against histone posttranslational modifications associated with gene silencing, active transcription, and enhancers. Direct counting of single molecules allowed quantification of modified nucleosomes, the ratio between specific histone modifications, and the percentage of nucleosomes concurrently modified by two chromatin marks. Moreover, this platform was adapted for single-molecule analysis of DNA methylation, as well as protein biomarkers clinically relevant in colorectal cancer (CRC), including carcinoembryonic antigen (CEA), tissue inhibitor of metalloproteinase-1, and mammalian sterile 20-like kinase 1 (MST1). Notably, reflecting the high sensitivity of TIRF, EPINUC was also able to detect wild-type and mutant p53 that originated directly from cells rather than being secreted. EPINUC analysis of plasma samples from healthy individuals and patients with early- or late-stage CRC demonstrated that CRC samples had higher ratios of CEA to MST1, higher levels of bivalent nucleosomes, and decreased levels of DNA methylation while revealing the utility of multiparametric analysis in differentiating stages of tumor progression. Moreover, EPINUC profiling distinguished between samples originating from patients with CRC or other tumor types, including pancreatic ductal adenocarcinoma. In summary, this work develops a single-molecule platform that concurrently assesses multiple epigenetic parameters and may improve the clinical applications of cfDNA testing. ■

Fedyuk V, Erez N, Furth N, Beresh O, Andreishcheva E, Shinde A, et al. Multiplexed, single-molecule, epigenetic analysis of plasma-isolated nucleosomes for cancer diagnostics. Nat Biotechnol 2022 Sep 8 [Epub ahead of print].

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