The American Journal of Pathology, Vol. 189, No. 6, June 2019

ELSEVIER

The American Journal of PATHOLOGY

ajp.amjpathol.org

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COMMENTARY

Uterine Epithelial Development and Enhancer of Zeste Homolog 2

It Is Important for More than Just Cancer

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Enhancer of zeste homolog 2 (EZH2) functions as a master regulator via epigenetic regulation of gene expression. EZH2 may function to methylate nonhistone proteins, methylate histone proteins, or act as a transcription factor, which up-regulates or down-regulates gene expression on the basis of context within the cell.[1] As a master regulator, EZH2 can have long-standing effects on development that lead to increased risk of disease later in life. For example, early work showed that treatment of mice with xenoestrogens, such as diethylstilbestrol, led to inactivation of EZH2, subsequent reprogramming of estrogen-responsive genes, and increased sensitivity to the proliferative effects of estradiol in the uterus.[2,3] However, the role of EZH2 in uterine development has not been described. In this issue of *The American Journal of Pathology*, Fang et al[4] report on the impact of conditional deletion of *Ezh2* in the mouse uterus, using a progesterone-driven *Cre* recombinase [*Ezh2* conditional knockout (cKO)].

Uterine Epithelial Development

Mouse and human endometrium is composed of luminal epithelium that invaginates to form glandular epithelium, which is surrounded by endometrial stromal cells.[5,6] Several genes, including catenin β 1 (*Ctnnb1*), forkhead box A2 (*Foxa2*), leucine-rich repeat-containing G-protein–coupled receptor 4 (*Lgr4*), wingless-related mouse mammary tumor virus (MMTV) integration site 4 (*Wnt4*), and *Wnt7a*, regulate uterine epithelium development and differentiation.[7] Fang et al[4] add *Ezh2* to the list of genes important in uterine epithelial cell development.

The key function of the endometrium is in the implantation process of the blastocyst. Each component of the endometrium plays a unique molecular role in embryo implantation. Both luminal epithelium and glandular epithelium are histologically columnar epithelial cells. However, they differ in shape, location, and molecular function, particularly regarding steroid hormone and non–steroid hormone signaling cascades.[5,8] Luminal epithelium is critical for uterine receptivity, embryo attachment, and furthering differentiation-signaling cascades. Glandular epithelium is important for angiogenesis and successful implantation and continuation of pregnancy.[5,8] The *Ezh2* cKO mouse uterine epithelium exhibits a unique morphologic change. Specifically, the normal single layer of columnar glandular epithelium is replaced by stratified columnar epithelium. Consistent with endometrial hyperplasia, the endometrium contains an increased number of dilated glands separated by normal stroma. More important, deletion of *Ezh2* in the uterus results in fertility defects.[4]

Molecular Impact

In *Ezh2* cKO mice, uterine epithelium development was dysregulated at the molecular level. In particular, there was an aberrant expression of basalis cell markers.[4] The endometrium is composed of the stratum compactum, stratum spongiosum, and stratum basalis. Both the stratum compactum

Supported by the Indiana University CTSI Strategic Research Initiative (S.M.H.) and the Indiana University Clinical and Translational Sciences Institute PDT/NIH grant UL1TR001108 (S.M.H.).

Accepted for publication March 29, 2019.

Disclosures: None declared.

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https://doi.org/10.1016/j.ajpath.2019.03.007

and stratum spongiosum make up the stratum functionalis, which exhibits large changes through the menstrual cycle.[9] In contrast, the stratum basalis remains more histologically constant and is changed with replacing tissue lost during menstruation, potentially through stem-like or progenitor cells in the basalis.[10–12] Transformation-related protein 63 (p63), alias tumor protein p63, is involved in the development of the epithelial basal layer.[13] The p63 gene contains two main isoforms by alternative promoters that contain a p53-like N-terminal transactivation domain and lack this domain.[14] The uterine epithelium of *Ezh2* cKO mice contained high levels of p63 that lacked the N-terminal transactivation domain, consistent with an increased basal cell phenotype.[4] Recent work has shown that neonatal exposure to estrogenic chemicals, such as diethylstilbestrol, affects p63 expression by increasing the number and location of basal cells and increasing the risk of uterine carcinoma in adults.[15] The p63 may be a marker of metaplastic differentiation and/or initiation of epithelial stratification.[16] EZH2 may play a similar role and/or regulate p63 expression.

The Future of EZH2 Inhibitors

EZH2 inhibitors hold significant promise as new therapies for switch/sucrose non–fermentable (SWI/SNF)-mutated cancers.[17] The SWI/SNF complex, involved in chromatin remodeling, is composed of approximately 15 subunits, such as *ARID1A* and *SMARCA4*. Next-generation sequencing studies across multiple different cancer types have shown that members of the SWI/SNF complex are frequently mutated in cancer, with a range of mutation frequency from 9% in triple-negative breast cancer to 75% in ovarian clear cell carcinoma.[18] Important translational studies have shown that EZH2 inhibitors demonstrate increased efficacy in *ARID1A*-mutated cancers.[17] The function of EZH2 in the endometrium is critical to understanding normal development, but also, it is critical to understanding the pharmacologic effects of EZH2 inhibitors on tissues other than cancer. Deletion of *Ezh2* in the mouse uterus results in simple hyperplasia that is histologically similar to the endometrial histology of women treated with ulipristal, a selective progesterone receptor modulator.[19] On the basis of data from this *Ezh2* cKO mouse model,[4] as clinical trials progress for EZH2 inhibitors, careful consideration for fertility and endometrial evaluation are critical.

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