PUBLIC ABSTRACT

ARID1A MUTANT PATHOGENESIS OF THE ENDOMETRIAL EPITHELIUM

By

Jake Jordan Reske

Women's health diseases represent an understudied, widespread medical concern with historically limited treatment options. Diseases of the endometrium, the innermost lining of the uterus, are a highly prevalent public burden. Endometriosis occurs in 1 in 10 women, and endometrial cancer is the most common gynecologic malignancy in the United States. Recent advances have revealed recurrent genetic causes of endometrial diseases, including gene mutations known to play a role in cancer development. *ARID1A* is one such gene that is commonly mutated in endometrial diseases, and it encodes a protein involved in regulating DNA packaging and activity in the cell nucleus within a large complex known as SWI/SNF. The focus of this dissertation is to improve our understanding of how ARID1A mutations promote endometrial diseases at multiple biological levels, with a particular focus on how disrupted chromatin regulation affects physiologically relevant gene expression. In these works, genetic engineering techniques are leveraged in mice and human cell-based models supported by public and clinical data to establish the consequences of ARID1A mutations in the endometrium and how they relate to other common genetic alterations in these diseases. These studies have revealed that ARID1A is a tumor suppressor in the endometrial epithelium, such that ARID1A loss drives cellular invasion into nearby tissue. ARID1A mutations also promote invasive metastasis and squamous metaplasia in the context of aggressive TP53 mutations. At the level of chromatin, ARID1A and SWI/SNF directly regulate endometrial epithelial identity genes through both promoter and distal enhancer chromatin interactions. Mechanistically, ARID1A mutant invasion is driven by cell

identity control regions known as super-enhancers that become hyperactivated, which can be reversed pharmacologically. Moreover, ARID1A physically and genomically interacts with other nuclear chromatin regulators to govern gene activation states through variant histone regulation. These works have contributed in multiple aspects toward deciphering how ARID1A mutations promote disease in the endometrium, including pre-clinical support for using epigenetic therapies to treat invasive ARID1A mutant endometrial conditions. Future efforts will aim to further identify and understand molecular and biochemical mechanisms linking ARID1A and SWI/SNF chromatin remodeling activity to regulation of gene expression. Ongoing work seeks to explain roles of ARID1A and SWI/SNF epigenetic regulation in normal physiological processes of the endometrium, such as hormone signaling across the menstrual cycle.

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Subunits within the mammalian SWI/SNF chromatin remodeling complex are prone to mutation in human diseases such as cancer. The ARID1A (AT-rich interactive domain 1A; BAF250A) subunit serves as a large scaffold and DNA-binding module of certain SWI/SNF complexes, and deleterious *ARID1A* mutations are frequently observed in pathologies of the endometrial epithelium, including endometriosis, endometrial hyperplasia, and endometrial carcinoma. This dissertation aims to further our understanding of ARID1A mutant pathologies of the endometrium, spanning genetic interactions involving ARID1A and other disease-associated mutations, physiological consequences to ARID1A deficiency, cellular and molecular mechanisms of ARID1A mutant pathogenesis, chromatin and transcriptional alterations directly resulting from disruption of ARID1A regulation, and biochemical and genomic interactions interdependent upon normal ARID1A-SWI/SNF activity. In Chapter 1, endometrial pathophysiology and the role of ARID1A in chromatin regulation are reviewed as an introduction to the present works. In Chapter 2, a novel genetic mouse model displaying invasive endometrial hyperplasia is established through ARID1A haploinsufficiency in the presence of an oncogenic PI3-kinase pathway mutation specifically in the endometrial epithelium. In this model, transcriptome expression and genome-wide chromatin accessibility measurements indicate that ARID1A loss alters gene promoter chromatin activity leading to epithelial-to-mesenchymal transition and collective invasion *in vivo*. In Chapter 3, these *in vivo* genome-wide chromatin accessibility measurements are used to demonstrate that the choice of ATAC-seq normalization

method can significantly alter biological interpretation, and analytical strategies to direct genomic data interpretation are explored. In Chapter 4, a genetic interaction between ARID1A and tumor suppressor TP53 in endometrial cancer is investigated, and p53 signaling activation is indicated as a hallmark of ARID1A mutant tumors through disruption of ARID1A regulation at p53 target gene chromatin. In Chapter 5, functions of BRG1 (SMARCA4), a SWI/SNF catalytic subunit, are investigated and contrasted with that of ARID1A in the endometrial epithelium. BRG1 loss *in vivo* promotes spontaneous translocation of endometrial glands to the uterine myometrium, akin to adenomyosis. In Chapter 6, a genome-wide chromatin state map is constructed from epigenomic assays following the effects of ARID1A loss, and ARID1A is observed to regulate highly active enhancer-like chromatin regions. Unexpectedly, ARID1A oppositely regulates typical vs. super-enhancers, whereby ARID1A loss causes H3K27-hyperacetylation and hyperactivation of the *SERPINE1* super-enhancer loci that is required for ARID1A loss-driven invasion and dependent on P300 histone acetyltransferase activity. Finally, in Chapter 7, genome-wide assays reveal that ARID1A is required for maintenance of histone variant H3.3 in active chromatin. Mechanistically, ARID1A physically interacts with H3.3-interacting remodeler CHD4 (NuRD) associated with H3.3 maintenance, and this H3.3 chromatin regulation is further guided by histone reader ZMYND8 to specify repression of hyperactivation at H4(K16)ac+ super-enhancers. Major conclusions and future directions of these studies are summarized in Chapter 8. Altogether, these works have elucidated numerous aspects of ARID1A biology from pathophysiology to genetics to chromatin and transcriptional regulatory mechanisms. Further efforts will aim to explain how ARID1A and SWI/SNF regulate other context-specific chromatin through alternative co-factor interactions. Similar functional genomic experimental frameworks will be applied to demonstrate how these processes are governed during normal endometrial physiology.