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SWI/SNF Complex Mutations in Gynecologic Cancers: Molecular Mechanisms and Models

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SCCOHT, small cell carcinoma of the ovary hypercalcemic type, DDEC, dedifferentiated endometrial carcinoma, clear cell carcinoma of the ovary, SWI/SNF, SMARCA4, ARID1A

Abstract

The SWI/SNF (mating type SWItch/Sucrose NonFermentable) chromatin remodeling complexes interact with histones and transcription factors to modulate chromatin structure and control gene expression. These evolutionarily conserved multisubunit protein complexes are involved in regulating many biological functions, such as differentiation and cell proliferation. Genomic studies have revealed frequent mutations of genes encoding multiple subunits of the SWI/SNF complexes in a wide spectrum of cancer types, including gynecologic cancers. These SWI/SNF mutations occur at different stages of tumor development and are restricted to unique histologic types of gynecologic cancers. Thus, SWI/SNF mutations have to function in the appropriate tissue and cell context to promote gynecologic cancer initiation and progression. In this review, we summarize the current knowledge of SWI/SNF mutations in the development of gynecologic cancers to provide insights into both molecular pathogenesis and possible treatment implications for these diseases.

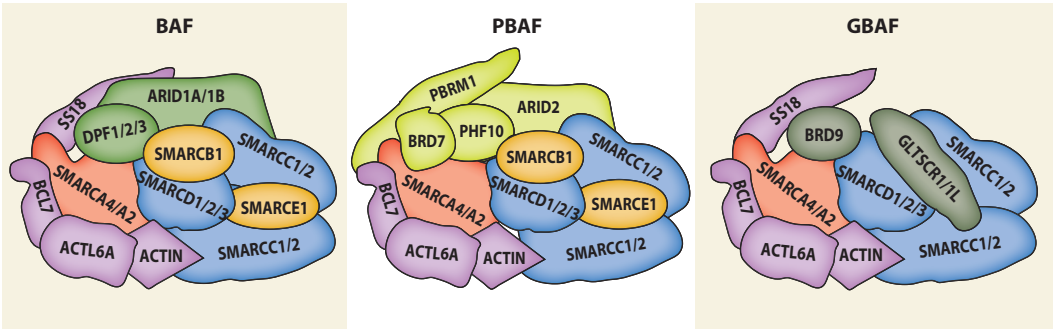
1. INTRODUCTION

Gynecologic cancers, also referred to as cancers of the female reproductive tract, include malignancies arising from the cervix, fallopian tubes, ovaries, uterus, vulva, and vagina. Among these cancers, although uterine cancer is the most common, ovarian cancer is the most common cause of death. Both ovarian and uterine cancers are composed of several distinct histologic subtypes. Many of these subtypes are believed to arise from distinct cellular origins in addition to having distinct and sometimes pathognomonic mutations. Recent genomic studies have revealed that each histologic subtype is often associated with a distinct mutational landscape and signature, such as the homologous recombination signature characteristic of BRCA-deficient high-grade serous cancer of the ovary. In addition to finding common, well-studied tumor suppressors and oncogenes, genomic analyses of gynecologic cancers have identified frequent mutations of genes encoding subunits of the SWI/SNF (mating type SWItch/Sucrose NonFermentable) chromatin remodeling complex. Some mutations, such as those in *SMARCA4* (encoding the BRG1 protein), occur as a germline event and function as a classic tumor suppressor and key driver in small cell carcinoma of the ovary, hypercalcemic type (SCCOHT). Other mutations occur either early during transformation or as a late event during the progression of a gynecologic cancer. These studies suggest specific roles for mutations in the SWI/SNF complex during multiple steps of development of gynecologic cancer. In this review, we summarize the current knowledge of SWI/SNF mutations in the development of gynecologic cancer to provide insights into both molecular pathogenesis and the potential treatment implications for these diseases.

2. BIOLOGY OF THE SWI/SNF COMPLEXES

The SWI/SNF complexes were first discovered in *Saccharmomyces cerevisiae*. They are evolutionarily conserved, multisubunit protein complexes that interact with histones and transcription factors. They are composed of 10 to 15 subunits, with many possible combinations of subunits, resulting in the presence of numerous distinct subcomplexes in a single cell (1–5) (**Figure 1**). Two major types of SWI/SNF complexes have been well documented in mammalian cells: the canonical BAF (BRG1-associated factors) complex, which contains a mutually exclusive ARID1A (AT-rich interaction domain 1A) or ARID1B subunit, and the PBAF (polybromo-associated BAF) complex, which incorporates ARID2, PBRM1 (polybromo 1), and BRD7 (bromodomain-containing protein 7) as its signature subunits (6–8). Recently, a third type of SWI/SNF complex was discovered independently by Alpsoy et al. (9) and Mashtalir et al. (8), and it does not include ARID1A or ARID1B and ARID2 and instead contains BRD9 and either GLTSCR1 (glioma tumor suppressor candidate region gene 1) or GLTSCR1L (GLTSCR1-like) as its unique subunit and, therefore, is called either GBAF or the noncanonical BAF complex (8, 9). All of these SWI/SNF complexes include a catalytic ATPase subunit (mutually exclusive SMARCA4/BRG1 or SMARCA2/BRM) and core subunits, including SMARCC and SMARCD proteins (10). Assembly of these complexes occurs in a highly ordered and modular fashion through which the core module acts as an essential platform for recruiting complex-specific subunits and, subsequently, the preassembled ATPase module caps (8).

The SWI/SNF complexes are believed to bind to DNA regions via ARID1A and ARID1B, two mutually exclusive accessory subunits of the complex with nonselective DNA binding activities (11), or via interaction with general or specific transcription factors (12), or both. They utilize the energy of ATP hydrolysis to mobilize nucleosomes to control chromatin accessibility (1–5). Therefore, these complexes are crucial for gene transcription, cell fate control, and lineage specification, all of which are frequently perturbed in cancer (13–15). Although it remains unclear whether and how each class of the SWI/SNF complex functions differently, Pan et al. (16) have



		BAF	PBAF	GBAF
ATPase cap	ATPase	SMARCA4/A2*	SMARCA4/A2*	SMARCA4/A2*
	Others	ACTIN ACTL6A SS18 BCL7A/B/C*	ACTIN ACTL6A PBRM1 BCL7A/B/C*	ACTIN ACTL6A SS18 BCL7A/B/C*
Core subunits		SMARCC1/2* SMARCD1/2/3* SMARCE1 SMARCB1	SMARCC1/2* SMARCD1/2/3* SMARCE1 SMARCB1	SMARCC1/2* SMARCD1/2/3* GLTSCR1/1L*
Accessory subunits		ARID1A/1B* DPF1/2/3*	ARID2 BRD7 PHF10	BRD9

Figure 1

Scheme of three classes of the SWI/SNF complex. For each class, there are distinct SWI/SNF complexes containing unique combinations of mutually exclusive subunits (indicated by asterisks) of several components. Abbreviations: BAF, BRG1-associated factors; GBAF, GLTSCR1/1L-associated BAF; PBAF, polybromo-associated BAF; SWI/SNF, SWItch/Sucrose NonFermentable.

recently demonstrated that both the noncatalytic and the ATPase activities of the ATPase module cap determine the divergent localization of each class of SWI/SNF complex to its genomic loci. Furthermore, Michel et al. (17) mapped SWI/SNF complexes onto chromatin and revealed that each class of the SWI/SNF complex has a differential preference for chromatin localization. The BAF complex displayed strong enrichment in active enhancers, marked by the presence of both H3K27ac and H3K4me1 histone modifications, and primed enhancers, marked by the presence of only the H3K4me1 modification, suggestive of its key roles in enhancer regulation, whereas the PBAF complex had the strongest enrichment in active promoters, marked by the presence of both H3K27ac and H3K4me3 modifications (17). In contrast, a great portion of the GBAF complex binds to CTCF (CCCTC binding factor) sites (17), which are known to play important roles in maintaining DNA architecture (18). These findings highlight the diverse functions of the SWI/SNF complexes.

Given its pivotal role in regulating diverse pathways, it is not surprising that mutations impacting SWI/SNF function occur in a broad spectrum of cancers. Indeed, data from The Cancer Genome Atlas (TCGA) have shown that mutations in SWI/SNF subunits are found in approximately 20% of all cancers (19, 20), a rate that approaches the frequency of *TP53* mutations. At least nine subunits of this complex are recurrently mutated, suggesting a conserved or shared mechanism at the heart of its tumor suppressor activity. Genomic, functional, and genetically engineered mouse model (GEMM) studies all strongly support SWI/SNF subunits as bona fide tumor suppressors (21–24). However, specific subunits are mutated in different malignancies across the body, suggesting there are subunit-, complex-, or context-dependent functions, or

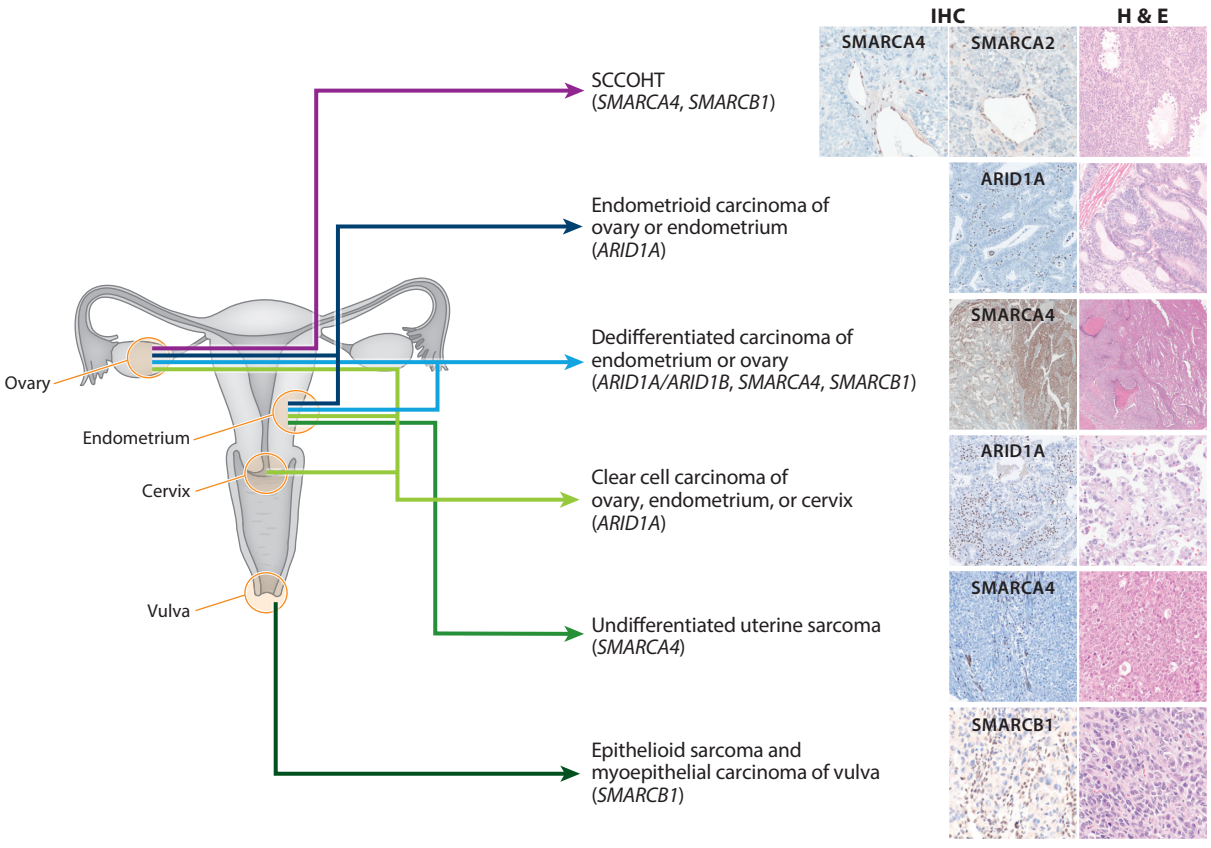


Figure 2

SWI/SNF mutations and protein loss in gynecologic cancers. Specific mutations of the SWI/SNF complex are indicated for each gynecologic cancer described. Representative H & E and IHC staining of relevant SWI/SNF subunits is shown. Abbreviations: H & E, hematoxylin and eosin; IHC, immunohistochemistry; SCCOHT, small cell carcinoma of the ovary, hypercalcemic type; SWI/SNF, SWItch/Sucrose NonFermentable.

some combination of these, of the SWI/SNF complexes. The mechanisms by which inactivating mutations in distinct SWI/SNF subunits promote oncogenic transformation and progression remain largely unaddressed. Investigating the function of specific mutations in relevant contexts with appropriate biological models will help in designing novel strategies for managing a large number of malignancies associated with defects in this complex.

3. MUTATIONS IN THE SWI/SNF COMPLEXES IN GYNECOLOGIC CANCERS

Mutations in SWI/SNF complexes have been reported in multiple types of gynecologic cancers (**Figure 2**). These mutations, which impact different subunits of the complex, arise at different stages of tumor development and thus may play distinct roles in each tumor type. For example, the inactivating mutations of the *SMARCA4* gene, the only recurrent genetic event in SCCOHT, are considered to be the driver event of SCCOHT development. In contrast, the inactivating mutations in *SMARCA4*, *SMARCB1*, *ARID1A*, and *ARID1B* genes are late events in the development of endometrial cancer that may promote disease progression. In this section, we summarize the

current understanding of SWI/SNF mutations in gynecologic cancers. This review is ordered by the point in time in which the SWI/SNF deficiencies operate during disease development and progression: whether as a driver event, an early event, or a progression event.

3.1. Gynecologic Cancers with SWI/SNF Deficiency as a Driver Event

SCCOHT is a rare type of ovarian cancer that primarily affects young women, with a median age of diagnosis in the mid-twenties (25–29). About two-thirds of SCCOHT patients display hypercalcemia, which resolves upon removal of the tumor. Various mechanisms have been proposed that may account for the paraneoplastic hypercalcemia, including elevated levels of parathyroid hormone and parathyroid hormone–related peptide, but none have yet been fully substantiated (30, 31). Patients' outcomes are abysmal, with a 65% recurrence rate and a 2-year survival rate of less than 35% (32–34). While standard treatment for SCCOHT includes surgery and aggressive multiagent chemotherapy, conventional treatment is often ineffective, which highlights the urgent need to develop new tumor-specific treatment strategies.

Histologically, SCCOHT tumors most often grow as sheets of closely packed, small-to-intermediate-sized cells with scant cytoplasm, but with hyperchromatic nuclei and prominent nucleoli. Follicle-like spaces containing eosinophilic fluid are seen in 80% of cases. A minority of cases will have mixed architectural patterns, including nests, cords, and single cells. Glands or cysts lined by mucinous epithelium are seen in 10% of cases, and signet ring cells are seen in 1% of cases. The stroma varies from fibrous to myxoid to edematous. Approximately half of these tumors contain larger cells, some of which have rhabdoid features (e.g., eccentric nucleus, prominent nucleolus, and abundant eosinophilic cytoplasm). Contrary to what its name implies, SCCOHT tumors are actually composed of cells that are mostly intermediate in size. Scully coined the name SCCOHT to highlight the relatively small size of the cells compared with typical undifferentiated carcinoma of the ovary, which is composed of conspicuously large cells (35). In addition, some SCCOHT tumors are composed exclusively of large or rhabdoid cells and, thus, are given the paradoxical name of SCCOHT, large cell variant (29). Historically, diagnosis was challenging due to nonspecific, poorly differentiated histology and a lack of diagnostic immunohistochemistry (IHC) markers (32, 34–36). The majority of SCCOHT tumors are positive for WT1 (Wilms' tumor 1) and CD10, and they are variably positive for cytokeratins, EMA (epithelial membrane antigen), and calretinin. However, none of these markers reliably distinguishes SCCOHT from other ovarian tumors. It was not until the molecular underpinnings of SCCOHT were discovered that specific IHC markers were revealed for diagnostic use.

We and others have discovered that unlike most common ovarian malignancies, the genome of SCCOHT is diploid, with inactivating germline and/or somatic mutations of the *SMARCA4* gene, as the only recurrent feature and the likely driver event in approximately 90% of SCCOHT tumors (37–40). In addition, SCCOHT tumors do not express SMARCA2 (41, 42), the alternative ATPase of the SWI/SNF chromatin remodeling complex, a surprising finding given that SMARCA2 is essential for the survival of most other SMARCA4-deficient cancer cells (43, 44). Through IHC analysis of more than 3,000 primary gynecologic tumors, we further demonstrated that the loss of SMARCA4, either alone or together with SMARCA2, is highly sensitive and specific for the diagnosis of SCCOHT (41), thus providing a definitive tool for SCCOHT diagnosis. Furthermore, claudin-4, an epithelial differentiation marker that distinguishes tumors of epithelial origin from sarcomas exhibiting epithelioid morphology, was not expressed in any of the 10 SCCOHT tumors examined (45), suggesting that SCCOHT does not fit the classification of a bona fide epithelial tumor. SCCOHT tumors share both histologic and genetic similarities with malignant rhabdoid tumors of the kidney, soft tissues, and brain, which are tumors caused by

mutations in *SMARCB1* (46). As such, SCCOHT has been proposed to be essentially a rhabdoid tumor of the ovary (47) and has now been included in rhabdoid tumor predisposition syndrome 2 (MIM numbers: * 603254, # 613325; the asterisk indicates a gene and the number sign indicates a descriptive entry). Although the removal of the term small cell may circumvent confusion with neuroendocrine small cell carcinomas, it is our opinion that changing the name to rhabdoid tumor of the ovary will still lead to confusion rather than clinical clarity unless SCCOHT shares a unique and effective treatment with rhabdoid tumors.

3.2. Gynecologic Cancers with SWI/SNF Deficiency as an Early Event

Mutations in *ARID1A*, which encodes an accessory subunit of the SWI/SNF BAF complex, are prevalent in both endometriosis-associated ovarian cancer and multiple subtypes of endometrial cancer. However, *ARID1A* inactivation alone is not sufficient to drive oncogenic transformation of either the endometrium or ovarian surface epithelium. Instead, additional oncogenic mutations, such as *PIK3CA*-activating mutations, cooperating with *ARID1A* mutations are required to drive cancer development, suggesting that *ARID1A* mutation is an early event, but not a sufficient driver event, during cancer initiation.

3.2.1. *ARID1A* mutations in endometriosis-associated ovarian cancer. Clear cell ovarian carcinoma (CCOC) and endometrioid ovarian carcinoma (ENOC) are strongly associated with endometriosis (48), a common complication affecting 10% of women who have functional endometrial tissue outside the endometrial cavity of the uterus that likely arises from retrograde menstruation (49). ENOC represents approximately 10% of ovarian carcinomas, while CCOC represents 5–12% of ovarian carcinomas, with CCOC having a higher frequency in some Asian countries (50–53). CCOC tends to be diagnosed at a younger age and an earlier stage than the more common high-grade serous ovarian carcinoma, but unlike the majority of other ovarian cancer subtypes, CCOC is inherently resistant to standard platinum and taxane chemotherapy (54). In the absence of an alternative, however, platinum- and taxane-based chemotherapy is still the standard of care for this disease.

Although both CCOC and ENOC arise from ovarian endometriotic cysts, these two cancers are quite distinct. ENOC forms glandular or solid architecture and is composed of columnar cells with eosinophilic cytoplasm, and it is mostly estrogen receptor (ER) positive; while CCOC often has tubulocystic or papillary architecture and is composed of cuboidal cells, usually with clear cytoplasm, and it is predominantly ER negative. Our genomic interrogation of these cancers revealed that although there are no specific mutations found exclusively in either subtype, there are some genomic features that differentiate these cancers, such as the APOBEC (apolipoprotein B mRNA editing enzyme catalytic polypeptide-like) signature found in 26% of CCOCs and the microsatellite instability found in 28% of ENOCs (55). Interestingly, CCOC and its atypical endometriosis precursor have histologic and immunophenotypic similarities to the Arias-Stella reaction (56), a distinct and benign morphologic change to the endometrium induced by high levels of estrogen or progesterone from pregnancy or exogenous hormone use that cytologically resembles a malignancy. It has also been observed that the Arias-Stella reaction and CCOCs have similar gene expression patterns (56).

Research from our group and others has shown that somatic mutations in the *ARID1A* gene commonly occur in both CCOC and ENOC, resulting in complete loss of ARID1A protein expression in approximately 50% of CCOCs and 30% of ENOCs (57, 58). ARID1A protein can also be lost in the endometriotic cysts adjacent to ARID1A-deficient CCOCs (58). Yamamoto and colleagues (59) confirmed that ARID1A protein loss occurred frequently in precancerous

lesions adjacent to ARID1A-deficient CCOCs, including in 86% (12/14) of nonatypical endometrioses, and 100% of atypical endometrioses (14/14), benign (3/3), and borderline (6/6) clear cell adenofibroma components, but ARID1A loss was absent in 22 solitary endometrioses and 10 endometrioses distant from the carcinomas. Another study from the same group further confirmed that ARID1A was also lost in endometriotic cysts adjacent to ARID1A-deficient ENOCs (60). Furthermore, ARID1A loss has also been recently discovered in 4.5% (3/66) of histologically benign ovarian endometriotic cysts (61) and at a low frequency in deep infiltrating endometrioses (62, 63), which have nearly no risk of malignant transformation. Thus, although *ARID1A* inactivation occurs early during transformation, it appears that ARID1A loss by itself is insufficient for malignant transformation. Moreover, ARID1A loss is significantly associated with genetic alterations that activate the PI3K/AKT signaling pathway in CCOC, such as the loss of PTEN (phosphatase and tensin homolog) or gain-of-function mutations of the *PIK3CA* gene (59, 64, 65), suggesting a cooperative role for ARID1A inactivation and PI3K/AKT activation in malignant transformation of premalignant lesions.

3.2.2. *ARID1A* mutations in endometrial cancer. Endometrial cancer is the most common and second most lethal gynecologic cancer. The term refers to endometrioid endometrial carcinoma (EEC), the most common subtype and an estrogen-dependent cancer type, and estrogen-independent subtypes including serous endometrial carcinoma (SEC) and clear cell endometrial carcinoma (CCEC). Mutations of *ARID1A* occur in 30–50% of low-grade EECs and 40–60% of high-grade EECs, and at a much lower frequency in SECs and endometrial carcinosarcomas (66, 67), as highlighted by TCGA and ourselves. We further validated that ARID1A protein is lost in 29% (29/101) of low-grade EECs, 39% (44/113) of high-grade EECs, 18% (17/95) of SECs, and 26% (6/23) of CCECs (68). Similarly, Guan et al. (69) reported a comparable rate of ARID1A loss in 26% (15/58) of low-grade EECs, but this loss was not seen in any of the 12 SECs or 5 carcinosarcomas. The mutation landscape of pure CCECs has been described by DeLair et al. recently (70). Mutation of *ARID1A* was observed in 22% (7/32) of CCECs along with protein loss (70), in agreement with an earlier report by Fadare et al. (71). Thus, *ARID1A* mutations occurs at different frequencies in most common types of endometrial carcinomas, with a significant enrichment in EECs.

EEC is believed to arise from atypical hyperplasia (AH), which is sometimes referred to as endometrial intraepithelial neoplasia (EIN). Mao et al. (72) analyzed ARID1A expression by IHC in 246 cases of benign endometrium, hyperplasia, and endometrioid carcinoma at different stages of progression, of which ARID1A was intact in all 65 benign endometrial tissues, but was lost clonally in 16% (6/38) of EIN (AH) and either completely lost or clonally lost in, respectively, 25% (22/88) and 24% (21/88) of low-grade EECs and completely or clonally lost in, respectively, 44% (24/55) and 9% (5/55) of high-grade EECs. This was supported by a study from Werner et al. (73) that reported a similar frequency of ARID1A loss in EIN (AH) but not in benign hyperplasia, suggesting that ARID1A loss is likely an important driver event for the transition of EIN (AH) to carcinoma. Accordingly, Yen et al. (74) revealed that the detection of complete or heterogeneous ARID1A loss from tissue samples from biopsy or curettage by IHC predicted EEC in subsequent hysterectomy in 94% (15/16) of patients, but only 15% of patients with retained ARID1A developed EEC, thus supporting a predictive value for ARID1A loss in the diagnosis of sporadic cases of EEC. Furthermore, Mao et al. (72) uncovered a significant increase in the complete loss of ARID1A in the high-grade component of 19 cases of high-grade EECs that contained concurrent low-grade carcinomas, with 26% (5/19) of them showing progressive loss of ARID1A from retention or clonal loss in low-grade components and complete loss in high-grade areas, thus indicating that ARID1A loss may also play an important role in the progression of EECs, which requires further validation.

Patients with Lynch syndrome, one of the most prevalent hereditary cancer predisposition syndromes arising from a germline mutation in one of the four mismatch repair (MMR) genes (i.e., *MLH1*, *MSH2*, *MSH6*, or *PMS2*), have a 40–60% lifetime risk of developing ECs (75, 76). The value of ARID1A loss in predicting malignant transformation in patients with Lynch syndrome has also been studied. Using IHC, Niskakoski et al. (77) discovered that ARID1A loss occurs in approximately 60% (14/23) of patients with Lynch syndrome EECs in Finland and appears in approximately 20% of the EINs (AHs) preceding the development of EECs, suggesting that ARID1A loss may predict the risk of EC development in these patients. However, Bosse et al. (78) reported that ARID1A loss was seen in only 14% (5/36) of patients in the Netherlands with Lynch syndrome EECs. This discrepancy may be attributed to the size and origin of different Lynch syndrome kindreds that may carry distinct mutations of MMR genes. For example, 79% (52/66) of the Finland cohort carriers harbored the *MLH1* germline mutation (77), whereas the Netherlands cohort carriers had germline mutations in any of the four MMR genes, with the frequency being unknown (78). Therefore, additional studies are required to validate the significance of ARID1A loss in predicting the likelihood of malignant transformation of the gynecologic tract in patients with Lynch syndrome with distinct germline mutations.

3.3. Gynecologic Cancers with SWI/SNF Deficiency as a Progression Event

Dedifferentiated endometrial carcinoma (DDEC) occurs when an undifferentiated carcinoma arises abruptly within a clonally related low-grade EEC, which is often a microsatellite instable (MSI) tumor, as seen both in Lynch syndrome and in sporadic cancer (79–81). The undifferentiated component shows sheet-like proliferations of monomorphic round-to-polygon-shaped cells that lack cellular cohesion and evidence of epithelial architecture (i.e., an absence of glands, nests, or trabeculae). Rhabdoid-like cells are seen in 20% of cases (82). In about 40% of cases, the low-grade component is eclipsed, likely due to an outgrowth of the undifferentiated component, and the overall appearance of the tumor is that of a pure undifferentiated endometrial carcinoma (79, 83). The endometrioid components usually express ERs, progesterone receptors, and PAX8 (paired box 8), whereas the undifferentiated components are negative for ERs and PAX8 and express stem cell markers, such as SOX2 (SRY-box transcription factor 2). DDEC and undifferentiated endometrial carcinoma (hereafter referred to only as DDEC) occur in women, with a peak of age of diagnosis at 55 years. DDEC is clinically aggressive, and most patients quickly succumb to their disease, even when the undifferentiated component is only a minor fraction of the tumor (79, 81). Its clinical behavior is worse than high-grade EEC, the entity with which DDEC is most often confused. Although DDEC is relatively rare, its actual incidence is largely undetermined due to its late recognition, as it was described by Silva and colleagues (79) at the University of Texas M.D. Anderson Cancer Center only in 2006. By reviewing 633 cases of endometrial adenocarcinoma accessioned in the department of pathology at the cancer center during 2003 and 2004, Silva et al. (79) found that DDEC accounted for approximately 9% of cases (83), suggesting that the incidence of DDEC may be underestimated due to misdiagnosis or underrecognition.

The genomic landscape of DDEC remains largely undetermined. Espinosa et al. (84) recently analyzed the immunophenotypic features and mutational status of a small panel of genes in 21 cases of DDEC and revealed that DDECs with *POLE* exonuclease domain mutations were more often stage I disease [78% (7/9) versus 25% (3/12); $p = 0.023$] with favorable disease-specific survival compared with DDECs without *POLE* mutations. This finding has yet to be validated in larger series of DDECs. We and others have discovered three types of inactivating mutations occurring in subunits of the SWI/SNF complex in DDECs (85–88). These include inactivating mutations in *SMARCA4* resulting in the loss of SMARCA4 protein in 30–40% of DDECs, inactivation of

SMARCB1 resulting in the loss of SMARCB1 protein in 2–7% of DDECs, or co-inactivation of *ARID1A* and *ARID1B* resulting in concurrent loss of ARID1A and ARID1B proteins in 28% of DDECs (82, 85, 86, 88, 89). Loss of SMARCA4 or SMARCB1 is restricted to the undifferentiated component of DDECs. In such instances, about half of the cases also exhibit a loss of ARID1A expression in both components (86, 89). While the loss of ARID1B is also restricted to the undifferentiated component of DDECs, it occurs concordantly with ARID1A loss, which usually occurs in both low-grade and undifferentiated components (86, 89). Furthermore, mutational loss of *SMARCA4*, *SMARCB1*, or *ARID1A* or *ARID1B* is often associated with the loss of expression of SMARCA2 protein, which is the alternative ATPase of the SWI/SNF complex, through non-genetic mechanisms (82, 86, 89). Such SWI/SNF mutations are also implicated in undifferentiated carcinomas of the ovary (86), urinary tract (90), gastrointestinal tract (91), and kidney (92), suggesting that the SWI/SNF deficiency acquired during tumor progression may be a widespread mechanism that promotes cancer dedifferentiation. Furthermore, no apparent histologic differences have been observed between ARID1A and ARID1B co-inactivated tumors and SMARCA4- or SMARCB1-inactivated tumors, as the inactivation of these SWI/SNF proteins coincided with an undifferentiated histology (86). However, ARID1A–ARID1B dual-deficient DDEC has intact PBAF and GBAF complexes, and SMARCB1-deficient DDEC has a functional GBAF complex, whereas SMARCA4-deficient DDEC likely loses the ATPase activity of all SWI/SNF complexes completely. Thus, it remains possible that DDEC driven by these distinct modes of mutations in the SWI/SNF complexes may result in abrogation of cell lineage determination differentially. Future digital histology analysis using advanced technologies, such as artificial intelligence, may help identify subtle difference among these cancers. Last, it has been shown that there is a greater propensity for MMR protein–deficient endometrioid carcinomas to give rise to either ARID1A–ARID1B co-deficient or SMARCA4- or SMARCB1-deficient DDECs, with nearly 75% of them being MMR protein deficient (86, 89). Given the reported reliance of ARID1A-deficient cells on the presence of ARID1B (93), we speculate that an MSI tumor background may facilitate the acquisition of the additional molecular aberrations required for the successful emergence of an ARID1A–ARID1B dual-deficient subclone.

The phenomenon of dedifferentiation has yet to be described in the context of CCEC or CCOC. Further studies are needed to determine why this phenomenon either does not occur or, alternatively, is lethal to CCEC and CCOC cells.

3.4. Gynecologic Cancers with SWI/SNF Deficiency Not Mapped to Disease Progression

In addition to playing crucial roles in the development of the multiple types of gynecologic cancers described above, SWI/SNF mutations have also been discovered in several gynecologic cancers in which their significance is unclear.

3.4.1. *SMARCA4* mutations in undifferentiated uterine sarcoma. Undifferentiated uterine sarcoma is a diagnosis of exclusion, and limited molecular genetic data are available. Recently, Kolin et al. (94) discovered *SMARCA4* mutations along with SMARCA4 protein loss in five cases of undifferentiated uterine sarcoma, who had a median age at diagnosis of 33 years, which is close to that of SCCOHT. These tumors were composed of sheets of large, atypical epithelioid cells with prominent rhabdoid morphology, indistinguishable from the large cell variant of SCCOHT. They were uniformly aggressive, and all patients died of their disease, having a median survival of 7 months (range 1–43 months). Given that these tumors share a similar age of diagnosis and histologic and molecular features with SCCOHT, Kolin et al. (94) suggested giving these tumors a new

identity: SMARCA4-deficient undifferentiated uterine sarcoma or malignant rhabdoid tumor of the uterus. Aside from the age at diagnosis, SMARCA4-deficient uterine stromal tumors, which occur in younger women, are challenging to distinguish from SMARCA4-deficient DDECs with no identifiable low-grade components, which occur in older women. The SMARCA4-deficient undifferentiated uterine sarcomas did not express claudin-4, which is noted to be a marker of epithelial differentiation that distinguishes tumors of carcinoma origin from sarcomas in SWI/SNF-deficient malignancies (45). Kolin et al. suggest that claudin-4 expression can be a useful feature for differential diagnosis. However, a recent study by Tessier-Cloutier et al. (95) discovered that the undifferentiated components of DDECs always lost the expression of claudin-4, indicating that claudin-4 cannot be used to infer mesenchymal or epithelial tumor origin in the endometrium. Furthermore, all three SMARCA4-deficient undifferentiated uterine sarcomas with known MMR protein status were microsatellite stable (MSS) tumors (94), whereas MSI tumors comprise the majority of SMARCA4-deficient DDECs (89). Whether the SMARCA4-deficient uterine stromal sarcoma has a stable genome requires further study. Therefore, a large number of cases are required to fully characterize the clinicopathologic, immunophenotypic, and genomic features of this entity and understand the role of SMARCA4 loss in the development of SMARCA4-deficient uterine stromal sarcomas.

3.4.2. *SMARCB1* mutations in epithelioid sarcoma and myoepithelioma-like tumors of the vulvar region. Epithelioid sarcoma and myoepithelial carcinoma are rare malignancies of the vulvar region. Recently, Folpe et al. (96) studied the clinicopathologic, IHC, and molecular genetic features of 14 SMARCB1-deficient vulvar neoplasms and discovered that the proximal-type epithelioid sarcoma was the predominant subtype of SMARCB1 deficiency, followed by myoepithelial carcinoma. The mean age at diagnosis was 46 years, with a range from 22 to 62 years. Follow-up data on 13 patients (at 4–72 months; mean, 31 months) indicated that 3 patients had died of their disease, 1 was alive with unresectable metastatic disease, 1 was alive with radiographic evidence of extensive lymph node disease, and 8 were alive without disease (96). SMARCB1 deficiency in myoepithelial carcinoma was further supported by a study from Yoshida et al. (97), in which such tumors, referred to as myoepithelioma-like tumors of the vulvar region, were diagnosed at a median age of 41 years (range, 24–65 years). The cells of these tumors of the vulvar region were well circumscribed, focally encapsulated, and lobulated, and follow-up data demonstrated that all nine patients were alive without metastases at a mean follow-up of 66 months (97). Despite this, future study of a large number of cases is required to fully characterize the clinicopathologic, immunophenotypic, and genomic features of these two entities and address whether *SMARCB1* inactivation is the only recurrent driver event, as seen in epithelioid sarcomas at other anatomic sites.

4. PROGNOSTIC AND CLINICOPATHOLOGIC SIGNIFICANCE OF SWI/SNF MUTATIONS IN GYNECOLOGIC CANCERS

4.1. Does ARID1A Loss Have Prognostic Value in Endometrial Carcinoma or Endometriosis-Associated Ovarian Cancer?

As ARID1A inactivation appears in a portion of endometriosis-associated ovarian cancer and endometrial cancer, it has been of great interest to investigate the prognostic value of ARID1A loss. We initially analyzed ARID1A expression in 132 CCOCs and 125 ENOCs by IHC and did not identify any correlation between ARID1A loss and a patient's age at diagnosis, disease stage, and disease-specific survival in either cancer type (58). Accordingly, Maeda (98), Yamamoto (99),

and Lowery (100) and their colleagues independently analyzed the expression of ARID1A by IHC in, respectively, 149 CCOCs, 90 CCOCs, and 82 CCOCs and 130 ENOCs; they did not identify significant differences between ARID1A expression status and histopathologic features, such as age at diagnosis, clinical stage, tumor grade, or overall survival. Supporting these findings, analyses of transcriptomic profiles from the *ARID1A* wild-type and mutant CCOCs failed to identify a canonical signaling process that was distinct between wild-type and *ARID1A* mutant tumors (101). In contrast, Katagiri et al. (102) found that loss of ARID1A was significantly correlated with advanced FIGO (International Federation of Gynecology and Obstetrics) stage and high CA125 levels and a shorter progression-free survival in 60 patients with CCOC who received platinum-based chemotherapy. However, only 15% (9/60) of CCOCs in the Katagiri study were deficient in ARID1A expression, raising the possibility that some cases in that cohort were misdiagnosed. Interestingly, in a cohort of CCOCs that included 39% (44/112) of the cases being negative for ARID1A by IHC, Itamochi et al. (103) discovered that although ARID1A loss was not correlated with a patient's age at diagnosis, FIGO stage, or status of the residual tumor, the 5-year overall survival rate was significantly lower in stage I or II CCOCs with intact ARID1A than in those without ARID1A expression [74% (15/20) versus 92% (23/25)]. This observation indicates that the loss of ARID1A protein expression may have some prognostic value among patients with early-stage CCOC. Liu et al. (104) recently performed a meta-analysis of more than 600 CCOCs from seven studies that mostly included patients from Japan and China, and they concluded that ARID1A loss correlated with a shorter progression-free survival (hazard ratio, 1.97; 95% confidence interval, 1.37 to 2.83; $p = 0.000$) but not with overall survival. To validate these findings in independent cohorts, most recently our group studied 1,024 ENOCs from the Ovarian Tumor Tissue Analysis consortium and 595 CCOCs from the Canadian Ovarian Experimental Unified Resource. In this study, we found no evidence that ARID1A expression status is prognostically significant in either CCOC or ENOC (105).

The prognostic value of ARID1A loss in endometrial cancer has also been analyzed by several groups. Werner et al. (73) analyzed a collection of 535 primary endometrial cancers as well as 77 metastatic endometrial cancers and documented a significant correlation between ARID1A loss and younger patient age and deeper myometrial infiltration, but not survival. Zhang et al. (106) failed to detect any association between ARID1A loss and clinical stage, the depth of myometrial invasion, presence of lymph node metastasis, or overall survival in patients with endometrial carcinoma. In contrast, Heckl et al. (107) determined the expression of ARID1A in 59 EECs by immunohistochemistry and reported a significant association between ARID1A loss and worse 5-year survival. However, this EEC cohort had an unusually high rate of ARID1A loss [90% (53/59)] (107), calling into question whether such an observation was a consequence of an unusual collection of patients with EEC. Interestingly, using data from the molecularly defined endometrial carcinoma cohort from TCGA, Shen et al. (108) demonstrated that *ARID1A* mutations correlated with a better outcome in endometrial carcinoma. Because patients with MSI endometrial cancer had a better prognosis than patients with MSS endometrial cancer, Shen et al. (108) further investigated whether such correlation reflects the enrichment of *ARID1A* mutations in MSI tumors. It was revealed that *ARID1A* mutation status was not significantly associated with survival in patients with MSI tumors, but it was associated with a better prognosis in patients with MSS tumors, implying that *ARID1A* mutation status may predict patients' outcomes in MSS endometrial cancer (108). Furthermore, Fadare et al. (71) investigated 22 CCECs and found that ARID1A loss occurred only in stage III and IV and was associated with a worse outcome. However, the sample volume in the Fadare study was small, highlighting a need for further investigation.

4.2. Prognostic Values of SWI/SNF Deficiency in Dedifferentiated Endometrial Carcinoma

Although patients with DDEC had a worse outcome than those with grade 3 EEC, our initial comparison between 15 cases of DDEC with intact SMARCA4 and SMARCB1 and 15 DDECs with a loss of SMARCA4 or loss of SMARCB1 did not identify significant differences in disease-specific survival (89). This may be due to the presence of ARID1A–ARID1B dual-deficient cases in the DDECs with intact SMARCA4 and SMARCB1. Subsequently, we found that DDEC patients with any of the three types of SWI/SNF deficiency had worse outcomes than those with intact SMARCA4, SMARCB1, and ARID1B (86). DDECs with any of the three modes of SWI/SNF deficiency exhibited similarly aggressive clinical behavior, with more than 50% of the patients in both groups dying of their disease within 2 years of initial diagnosis (86). In another study that examined only DDEC with no identifiable low-grade components, Kobel et al. (87) confirmed that patients with any of the three types of SWI/SNF deficiency had much worse 1-year disease-specific survival [26% (4/15)] than did those with intact SMARCA4, SMARCB1, and ARID1B [75% (12/16)]. Therefore, clinically, SWI/SNF complex deficiency defines a highly aggressive subset of DDEC.

5. UNDERSTANDING THE PATHOGENIC ROLES OF SWI/SNF MUTATIONS IN GYNECOLOGIC CANCER USING IN VITRO MODELS

5.1. Role of SMARCA4 Loss in Small Cell Carcinoma of the Ovary, Hypercalcemic Type

Despite the rarity of SCCOHT, there are three cell line models available for studying its molecular pathogenesis. BIN-67 cells were derived from a metastatic pelvic nodule of a primary SCCOHT (109). SCCOHT-1 cells were established from a recurrent chemotherapy-naive SCCOHT (110). COV434 was established from a primary tumor that was initially diagnosed as a granulosa cell tumor (111) and later reclassified as SCCOHT due to the absence of SMARCA4 and SMARCA2 and through reexamination of the original tumor material (A.N. Karnezis, Y. Wang, S.Y. Chen, W.P.D. Hendricks, P. Ramos, et al., manuscript in preparation). Reexpression of either SMARCA4 or SMARCA2 dramatically suppressed the growth of all three cell lines (41, 112), supporting the tumor-suppressive role of SWI/SNF complexes in the development of SCCOHT.

In order to understand the oncogenic pathways that sustain the proliferation of SMARCA4-deficient SCCOHT cells, we and others have performed genetic screens and identified key oncogenic features of SCCOHT. In agreement with the notion that SWI/SNF complexes function antagonistically to PRC2 (Polycomb repressive complex 2) (113, 114), the master regulator depositing the repressive histone marker H3K27me3, we discovered that SMARCA4 loss in SCCOHT leads to increased expression of EZH2 (enhancer of zeste homolog 2), the catalytic subunit of PRC2 (115), resulting in a dependency of SCCOHT cells on the enzymatic activity of PRC2 and its companion histone deacetylases for maintaining their survival (112, 115, 116). Lang et al. (117) performed a screen in BIN-67 cells using a druggable genome small interfering RNA library and uncovered receptor tyrosine kinase (RTK) signaling as the top supportive oncogenic pathway in SCCOHT, including fibroblast growth factor receptors, platelet-derived growth factor receptors, and epidermal growth factor receptors alongside the AKT (protein kinase B) and MAPK (mitogen-activated protein kinase) signaling cascades downstream of these RTKs. These findings suggest that SMARCA4 loss mediates malignant transformation of precursor cells in part through PRC2-dependent epigenetic rewiring and activation of RTKs. Furthermore, although the cell origin of SCCOHT remains to be determined, extensive sectioning of two SMARCA4-deficient SCCOHT primary tumors identified minor foci of immature teratoma or

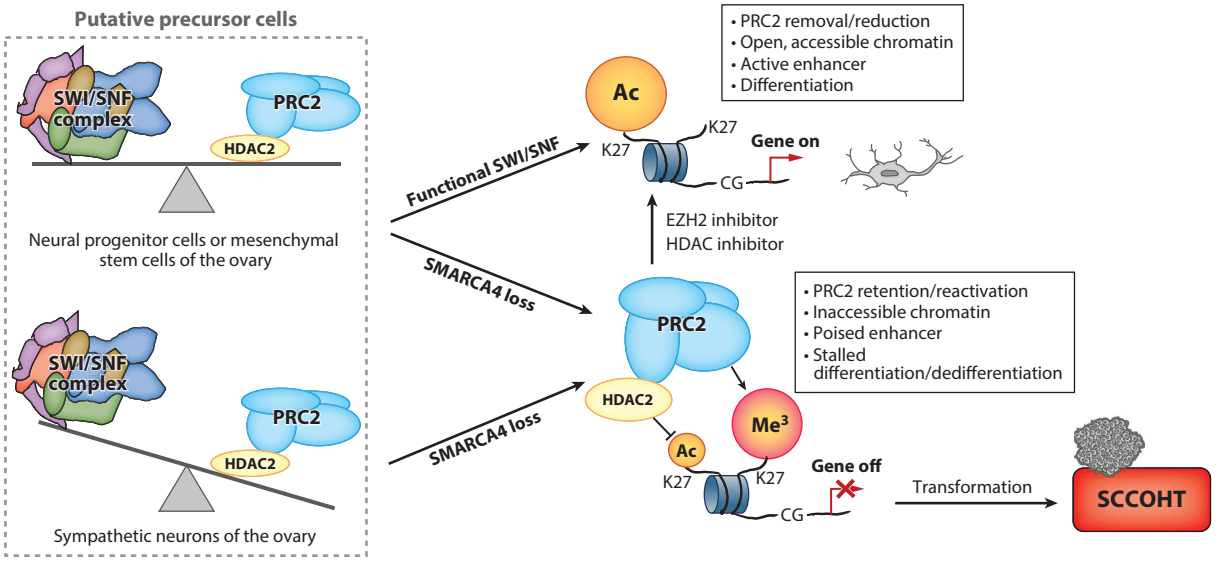


Figure 3

A hypothetical model of the development of SCCOHT. SCCOHT may arise from progenitor cells of the ovary that hold the potential to undergo differentiation into neuronal lineages, in which SMARCA4 inactivation causes hyperactivation of PRC2, reduces chromosomal accessibility of the promoters and/or enhancers of genes that promote differentiation, and drives the development of poorly differentiated cancer. Alternatively, SMARCA4 loss in sympathetic neurons of the ovary may alter the chromosomal structure, unleash the suppression of genes required for maintaining cell stemness (e.g., *EZH2*), and thereby drive cellular dedifferentiation and malignant transformation. Abbreviations: Ac, acetylation; CG, cytosine–guanine sites; EZH2, enhancer of zeste homolog 2; HDAC, histone deacetylase; K27, histone H3 lysine residue 27; Me^3, histone H3 trimethylation; PRC2, Polycomb repressive complex 2; SWI/SNF, SWItch/Sucrose NonFermentable; SCCOHT, small cell carcinoma of the ovary, hypercalcemic type.

both immature teratoma and yolk sac tumor that displayed the reduced protein expression of SMARCA4 in comparison to stromal cells (38). Restoring the expression of SMARCA4 triggered neuronal-like differentiation of SCCOHT cell lines (41, 112). Therefore, it is plausible that some SCCOHTs may arise from the neuroepithelium of immature teratomas or from adult mesenchymal progenitor cells of the ovary, which hold the potential to differentiate into multiple lineages, including neurons (118, 119), where complete inactivation of SMARCA4 may pause the differentiation of these putative precursors and drive their malignant transformation (**Figure 3**). Alternatively, as terminally differentiated neurons can undergo dedifferentiation upon activation of oncogenes (120), it is possible that SMARCA4 loss may unleash the suppression of genes essential for maintaining cell stemness (e.g., *EZH2*), thereby initiating the dedifferentiation and malignant transformation of sympathetic neurons in the ovary. However, because SMARCA4 is required to maintain adult neural stem and progenitor cells and is only rarely mutated in brain cancers, such as atypical teratoid rhabdoid tumors, the unique microenvironment of the ovary may be crucial for sustaining the viability of SMARCA4-deficient mesenchymal progenitor cells, neuronal cells, or neural progenitor cells and fostering their transformation.

5.2. Role of ARID1A Loss in Endometriosis-Associated Ovarian Cancer and Endometrial Cancer

In order to understand the role of ARID1A loss in the development of gynecologic cancers, Guan et al. (121, 122) reexpressed ARID1A in an OVISE cell line, which is an ARID1A-null

CCOC cell line, and demonstrated that restoring the expression of wild-type *ARID1A*, but not mutant *ARID1A*, with naturally occurring in-frame insertions or deletions suppressed cell proliferation, partly through upregulation of the expression of p21, and inhibited the growth of OVISE xenografted tumors in mice. Another study from the same group further revealed that ARID1A loss reversed the suppression of hTERT (human telomerase reverse transcriptase) in endometrial epithelial cells and conferred a survival advantage on tumor cells by maintaining their telomeres (123). Furthermore, Bitler et al. (124) discovered that reexpression of ARID1A in OVISE cells reactivated the expression of PIK3IP1, a known negative regulator of the PI3K/AKT pathway, by recruiting the SWI/SNF complex. Although it was shown that the induction of PIK3IP1 was crucial for apoptosis of OVISE cells induced by GSK126 (124), an EZH2 inhibitor, it remains undetermined whether suppression of PIK3IP1 is required for the proliferation of ARID1A-deficient CCOC cells.

Utilizing a drug screen combined with target identification in a panel of CCOC cell lines with intact or no ARID1A expression, Miller et al. (101) discovered that the loss of ARID1A expression in CCOCs created a dependency on YES1, an SRC tyrosine kinase that is involved in cell proliferation, migration, and invasion. Lakshminarasimhan et al. (125) knocked down ARID1A expression in an immortalized endometriosis cell line and identified altered expression of genes enriched in the integrin signaling and the paxillin pathways. Furthermore, Goldman et al. (126) performed proteome analyses of an isogenic CCOC cell pair (OVCA429) with or without ARID1A expression and revealed that *ARID1A* depletion impacted only 5% of the detected proteome, and there was a significant enrichment in proteins of the mevalonate pathway, an important metabolic pathway involved in isoprenoid synthesis, cholesterol synthesis, and other downstream pathways. Mechanistically, it has been demonstrated that downregulation of ARID1A in an immortalized endometriosis cell line did not markedly alter global chromatin accessibility or DNA methylation, but it increased the active H3K27ac mark in promoter regions and decreased H3K27ac at potential enhancers, suggesting a role for ARID1A loss in impacting the distribution of H3K27ac histone marks (125). In agreement with this observation, depletion of *ARID1A* played a dominant role in limiting chromatin accessibility at enhancers in an HCT116 colon cancer cell line (127). Therefore, although the significance of these findings warrants further investigation, these studies suggest that ARID1A loss may reactivate hTERT activity, unleash cell cycle control, and alter cell metabolism and cell-to-cell communication in precursor cells by reducing chromatin accessibility in enhancer regions to allow malignant transformation.

Furthermore, to address whether ARID1A loss is sufficient for the transformation of precursor cells, Guan et al. (121) depleted ARID1A in nontransformed human ovarian surface epithelial cells using short hairpin RNAs and observed increased cellular proliferation and tumorigenicity, suggesting that ARID1A loss alone is sufficient for the transformation of human ovarian surface epithelial cells. However, a subsequent study from the same group demonstrated that Arid1a loss alone was insufficient for the transformation of mouse ovarian surface epithelium (128). Accordingly, simultaneous *ARID1A* deletion and *PIK3CA* activation drove the transformation of human ovarian epithelial surface cells, while concordant deletion of both *Arid1a* and *Pten* is also required for the transformation of mouse ovarian epithelial surface cells (128–130). Nevertheless, as both CCOC and ENOC are believed to have an endometrial cell origin, these studies did not address whether ARID1A loss is sufficient for the transformation of endometrial cells. Recently, Lakshminarasimhan et al. (125) knocked down ARID1A expression in an immortalized endometriosis cell line and observed signs of neoplastic transformation, such as higher efficiency of anchorage-independent growth, increased adhesion to collagen, and increased invasion into basement membrane extract, leading the authors to conclude that ARID1A loss alone is sufficient for the transformation of endometrial cells in endometriosis. However, the genomic background

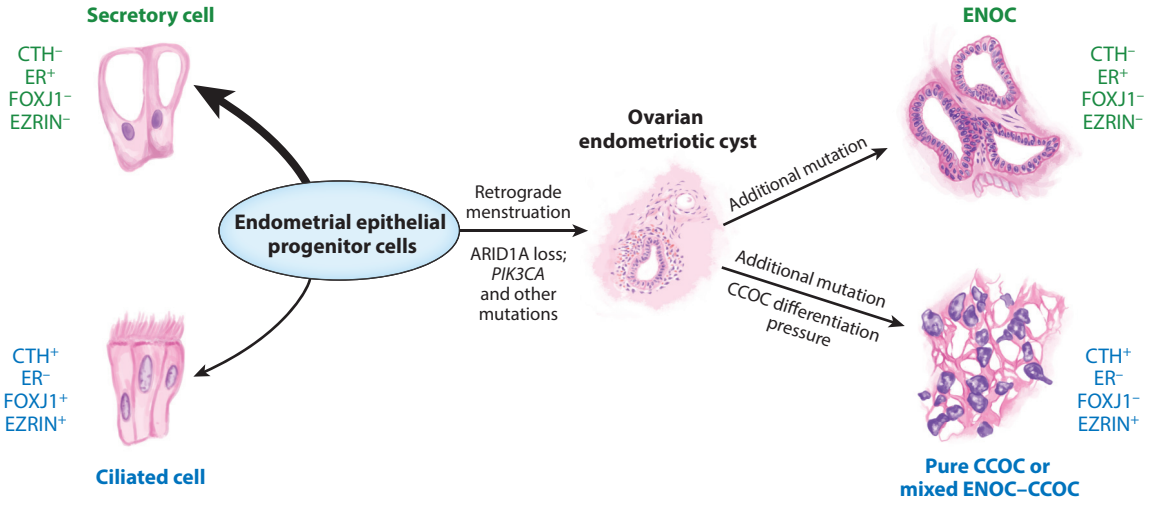


Figure 4

A hypothetical model of the development of ENOC and CCOC. Endometrial epithelial progenitor cells can differentiate into ciliated cells or secretory cells in normal endometrium or typical endometriosis. The local ovarian microenvironment of each patient, which holds distinct differentiation pressure, may determine the differentiation of ARID1A-deficient bipotent premalignant progenitor cells of endometriotic cysts and, together with accumulated additional mutations, drive malignant transformation toward either ENOC or CCOC. Such CCOC development pressure may include exposure to excessive hormones. The thicker arrow indicates a preferential differentiation pathway. Abbreviations: CCOC, clear cell ovarian carcinoma; CTH, cystathionine γ-lyase; ENOC, endometrioid ovarian carcinoma; ER, estrogen receptor; FOXJ1, forkhead box J1.

of this immortalized endometriosis cell line was not determined. Given that *PIK3CA* mutations and PTEN loss occur in both benign endometriotic tissues and endometriotic lesions with a low risk of cancer development (62, 131), it is plausible that such mutations may be present in this endometriosis cell line.

As no specific mutations have been found exclusively in either ENOC or CCOC, it remains unclear how a common premalignant lesion develops into two histologically distinct entities. Through proteomic analysis of epithelial carcinoma followed by IHC validation, we have recently discovered that ENOC shares many immunophenotypic features with secretory cells of normal endometrial epithelium, such as ERs, whereas CCOC expresses some markers of ciliated cells of the endometrial epithelium, such as cystathionine γ-lyase and EZRIN (132, 133). Since within the normal uterine environment endometrial epithelial progenitor cells undergo terminal differentiation with a preference toward secretory cells, it is speculated that the local ovarian microenvironment of each individual patient, which holds distinct differentiation pressure, may determine the differentiation of ARID1A-deficient bipotent premalignant progenitor cells in endometriotic cysts and together with accumulated additional mutations or epigenetic changes, drive their malignant transformation toward either ENOC or CCOC (**Figure 4**). Thus, it will be crucial to identify the factors that alter the differentiation of bipotent endometrial progenitor cells. It will also be interesting to determine whether the introduction of simultaneous mutations in *ARID1A* and *PIK3CA* will drive the malignant transformation of endometrial progenitor cells that have committed to differentiation into either secretory or ciliated cells.

5.3. Role of SWI/SNF Mutations in Dedifferentiated Endometrial Carcinoma

Although a worse outcome has been associated with SWI/SNF defects in DDEC, which establishes an unmet need for an expanded understanding of DDEC biology and treatment paradigms,

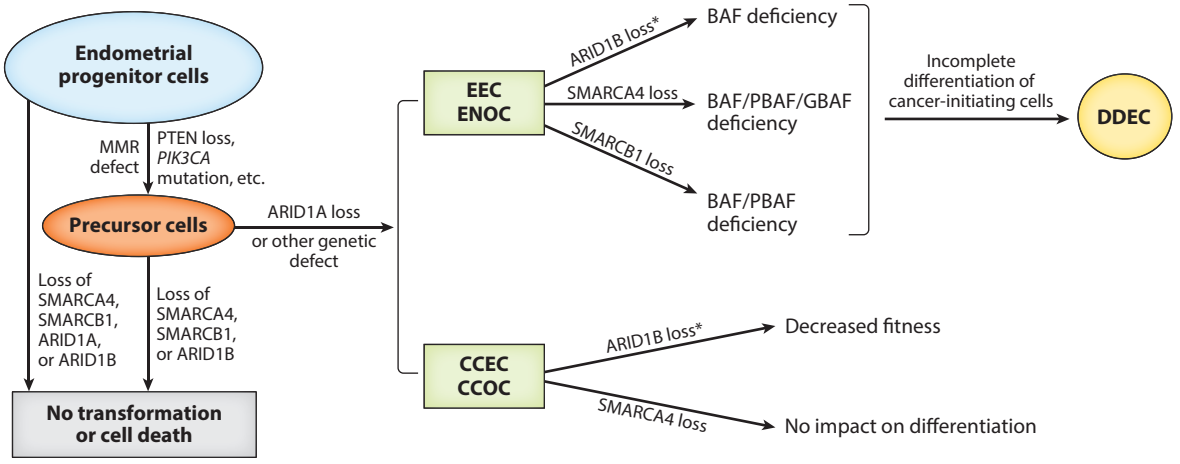


Figure 5

A hypothetical model of the development of dedifferentiated carcinoma of the endometrium and ovary. The accumulation of genetic alterations, including *ARID1A*-inactivating mutations, in endometrial epithelial progenitor cells drives their malignant transformation and the development of either endometrioid or clear cell carcinoma. In endometrioid carcinoma, but not in clear cell carcinoma, the subsequent loss of additional SWI/SNF components, such as ARID1B (in the presence of ARID1A loss), SMARCA4, or SMARCB1, leads to the compound inactivation of SWI/SNF complexes and the development of dedifferentiated carcinoma, possibly through stalling the differentiation of cancer-initiating cells. The asterisk indicates ARID1B loss in the presence of ARID1A loss. Abbreviations: BAF, BRG1-associated factors; CCEC, clear cell endometrial carcinoma; CCOC, clear cell ovarian carcinoma; DDEC, dedifferentiated endometrial carcinoma; EEC, endometrioid endometrial carcinoma: ENOC, endometrioid ovarian carcinoma; GBAF, GLTSCR1/1L-associated BAF; MMR, mismatch repair; PBAF, polybromo-associated BAF; PTEN, phosphatase and tensin homolog.

neither a cell line nor a genetic model of DDEC has been reported. Recently, we and others have demonstrated that TOV112D and OVK18, two ENOC cell lines, are likely DDEC cell lines derived from dedifferentiated carcinomas of the ovary based on their lack of expression of both SMARCA4 and SMARCA2 and histologic reevaluation of the original tumor materials (116; A.N. Karnezis, Y. Wang, S.Y. Chen, W.P.D. Hendricks, P. Ramos, et al., manuscript in in preparation). We and others have proven that both OVK18 and TOV112D cells behaved similarly to SCCOHT cells in their responses to reexpression of SMARCA4 or to EZH2 inhibitor treatment (112, 116), suggesting that SMARCA4–SMARCA2 dual-deficient DDEC cells may largely resemble SCCOHT cells. Because ARID1A–ARID1B dual-deficient DDEC has intact PBAF and GBAF complexes, and SMARCB1-deficient DDEC has a functional GBAF complex, whereas SMARCA4-deficient DDEC loses ATPase activity in all SWI/SNF complexes completely (**Figure 5**), it is plausible that inactivation of the BAF complexes is sufficient to stall the differentiation of cancer-initiating cells and drive histologic dedifferentiation. Future development of DDEC cell lines and in vivo models, particularly the ARID1A–ARID1B dual-deficient models, will be essential for understanding the molecular pathogenesis of the disease.

6. GENETICALLY ENGINEERED MOUSE MODELS OF SWI/SNF MUTATIONS IN GYNECOLOGIC CANCERS

GEMMs offer the opportunity to investigate the contribution of genetic mutations to cancer etiology and to test novel therapeutics in immunocompetent organisms. Therefore, there has been

great interest in developing SWI/SNF GEMMs of various human cancer types. Efforts from several teams have attempted to determine whether inactivation of *ARID1A* is sufficient to drive the development of ovarian cancer and whether coexisting mutations are required to synergize with ARID1A loss to promote tumorigenesis. Guan et al. (128) reported that adenoviral-mediated co-depletion of *Pten* and *Arid1a* in the ovarian surface epithelium of $Pten^{fl/fl}$;$Arid1a^{fl/fl}$ mice induces ovarian hyperplasia as early as 2 months after genetic depletion, which drives the development of undifferentiated or endometrioid carcinoma of the ovary in 46% (6/13) of mice examined at 6 months and 78% (7/9) of mice at 8–9 months. Notably, inactivation of one or both alleles of *Arid1a* in ovarian surface epithelium prolonged the survival of tumor-bearing *Apc/Pten*-deficient mice and promoted cancer cell differentiation to more closely resemble the histology of human ENOC (134). Chandler et al. (11) found that inactivation of *Arid1a* and expression of the *Pik3ca* H1047R–activating mutation in ovarian surface epithelium of $Arid1a^{fl/fl}$;$(Gt)Rosa26Pik3ca^{*H1047R}$ mice led to the rapid development of primary ovarian tumors, with a 7.5 week latency and CCOC-like histopathology. In contrast, mice with a deletion of *Pten* or *Arid1a* or activation of $Pik3ca^{H1047R}$ alone did not develop ovarian lesions except that activation of $Pik3ca^{H1047R}$ led to the development of hyperplasia in ovarian surface epithelium. These studies support the idea that ARID1A loss plays a critical role in driving the development of ENOC and CCOC. However, these models do not faithfully recapitulate the pathogenesis of ARID1A-deficient ovarian cancers in human patients.

Both ENOC and CCOC are highly associated with endometriosis (48). Recent genomic studies have demonstrated clonal relationships between these ovarian tumor types and the adjacent endometriosis (135, 136). In agreement with the robust protective effect of bilateral tubal ligation against endometriosis-associated cancer, these observations suggest that both ENOC and CCOC likely arise from endometrial cell lineages during endometriosis. Therefore, faithful GEMMs should be developed to reflect the pathogenesis of these diseases. Because *ARID1A* is also frequently mutated in endometrial carcinomas, inactivation of *Arid1a*, alone or in combination with genetic mutations of other genes (such as *Pten* or *Pik3ca*), in specific mouse Cre strains in which Cre expression is driven by endometrial tissue–specific promoters (e.g., *Pax8-Cre*, *Ksp-Cre*) will likely lead to the development of endometrial cancer that resembles human endometrial carcinoma with ARID1A loss. The injection of untransformed menstrual endometrial tissue from these GEMMs into the peritoneum of syngeneic recipient immunocompetent mice, which mimics human endometriosis (137), will likely drive the development of ovarian cancers that resemble ENOC or CCOC. These putative tissue-specific GEMMs will provide the opportunity to gain a better understanding of how the inactivation of *ARID1A* interacts with other genetic mutations as well as with the ovarian microenvironment to drive the transformation of precursor cells by enabling comparison of the gene expression profiles and epigenetic states of tumor cells to those of the precursor cells that can be identified by using lineage tracking markers.

Although *SMARCA4* inactivation appears to be the only driver event in SCCOHT, no SCCOHT GEMM has been developed. This is mainly due to the unknown origin of SCCOHT. Serber et al. (138) reported that the inactivation of Smarca4 using a *Wap-Cre* line, which activates Cre expression in granulosa cells of mouse ovary and multiple lineages of mouse uterus, frequently led to the development of ovarian cysts and a low incidence of endometrial cancer. Tumor incidence was not altered when *Smarca2* was simultaneously silenced (138). Therefore, the inactivation of Smarca4 cannot drive the transformation of ovarian granulosa cells, but it has the ability to transform cells of an unknown lineage in the uterus. The histology of endometrial tumors developed in *Wap-Cre:*$Smarca4^{fl/fl}$ GEMM mice was not determined by Serber et al. However, as SMARCA4 loss can arise in undifferentiated endometrial stromal tumors, the endometrial tumors of *Wap-Cre:*$Smarca4^{fl/fl}$ mice may resemble undifferentiated endometrial stromal tumors histologically. Therefore, specific and effective targeting of *Smarca4* in the stromal lineages of

uterine cells may provide a valuable GEMM of undifferentiated endometrial stromal tumors. Furthermore, additional inactivation of *Smarca4* or the BAF complex through dual inactivation of *Arid1a* and *Arid1b* will likely promote the development of undifferentiated cancer resembling human DDEC in GEMMs of well-differentiated endometrial cancer, such as *Pgr-Cre:Pten*$^{fl/fl}$ GEMM mice (139). These models will offer great opportunities for better understanding the context-specific tumor-suppressive roles of distinct SWI/SNF mutations in gynecologic cancers.

7. THERAPEUTIC IMPLICATIONS

Although SWI/SNF-associated gene mutations occur in nearly 20% of all human cancers, most of these gene mutations are loss-of-function mutations, including nonsense, frameshift, and large deletions, that lead to concurrent loss of their protein expression. Therefore, these mutations are not targetable. Consequently, much effort has been invested in identifying synthetic lethal targets that are conferred by these SWI/SNF-associated mutations on cancer cells, and these efforts have been summarized in detail in several reviews (140–142). These putative therapeutic targets include two key hallmarks of human cancer: sustained proliferative pathways—that is, the PI3K/AKT/mTOR pathway and the YES1/SRC tyrosine kinase pathway in ARID1A-deficient CCOC (101, 129, 143, 144) or CDK4 and CDK6 and receptor tyrosine kinases in SCCOHT—and metabolic alterations—that is, the glutathione biogenesis pathway in ARID1A-deficient CCOC (145, 146). Furthermore, as a consequence of the loss of a specific SWI/SNF subunit, tumor cells have adapted and evolved to rely on the residual complex or rewired epigenetic programming, or both. Therefore, the residual complex member and certain epigenetic modifiers are attractive targets for therapeutic developments aimed at these cancers. For example, ARID1B has been reported to be a vulnerable target in ARID1A-deficient CCOC (93), and targeting epigenetic modifiers, such as EZH2, histone deacetylase, and BRD4, is feasible in SCCOHT (112, 115, 116, 147). Furthermore, ongoing clinical trials of EZH2 inhibitors, for example, tazemetostat, for both SMARCB1-deficient epithelioid sarcoma and SCCOHT, will demonstrate whether they can be utilized in the near future for clinical management of these diseases. Moreover, despite the fact that SCCOHT has a low mutation burden, Jelinic et al. (148) discovered that 73% (8/11) of SCCOHTs expressed PD-L1 (programmed death ligand 1), which was associated with strong T cell infiltration. Inspired by this finding, the authors conducted a pilot study in four additional SCCOHT patients, and all four patients responded to anti-PD-1 immunotherapy (148). Although this study involved only a small number of cases and warrants validation, it provides a strong rationale for evaluating immune checkpoint blockades in patients with SCCOHT and, potentially, other SWI/SNF-deficient tumors with a low mutation burden, such as ARID1A-deficient CCOC and, possibly, SMARCA4-deficient uterine stromal sarcoma. The implication of possible effectiveness of PD-1 blockade on CCOC is supported by a recent preclinical study that used an ARID1A-depleted ID8 mouse ovarian cancer cell tumor model by Shen and colleagues (108). However, since the origin of this ARID1A-depleted mouse tumor model does not reflect the origin of CCOC, whether ARID1A-deficient CCOC will respond to immunotherapy requires further investigation.

8. CONCLUSIONS AND PERSPECTIVES

Mutations in several SWI/SNF subunits have been discovered in multiple gynecologic cancers. The frequency and inactivating nature of these mutations supports the idea that SWI/SNF complexes are bona fide tumor suppressors in gynecologic tissues, and this also defines unique vulnerabilities that warrant further clinical investigation. These SWI/SNF-associated mutations may

function as the sole driver event (as in SCCOHT), a malignant transformation–permitting early event (as in CCOC, ENOC, and EEC), or a late-progression event (as in DDEC) in distinct gynecologic cancers. It remains to be further studied whether SMARCA4 loss or SMARCB1 loss functions as a sole driver or early driver event of undifferentiated uterine sarcoma or vulvar cancers. SWI/SNF-associated mutations appear to have a value in predicting a worse outcome for patients with DDEC, even though a large number of cases are required to validate their clinical utility. In contrast, there is unlikely to be a prognostic value for ARID1A loss in ENOC and CCOC, although it may hold a prognostic value in MSS endometrial cancers. Furthermore, the pathogenic roles of the SWI/SNF-associated mutations are far beyond being fully understood in gynecologic cancers. The fact that specific subunits are mutated in specific gynecologic malignancies implies that SWI/SNF complexes function in context-specific manners. Particularly, since ARID1A loss occurs predominantly in endometrial cancers and endometriosis-associated ovarian cancers, we believe that ARID1A loss promotes oncogenesis only in a permissive tissue and cell context, which is unlikely to be the ovarian surface epithelium or fallopian tube epithelium. Thus, results should be cautiously interpreted from studies using high-grade serous ovarian carcinoma cell lines or ovarian surface epithelial cells or tissues to determine the impact of ARID1A depletion on cellular processes. The development of in vitro and in vivo models that appropriately reflect the endometrial cell origin of these tumors will be essential to address the role of ARID1A loss in endometriosis-associated ovarian cancer. In addition, because ARID1A loss occurs as a gateway event that permits the malignant transformation of premalignant endometrial cells in AH, the role of ARID1A loss should also be investigated on an appropriated genetic background. The understanding of SWI/SNF complex mutations and their cellular interactions will be pivotal to developing novel treatment strategies for these gynecologic malignancies, particularly those that portend poor prognoses and often have only conventional platinum- and taxane-based chemotherapy as a therapeutic option.

DISCLOSURE STATEMENT

D.H. is the founder and chief medical officer of Contextual Genomics. The work of Contextual Genomics is unrelated to this review article.

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