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Pathogenesis and the role of ARID1A mutation in endometriosis-related ovarian neoplasms

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Abstract

Endometriosis-related ovarian neoplasms (ERONs) are a unique group of tumors as they are associated with endometriosis, especially endometriosis presenting as an ovarian endometriotic cyst (endometrioma). ERONs include clear cell carcinoma, endometrioid carcinoma, and seromucinous borderline tumor. A growing body of evidence from both clinicopathological and molecular studies suggests that most, if not all, ERONs develop from endometriotic cyst epithelium through different stages of tumor progression. The endometriotic cyst contains abundant iron-induced reactive oxygen species which are thought to be mutagenic, and chronic exposure of cystic epithelium to this microenvironment facilitates the accumulation of somatic mutations that ultimately result in tumor development. Molecular analyses of ERONs, including genome-wide screens, have identified several molecular genetic alterations that lead to aberrant activation or inactivation of pathways involving ARID1A, PI3K, Wnt, and PP2A. Among all molecular genetic changes identified to date, inactivating mutations of the *ARID1A* tumor suppressor gene are the most common in ERON. Understanding the molecular changes and pathogenesis involved in the development of ERON is fundamental for future translational studies aimed at designing new diagnostic tests for early detection and identifying critical molecular features for targeted therapeutics.

Keywords

endometriosis-related ovarian neoplasms; clear cell carcinoma; endometrioid carcinoma; endometrial carcinoma; ARID1A

Introduction

Ovarian epithelial tumors can be broadly classified into two major types of diseases. Type I ovarian carcinoma is composed of clear cell carcinoma (CCC), endometrioid carcinoma (EC), mucinous carcinoma, and low-grade serous carcinoma, while type II ovarian carcinoma mainly consists of high-grade serous carcinoma, the most common and lethal type of ovarian neoplasm.[1] Type I and type II ovarian tumors are characterized by different types of precursor lesions and distinct molecular genetic alterations that account for their unique pathobiological features and clinical behaviors. For example, endometriosis is associated with several type I diseases including ovarian cystic clear cell and endometrioid

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Conflict of interest
None declared.

carcinoma, whereas type II tumors are thought to develop from fallopian tubal epithelium through a putative precursor lesion called “serous tubal intraepithelial carcinoma”.[2, 3] Recognition of the role of endometriosis in the development of some ovarian cancers (type I carcinomas) dates back to as early as 1925,[4] and subsequent studies have demonstrated that endometriosis, especially when presenting as an ovarian endometriotic cyst (endometrioma), is associated with a risk for developing cystic CCC and EC, collectively known as “endometriosis-related ovarian neoplasms (ERONs)”.[5] In fact, ERON is the most serious complication of endometriosis.[6] Moreover, clinicopathological, molecular, and epidemiologic studies provide further evidence identifying endometriosis as the tissue of origin for both cystic CCC and EC.[3] Historically, morphologic studies have consistently demonstrated an association of CCC and EC with endometriosis, and it is now widely recognized that most of these tumors arise from endometriotic cysts. Furthermore, a morphological continuum of sequential stages during tumor progression can be observed from endometriosis to EC or CCC (Fig. 1). Common molecular genetic alterations in ERON such as *PTEN* deletion and microsatellite instability can also be detected in the normal-appearing epithelial cells of endometriotic cysts.[7, 8] Several reports have further delineated the clonal relationship between endometriosis and ERON.[9–12] More recently, gene expression profiling has shown that ovarian CCC and EC are molecularly more similar to normal uterine endometrium than to colonic epithelium, ovarian surface epithelium, or fallopian tube epithelium.[13]

In addition to ovarian cystic CCC and EC, a relatively rare ovarian tumor termed “seromucinous borderline tumor” (SMBT) or “endocervical-like mucinous borderline tumor” is also frequently associated with endometriosis. Like ovarian CCC and EC, SMBT is frequently located within an ovarian endometriotic cyst, an observation providing further evidence for the endometriotic origin for these tumors. Thus, we include SMBT along with CCC and EC as the known ERONs.

Genome-wide analyses have been performed in several types of gynecological neoplasms including ovarian high-grade serous carcinoma,[14] ovarian low-grade serous carcinoma,[15] ovarian clear cell carcinoma,[16] uterine serous carcinoma,[17] and uterine endometrioid carcinoma (TCGA, unpublished). These studies come to the conclusion that somatic *ARID1A* mutations are uniquely associated with ERONs.[18] In this review, we briefly summarize the clinicopathological features and discuss the pathophysiology of ERONs with special emphasis on molecular genetic alterations of *ARID1A*.

Clinicopathological features of ERON

Ovarian clear cell carcinoma

There is a significant racial difference in the incidence of ovarian CCC among ovarian carcinomas. CCC represents approximately 5–10% of ovarian cancers in the United States.[19–23] In contrast, CCC incidence among ovarian carcinoma is significantly higher in Japan (approximately 20%),[24, 25] and the incidence has risen in the past decade. Similarly, Chan et al. [23] performed an incidence analysis according to racial background among 28,082 U.S. ovarian cancer patients, and found that the incidence of CCC is higher in the Asian American population (11.8%) than in the White American population (4.8%) or African American population (3.1%).

Despite 60–70% of CCCs present in early stages (Stage I or II) with approximately 50% being stage I disease, [21, 23, 24, 26–28] CCC has widely been regarded as a subtype with poor prognosis when presenting at an advanced stage. The prognosis of early stage CCCs are generally much better than advanced stage CCCs. [20, 22, 24, 29, 30] One of the main explanations for the poor prognosis of advanced stage ovarian CCC patients is that their

tumors are more resistant to platinum-based chemotherapy. [21232431323328] Development of new target-based therapies thus remains an unmet need for these patients.

It has been well established that there is strong association between endometriosis and the development of CCC, and women with endometriosis are at a higher risk to develop CCC than those without. Approximately 30–35% of CCCs are associated with endometriosis either in the involved ovary or in other pelvic or peritoneal tissues. By separating CCC into either cystic or adenofibromatous lesions, Veras et al.[34] found that endometriosis is more frequently associated with cystic CCC (90%) than with adenofibromatous CCC (44%). A prospective study in Japan, which specifically focused on carcinomas arising from ovarian endometriotic cysts in a cohort of 6398 patients with endometriotic cysts, revealed a significantly increased ovarian cancer incidence in women with ovarian endometriotic cysts (standardized incidence ratio, 8.95), with 39% of cancers being CCC, and 35% being EC.[35] Thus, endometriosis, especially endometriotic cysts, should be considered not only as a risk factor for ERONs, but as potential precursors of ERONs.

Histologically, cystic CCC exhibits a unilocular or paucilocular cystic lesion with a solid component that protrudes into the cystic cavity. Stepwise transition from benign-appearing glandular epithelium, to so-called “atypical endometriosis”, to overt CCC can be commonly observed.[34,36,37,12,13] The tumor cells of CCC recapitulate endometrial glands during pregnancy (Arias-Stella reaction), and are characterized by clear (glycogen-rich) cytoplasm with hobnail morphology, forming tubulocystic, glandular, solid, and papillary patterns. Several tumors may be morphologically confused with ovarian CCC [38] but ovarian CCC has relatively specific immunostaining pattern including positive HNF-1β staining and negative (or focally positive) ER, PR, WT1 and p53 staining, that helps its differential diagnosis.[39]

Ovarian endometrioid carcinoma

EC is another subtype of ovarian epithelial cancer that is frequently associated with endometriosis, especially endometriotic cysts. The incidence of ovarian EC in the older literature is about 15–20%. However, if strict criteria are applied, requiring a close morphological resemblance to uterine endometrioid adenocarcinoma and exclusion of high-grade carcinomas, the figure is estimated to be much lower (7.5%). Up to 42% of ECs are associated with ipsilateral ovarian or pelvic endometriosis.[40,41,42,36] Similar to CCC, EC is frequently associated with atypical endometriosis (23% of cases).[36] Interestingly, 15–20% of ovarian ECs are associated with uterine endometrial carcinoma. [43–47] The favorable outcome in cases exhibiting cancer limited to both organs suggests independent primaries. However, it is sometimes difficult to distinguish metastatic uterine endometrioid carcinoma involving the ovary from independent primary tumors of both organs. Most ERONs are either CCC or EC, but occasionally there are cases exhibiting mixed CCC and EC within the same tumor, suggesting that they share a common precursor arising in the endometriotic cyst, which then differentiates into different histologic types, or that they arise from independent clones that separately evolve into CCC and EC.

On gross examination, most ECs show solid growth in the background of an endometriotic cyst. The tumor nodules may be solitary or multiple with papillary protrusions that contain invasive carcinoma components (Fig. 1A). The common criterion used to diagnose EC is that the glandular component of EC resembles the endometrioid carcinomas of the uterus. As a consequence, almost all ovarian ECs are well-differentiated or low-grade, and are characterized by a confluent or cribriform proliferation of glands lined by tall, stratified columnar epithelium with sharp luminal margins. Occasionally, high-grade endometrioid carcinoma is diagnosed in the ovary, but it is uncertain if high-grade endometrioid carcinoma represents a tumor progression from a low-grade endometrioid carcinoma or if it arises independently.

Seromucinous borderline tumor

Two types of ovarian mucinous borderline tumors of the ovary have been recognized; they are gastrointestinal-type and nongastrointestinal-type borderline tumors. The former is far more common than the latter, comprising approximately 85% of mucinous borderline tumors of the ovary. Both types of borderline tumors are distinct in their clinical presentation, morphology, immunophenotype, and molecular genetic alterations. The nongastrointestinal-type mucinous borderline tumors have been described as displaying both endocervical and serous differentiation. There are several terms used to describe this tumor entity from time to time; they include “endocervical-like” mucinous borderline tumors, “mixed-epithelial papillary cystadenoma of borderline malignancy of mullerian type,” or “atypical proliferative seromucinous (borderline) tumors,” reflecting the uncertainty about the biological nature of this disease. In this review, we group all of these tumors under the umbrella seromucinous borderline tumor (SMBT). Bilateral ovarian involvement is much more frequent in SMBTs than in gastrointestinal-type borderline tumors. Grossly, SMBTs are almost always unilocular or paucilocular cystic lesions that have numerous intracystic papillae. Approximately 20% of SMBTs are reported to have extraovarian spread at the time of diagnosis.[48] However, the malignant counterpart of SMBT, seromucinous adenocarcinoma, is extremely rare. Histologically, SMBTs frequently contain ciliated cells, endometrial-type cells, cells with abundant eosinophilic cytoplasm, and hobnail-shaped cells, all of which can be found in endometrioid tumors (Fig. 2). In addition, a prominent leukocyte infiltration is almost always present, and appears unique to SMBT among all ovarian borderline tumors. Coexistence of endometriosis is observed in 30–70% of SMBTs.[48, 49,50] Furthermore, there is accumulating evidence based on immunohistochemical studies to support the Mullerian-type nature of SMBT. Most SMBTs express estrogen receptor, progesterone receptor, CA-125, and vimentin, whereas gastro-intestinal types of mucinous borderline tumors are usually negative for these markers.[51, 52] Therefore, it is now widely accepted that SMBT is a tumor with a mixed Mullerian phenotype, and the endometriotic cyst is its likely origin.

Pathogenesis of ERON

The carcinogenic steps contributing to the development of ERONs from endometriotic cystic epithelium are unclear. “Incessant menstruation” and longstanding estrogen stimulation are likely contributing factors, as repeated epithelial damage and repair in an inflammatory tissue microenvironment (cyst content) rich in iron-induced oxygen free radicals facilitate the accumulation of DNA damage that predispose endometriotic cyst epithelial cells to neoplastic transformation.[53, 54] It has been thought that DNA damage is the underlying driving force propelling tumor development through creating molecular genetic alterations in several cancer-related genes and the pathways they control. We summarize the most common molecular genetic alterations in ERONs with special emphasis on *ARID1A* mutations in the next section (Fig. 3).

In ovarian CCC, somatic activating mutations of *PIK3CA* [55] are detected in nearly half of the affinity-purified fresh tumors and cell lines. Moreover, Yamamoto et al. report that mutations of *PIK3CA* occur not only in CCCs but also in the concurrent endometriotic epithelium. Since the mutation is detected even in the associated endometriosis, which lacks cytological atypia, it has been suggested that these mutations occur during the early stage of tumorigenesis in ovarian CCC, i.e., during malignant transformation of endometriosis.[11, 56] The relatively high frequency of *PIK3CA* mutations in ovarian CCC contrasts with rare *PIK3CA* mutations in ovarian high-grade serous carcinoma, the most common and aggressive type of ovarian cancer. Interestingly, expression of *PTEN*, a tumor suppressor gene involved in the PI3K signaling pathway (Fig. 3), decreases in approximately one third of CCCs,[57] supporting a role of an aberrant PI3K pathway in the development of CCC (Fig.

2). Loss of heterozygosity at the *PTEN* locus is reported in both the carcinoma and associated endometriotic cyst epithelium in some cases, suggesting that inactivation of the *PTEN* tumor suppressor, like mutation of *PIK3CA*, is a relatively early molecular event in the development of ovarian CCC.[12] Mutations that are commonly detected in other types of ovarian cancer such as *KRAS*, *BRAF,* and *TP53* have been found in only a few CCCs.[58] Although studies to date are rather limited, CCCs do not appear to share many other changes with ECs, as canonical Wnt signaling pathway defects and microsatellite instability have been rarely observed in CCC (Fig. 3).[58]

In addition to molecular genetic changes, ovarian CCC is characterized by a unique gene expression pattern as compared to other histological types of ovarian carcinomas.[58] Compared to normal tissues including colon, endometrium, and fallopian tube, the overall gene expression profile of CCC is most similar to that of normal endometrium, supporting the view that the cell of origin of CCC likely arises from endometriosis.[13] An increasing number of genes have been reported to be preferentially expressed in CCC as compared to other types of ovarian carcinoma. Based on a comprehensive gene expression analysis, Yamaguchi et al. proposed a “signature” of ovarian CCC that consists of HNF-1β, versican (VCAN), and several genes involved in oxidative stress.[59] Interestingly, expression of these CCC signature genes is induced by treatment of immortalized ovarian surface epithelial cells with the contents of endometriotic cysts, suggesting that the CCC signature may be dependent on the tumor microenvironment, and suggesting that CCC pathogenesis is related to endometriosis. In fact, several researchers hypothesize that the iron in endometriosis participates in the pathogenesis of CCC via generation of reactive oxygen species.[54, 60]

In ovarian EC, several molecular genetic alterations have been reported including *CTNNB1* and *PTEN* mutations along with microsatellite instability, of which alterations are also detected in uterine endometrioid carcinoma (Fig. 3).[58] *CTNNB1* encodes β-catenin, which plays a pivotal role in the Wnt/β-catenin signaling pathway. Dysregulation of Wnt/β-catenin signaling occurs in 16–38% of ovarian ECs, most often as a result of activating mutations of *CTNNB1*. Interestingly, *CTNNB1* mutation is highly characteristic of ECs, as *CTNNB1* mutation is not found in other types of ovarian carcinoma. On the other hand, *PTEN* is mutated in 14–20% of ovarian ECs and in 46% of those with loss of heterozygosity (LOH) of 10q23. Similar to *CTNNB1*, *PTEN* mutation is uncommon in other types of ovarian carcinomas. It has been reported that 10q23 LOH and *PTEN* mutations occurred in both endometrioid cysts and adjacent EC,[12] a result supporting a possible precursor role of endometriosis in the carcinogenesis of ovarian ECs. Experimentally, Wnt and PI3K/PTEN pathway alterations are sufficient to induce ovarian EC development as evidenced by an engineered mouse model.[61] The frequency of microsatellite instability (13–20%) in ovarian ECs is less frequent than in uterine endometrial carcinoma, and is usually associated with low levels of expression of proteins associated with mismatch repair, such as hMLH1 and hMSH2.[62, 63]

ARID1A is a new ERON-associated gene

Although the elucidation of the molecular changes discussed above has significantly advance our understanding of the pathogenesis of ERON, revelation of a comprehensive molecular landscape of ERON has only been made possible by genome-wide analyses. To this end, whole exome sequencing and RNA sequencing have been recently applied to detect genome-wide somatic mutations in ovarian CCC.[16, 64] The two studies not only confirmed previously known molecular genetic changes as discussed above, but also identified novel alterations. Among them, somatic *ARID1A* mutation was independently shown by both studies to be the major molecular genetic change in ERONs.[16, 64] *ARID1A* encodes BAF250a, which belongs to the SWI/SNF chromatin remodeling family. The BAF250a

containing chromatin remodeling complex is responsible for several nuclear activities involving transcription, DNA methylation, and DNA synthesis and damage repair. BAF250a interacts with Brg1 (encoded by *SMARCA4*), an ATPase, which serves as the motor to move the chromatin remodeling complex along the DNA strand. *ARID1A* mutation occurs in approximately 50% of ovarian CCC and 40% of ovarian EC, as well as in 30% of uterine endometrioid carcinomas.[16, 18, 64, 65] Although *SMARCA4* mutations are not detected in CCC, it has been recently shown that lung adenocarcinomas harboring *ARID1A* mutations usually do not have *SMARCA4* mutations and vice versa,[66] indicating the ARID1A/Brg1 complex is important for tumor suppression. *ARID1A* mutations occur randomly in the coding regions, the great majority being frameshift and nonsense, leading to lost expression of ARID1A, suggesting that *ARID1A* is a tumor suppressor gene (Fig. 4). Indeed, a recent functional study has elucidated a tumor suppressor role of *ARID1A*, by which ARID1A protein interacts with p53 and suppresses cellular proliferation through p53-dependent transcriptional regulation of several tumor suppressors including *CDKN1A* (encoding p21) and *SMAD3*.[67] As expected, inactivating mutations of *ARID1A* and *TP53* are functionally synonymous, as mutations in either *TP53* or *ARID1A* abolish the transcription of their target tumor suppressors such as *CDKN1A*, allowing uncontrolled cellular proliferation in ERONs.[67] Of note, co-occurrence of *ARID1A* and *TP53* mutations can be found in other cancer types [66] and thus the mutual exclusive nature of *ARID1A* and *TP53* mutations may be tumor type dependent.

The fact that frameshift and non-sense mutations result in loss of ARID1A expression is clear, as those mutants produce truncated mRNAs that are readily degraded. However, whether in-frame insert/deletion mutations also lose their tumor suppressor function is intriguing. A very recent study using biochemical assays demonstrates that, like frameshift mutations, the in-frame mutations that have been analyzed also lose their ability to inhibit cellular proliferation or to activate transcription of p21, a downstream effector of *ARID1A*.[68]

Given that *ARID1A* mutation is associated with loss of its expression,[18, 64] undetectable ARID1A immunoreactivity has been proposed as a surrogate marker for the presence of inactivating *ARID1A* mutations in tissues. This is important because mutational analysis from formalin-fixed and paraffin-embedded tissues is technically challenging, as *ARID1A* contains many exons. However, we have correlated *ARID1A* mutational status and immunoreactivity in a series of cases and show that all tumors harboring *ARID1A* mutations in one or both alleles lose ARID1A immunoreactivity either completely or clonally (Fig. 4B). Several reports have analyzed ARID1A staining patterns in a variety of human cancers and normal tissues, and have demonstrated that loss of ARID1A expression, like its mutation, occurs most frequently in ERONs as well as uterine endometrioid carcinomas.[18, 69–71] There is no correlation between ARID1A expression and clinical outcome in patients with ovarian CCC.[56, 69, 72] The role of ARID1A inactivation in early molecular pathogenesis of CCC is illustrated in two recent reports. Yamomoto et al. report that loss of ARID1A protein expression occurs in early stages in endometriotic cyst epithelium, and frequently coexists with *PIK3CA* mutations [56]. Similarly, another recent study compared *ARID1A* expression in endometriotic cysts and associated contiguous ovarian CCCs and well-differentiated ovarian ECs.[73, 74] The results demonstrated that *ARID1A* loss occurred in two thirds of carcinomas (therefore those cases were informative), but in the remaining one third of cases, ARID1A immunoreactivity was retained in both the endometriotic cyst and in the concurrent carcinoma, and thus these cases were not informative.[74] Importantly, all informative cases demonstrated loss of ARID1A immunoreactivity in both endometriotic cyst (including those termed “atypical endometriosis”) and associated carcinoma. Based on our experience (Shih, unpublished), loss of ARID1A staining is rarely seen in endometriotic cysts without associated ERON.

However, some reports document a loss of ARID1A expression in 15%–20% of such cases.[74, 75] Taken together, similarly to mutations in *PTEN* and *PIK3CA*, the evidence indicates that loss of ARID1A expression, presumably due to mutations, is an early molecular event in the development of the majority of ERONs.[11, 56]

In light of the important role of ARID1A in ERONs, Wu et al. analyzed ARID1A expression in different histological subtypes of ovarian borderline tumors including serous, gastrointestinal-type mucinous, seromucinous, and endometrioid borderline tumors using immunohistochemistry, and performed mutational analysis of *ARID1A* in selected cases.[74] Loss of ARID1A staining was observed in 33% of SMBTs. In contrast, ARID1A staining was retained in all other borderline tumors with the exception of a single endometrioid tumor. Moreover, somatic *ARID1A* mutations were detected in 2 representative SMBTs, which showed complete loss of ARID1A. The loss of expression of ARID1A and the presence of inactivating mutations of *ARID1A* further link this tumor to CCC and EC, and provide molecular evidence that SMBT is a member of ERON.

Conclusion

It has become clear that certain types of ovarian neoplasms are associated with endometriosis, and they most likely arise from a pre-existing endometriotic cyst of the ovary. These tumors, collectively known as ERONs, include ovarian CCC, ovarian EC, and ovarian SMBT. ERONs are characterized by common molecular genetic changes that involve ARID1A, PI3K, and PP2A pathways, but they also have unique molecular changes such as microsatellite instability and *CTNNB1* mutations, which occur in ovarian EC, and overexpression of HNF-1β, which is found in CCC. It would be of considerable interest to determine the molecular switch that dictates the development of different types of ERON, and to determine how those early molecular alterations including *ARID1A*, *PTEN*, and *PIK3CA* mutations collaborate in driving neoplastic transformation from an endometriotic cyst to an ERON. Given the availability of PI3K inhibitors, future clinical studies should determine if targeting the PI3K signaling pathway together with other therapeutic interventions has clinical benefit in advanced stage patients with ERON.

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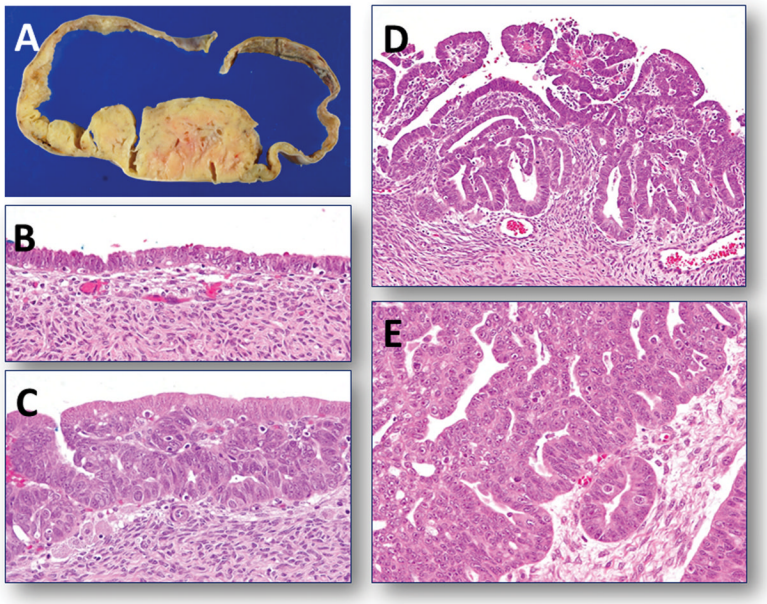

Fig. 1.

Gross appearance and morphological continuum in tumor progression in an endometrioid carcinoma. **A.** An ovarian endometriotic cyst containing an intracystic polyploid endometrioid carcinoma. **B–E**. Photomicrographs show a morphological continuum of different stages of tumor progression from normal-appearing endometriotic cyst epithelium to invasive endometrioid carcinoma.

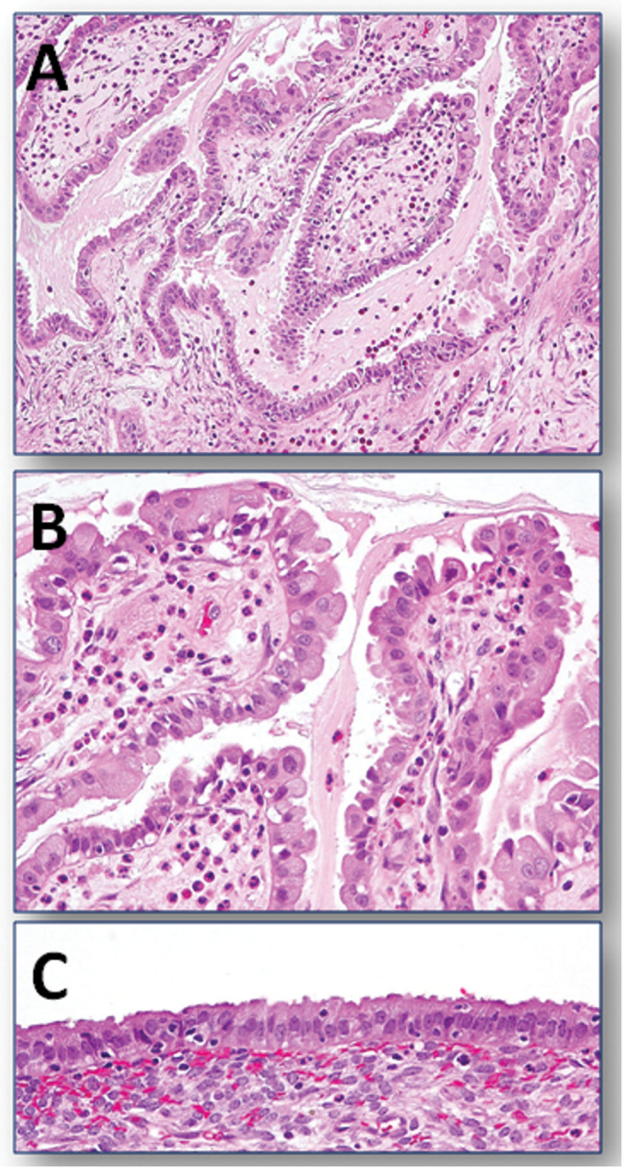

Fig. 2.

An example of a seromucinous borderline tumor of the ovary arising from an endometriotic cyst. **A.** A lower magnification view shows the typical papillary growth of the tumor with abundant mucinous material in the lumen. **B.** A higher magnification reveals the mixed histologic features of the tumor cells exhibiting serous, mucinous, and hobnail-shaped differentiation. A prominent leukocyte infiltration is also present. **C.** portion of endometriotic cyst adjacent to the ovarian tumor.

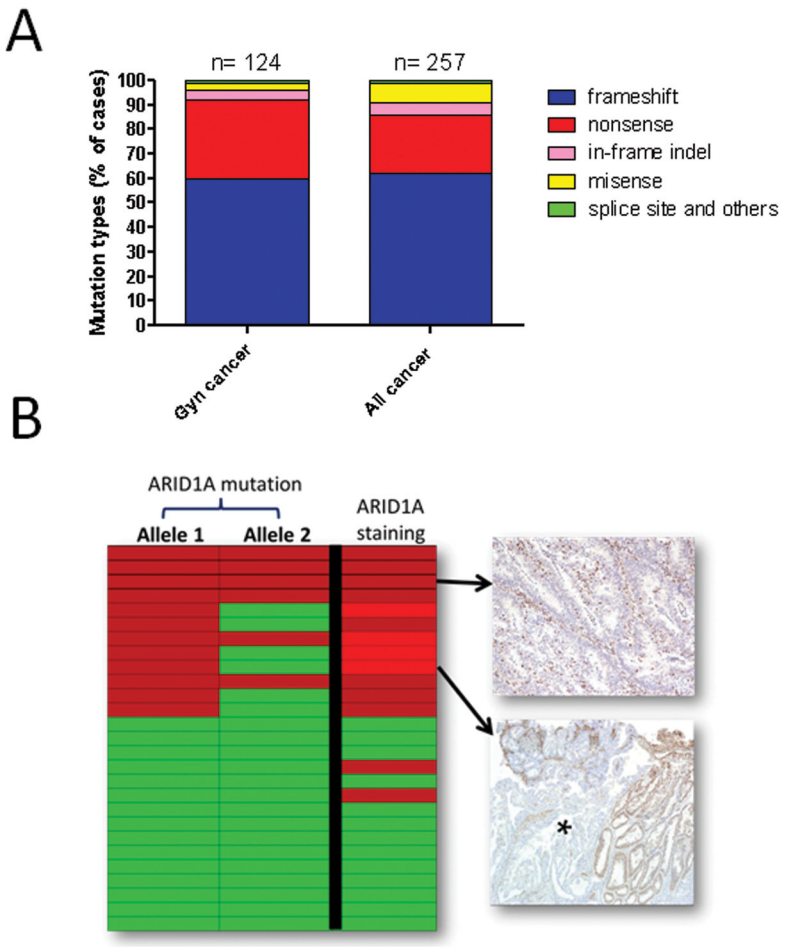

Fig 3. Molecular genetic alterations and pathway aberrations in ERON

The common molecular genetic changes shared by clear cell and endometrioid carcinomas include *ARID1A* mutation, *PIK3CA*/*PTEN* mutation, and *PP2R1A* mutation. Mutations in *CTNNB1* and mismatch repair genes such as *MHL1* and *MSH2* leading to microsatellite instability are almost always detected in endometrioid carcinoma but not in clear cell carcinoma. Genes in red indicate inactivating (loss of function) mutations, and genes in blue represent activating (gain of function) mutations. The larger the font, the greater the observed frequency of mutation of the gene. Asterisks indicate molecular changes that are predominantly found in ovarian endometrioid carcinoma.

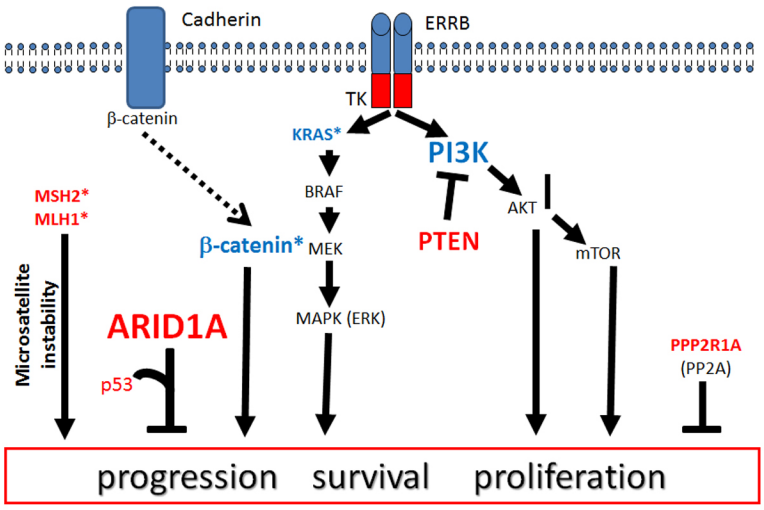

Fig. 4.

Types of *ARID1A* mutations and correlation of mutation and protein expression. **A.** Analysis of reported *ARID1A* mutations demonstrates that most of the mutation types belong to frameshift, nonsense, and in-frame mutations that are associated with loss of the tumor suppressor functions of *ARID1A*. **B.** *ARID1A* mutational status in 27 uterine endometrioid carcinomas. All the tumors with *ARID1A* mutation (in one or both alleles) have lost ARID1A immunoreactivity, and interestingly, two of 15 ARID1A wildtype cases also lost ARID1A staining probably due to epigenetic inactivation. Occasionally, clonal loss of ARID1A expression, as defined by undetectable ARID1A immunoreactivity contiguous to ARID1A positive tumor areas, is found in tumors harboring *ARID1A* mutations, probably as a result of intra-tumoral heterogeneity. Red boxes denote *ARID1A* mutation in both alleles (allele 1 and allele 2). Red boxes in the column of ARID1A staining indicate a complete loss (dark red) or clonal loss (light red) of ARID1A expression. Examples of ARID1A staining from two tumors are shown. Asterisk: the tumor area showing clonal loss of ARID1A staining.