The tubal epigenome – An emerging target for ovarian cancer

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ABSTRACT

Ovarian cancer is the most lethal gynecologic malignancy in the United States. The mortality of this disease is primarily attributed to challenges in early detection and therapeutic resistance. Recent studies indicate that the majority of high-grade serous ovarian carcinomas (HGSCs) originate from aberrant fallopian tube epithelial (FTE) cells. This shift in thinking about ovarian cancer pathogenesis has been met with an effort to identify the early genetic and epigenetic changes that underlie the transformation of normal FTE cells and prompt them to migrate and colonize the ovary, ultimately giving rise to aggressive HGSC. While identification of these early changes is important for biomarker discovery, the emergence of epigenetic alterations in FTE chromatin may also provide new opportunities for early detection, prevention, and therapeutic intervention. Here we provide a comprehensive overview of the current knowledge regarding early epigenetic reprogramming that precedes HGSC tumor development, the way that these alterations affect both intrinsic and extrinsic tumor properties, and how the epigenome may be targeted to thwart HGSC tumorigenesis.

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Contents

1. Introduction

Ovarian cancer is the most lethal gynecological malignancy in the United States. The term 'ovarian cancer' itself is a broad umbrella term describing a collection of heterogeneous neoplasms affecting the ovary. These neoplasms include germ-cell, sex cord-stromal, and epithelial tumors (Crum, Nucci, Haefner, & Peters, 2017)(Fig. 1).

Most ovarian tumors are epithelial in nature, comprising approximately 90% of ovarian cancer diagnoses, with only 20% of these patients diagnosed at early disease stages ("Cancer Statistics Center: Ovarian Cancer", 2019). Women with invasive epithelial carcinomas are often symptomatic, but the early manifestations of the disease are non-specific and not pathognomonic for ovarian cancer. The lack of specific symptomatology accounts, in part, for the low rates of early detection, and the typical diagnosis at an advanced metastatic stage. The American Cancer Society estimates the 5-year survival rate for patients with metastatic epithelial ovarian cancer (EOC) is less than 30%. This poor survival rate yields a daunting death-to-case ratio, especially

when compared with other female reproductive cancer types (International Agency for Research on Cancer, World Health Organization, et al., 2014). With such a striking threat to women's health, the pressing need to better understand disease pathogenesis, improve methods for early detection, and develop durable treatment options remains.

EOCs are stratified into four major histologic subtypes: clear cell, endometrioid, mucinous, and serous carcinomas (Fig. 1). Both clear cell and endometrioid carcinomas are often associated with endometriosis, whereas the origin of mucinous ovarian cancers has been recently attributed to non-gynecological precursors (Cheasley et al., 2019; Elias et al., 2018; Lheureux, Gourley, Vergote, & Oza, 2019; Vaughan et al., 2011).

Of the four subtypes of EOC, the majority are serous carcinomas that are further subdivided into high-grade or low-grade, depending on tumor dissemination and morphological characteristics. Low-grade serous carcinomas classified as 'Type I' carcinomas, are typically early stage tumors localized to the ovary and have more favorable clinical outcomes, accounting for only 10% of serous carcinoma deaths. High-grade serous ovarian carcinomas (Type II) are diagnosed at advanced stages and the highly proliferative nature of these disseminated tumors makes early diagnosis extremely challenging. Overall, high-grade serous carcinomas (HGSCs) are the most common form of epithelial ovarian carcinomas, comprising 75% of all EOC diagnoses (Karst & Drapkin, 2010; Kurman & Shih Ie, 2016; Lheureux, Gourley, et al., 2019).

The current standard-of-care for HGSC is cytoreductive surgery and treatment with a combination of taxanes and platinum-based chemotherapies. While patients tend to initially respond to this treatment regimen, many patients recur and develop chemoresistant tumors. Without a targeted treatment option, patients ultimately succumb to the disease (Agarwal & Kaye, 2003).

From a genomic standpoint, HGSC is primarily a copy number disease with few recurrent somatic mutations. In fact, the genomic landscape of HGSC displays more copy number alterations than any other tumor type studied to date and the only known recurrent somatic mutation in HGSC is in *TP53*, exhibited in nearly 100% of tumors (Cancer Genome Atlas Research, 2011; Vang et al., 2016). Analyses of copy number alterations in HGSC revealed many common loci of chromosomal amplification or deletion. Moreover, the aneuploidy exhibited in these cells ultimately arises from a high degree of chromosomal and genomic instability associated with HGSC tumor cells. In fact, gene breakage events have been shown to inactivate tumor suppressor genes (TSGs), including *RB1*, *NF1*, *RAD51B*, and *PTEN* (Patch et al., 2015). Approximately 50% of HGSCs are deficient in homologous recombination (HR) repair, with the most common defect being mutations in the *BRCA1* or *BRCA2* TSGs. Importantly, it is estimated that 15–20% of HGSC patients exhibit germline mutations in *BRCA1/2* that may be detected prior to

cancer development through germline testing (Alsop et al., 2012; Domchek & Robson, 2019). Identification of defects in the HR pathway has been essential for screening germline mutations in high-risk women as well as for guiding therapies targeting acquired *BRCA1/2* somatic mutations in HGSCs. These HR defects portend a vulnerability to DNA damaging agents, a heavily investigated therapeutic avenue; targeted therapeutics, particularly poly(adenosine diphosphate-ribose) polymerase (PARP) inhibitors, have been widely utilized in the clinic to take advantage of HR-deficient ovarian cancers (Ledermann, 2019; Lheureux, Gourley, et al., 2019; Mirza, Pignata, & Ledermann, 2018). PARP inhibitors, of which there are now four FDA approved compounds, trap PARP molecules on DNA lesions and encourage double strand break repair *via* HR. In the absence of functional BRCA1/2, repair cannot be achieved, and the accumulated DNA damage forces the cells to undergo apoptosis. Much like the chemotherapeutic resistance exhibited by platinum and taxane treated tumors, acquired and *de novo* PARP inhibitor resistances challenge the efficacy of this alternative targeted therapy. With this in mind, many groups have set out to identify mechanisms of PARP inhibitor resensitization utilizing various techniques including whole genome CRISPR screens and microRNA classification (Choi et al., 2016; Meghani et al., 2018; Pettitt et al., 2018; Zimmermann et al., 2018). Identification of such vulnerabilities has prompted optimization of PARP inhibitor efficiency by either treating upfront with standard-of-care chemotherapy regimens or in combination with other drugs targeting the DNA repair pathway including ATR and CHK1 inhibitors (H. Kim et al., 2017; Lheureux, Braunstein, & Oza, 2019; Parmar et al., 2019; Yazinski et al., 2017). While classic alterations like *BRCA1/2* mutations currently identify patients that might respond to targeted therapy, the point at which these somatic aberrations are acquired during early tumor development is less well studied. Understanding the genesis of these alterations would provide an opportunity for risk-reduction and chemoprevention by targeting malignant cells before full tumor onset.

2. Origins of high-grade serous carcinomas (HGSCs)

For many years, it was generally accepted that HGSCs were the product of genetic changes in the ovarian surface epithelium itself (Fathalla, 1971). However, more recent analysis of surrounding tissues revealed that this may not be the case for the majority of HGSCs. Shortly after the discovery of the *BRCA1/2* genes in the mid-1990s, at-risk patients were offered risk-reducing surgery in the form of salpingo-oophorectomy for ovarian cancer prevention. As early as 2000–2001, studies emerged showing that these prophylactic specimens occasionally harbored abnormal cells in the fallopian tube rather than the ovary (Piek et al., 2001; Zweemer et al., 2000) (Fig. 2). An understanding of these tubal lesions emerged with the development of the SEE-FIM

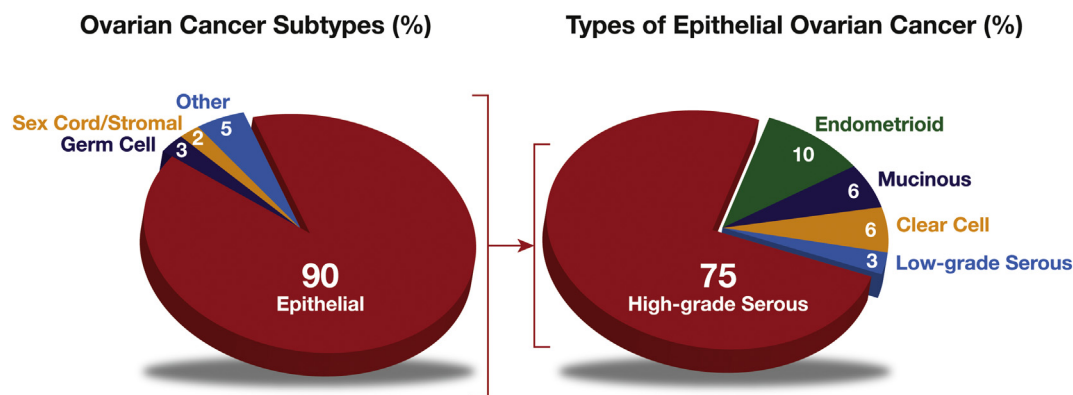**Fig. 1.** Ovarian cancer statistics. Ovarian cancer is a heterogeneous disease with epithelial tumors accounting for the majority of cases. The major histologic subtypes of epithelial ovarian carcinoma include serous, clear cell, mucinous and endometrioid tumors. High-grade serous carcinomas are the most common and are typically diagnosed after they have spread beyond the confines of the fallopian tube and ovary.

(Sectioning and Extensively Examining the FIMbriated end) protocol to comprehensively examine the fallopian tube (Kindelberger et al., 2007; Y. Lee et al., 2007; Medeiros et al., 2006). In otherwise healthy *BRCA1/2* mutation carriers, the SEE-FIM protocol showed that approximately 5% of these women harbored an occult precursor to HGSC in the FTE, termed Serous Tubal Intraepithelial Carcinoma (STIC) (Medeiros et al., 2006; Mingels et al., 2012; Shaw, Rouzbahman, Pizer, Pintilie, & Begley, 2009; Wethington et al., 2013). The subsequent evaluation of the fallopian tubes in patients with HGSC showed that up to 60% harbor precursor lesions in the fallopian tube, suggesting that preneoplastic events in the fallopian tube are the progenitors of HGSC in the majority of HGSC patients, regardless of *BRCA1/2* mutation status (Chen et al., 2017; Kindelberger et al., 2007; Kroeger Jr. & Drapkin, 2017). In the setting of advanced disease, HGSC cells can colonize the FT and appear as a STIC but in reality are metastatic deposits (Chen et al., 2017; Eckert et al., 2016; Kroeger Jr. & Drapkin, 2017; Labidi-Galy et al., 2017; McDaniel et al., 2015). Altogether, this histopathological evidence ultimately prompted a change in the HGSC origin hypothesis to suggest that early aberrations in the epithelium of fallopian tube fimbriae may result in HGSC tumorigenesis.

One of the main markers of the link between the fallopian tube and ovarian tumors is the early loss of the universal marker of HGSC, *TP53*. Mutations in this TSG likely determine the FTE cell fate, as it becomes vulnerable to further genomic instability that will eventually give rise to STIC and the primary tumor. This concept of early TP53 loss of function was originally dubbed a 'p53 signature' when first observed as high p53 immunohistochemical staining of tubal secretory epithelial cells in *BRCA* mutant carrier patients (Y. Lee et al., 2007). Following the mutation of *TP53* in the fallopian tube, cells undergo malignant transformation to form dysplastic STIC lesions in the FT. Whether all *TP53* mutations are capable of perpetuating subsequent aberrations is unclear (Tuna et al., 2020). However, not all HGSCs have an identifiable STIC lesion in the FT (Chen et al., 2017; Kroeger Jr. & Drapkin, 2017). While some animal studies suggest that HGSC can arise from the ovarian surface epithelium, we currently lack human data supporting the presence of precursors in the OSE (Flesken-Nikitin et al., 2013; Zhang et al., 2019). In addition to technical difficulties in identifying precursor lesions, which include sampling error, inter-observer variability, and consumption of the precursor by advanced disease (Carlson et al., 2010; Mahe et al., 2013; Rabban, Garg, Crawford, Chen, & Zaloudek, 2014; Vang et al., 2012; Visvanathan et al., 2011), a more recent concept suggests that *TP53* mutant cells have undergone 'precursor escape,' whereby benign-appearing but *TP53* mutated FT cells are shed to the ovary or peritoneum and undergo transformation at a later time (Soong, Kolin, Teschan, & Crum, 2018). In this scenario, transformation occurs at a non-tubal site but the cell of origin is still a *TP53* mutated FT cell. Douglas Levine and colleagues recently tested the 'precursor escape' hypothesis by profiling HGSC with and without associated STIC lesions using copy number alterations and transcriptomics for RNA and microRNA expression. They argued that if HGSCs without STICs have a non-tubal origin then the expression profiles of those HGSCs should differ from those with a tubal origin. Analyses of the data from copy number alterations, messenger RNA sequencing and microRNA profiling failed to identify any significant differences between HGSCs with or without precursor STIC lesions (Ducie et al., 2017). To infer the most probable site of origin, they calculated the similarity between HGSC
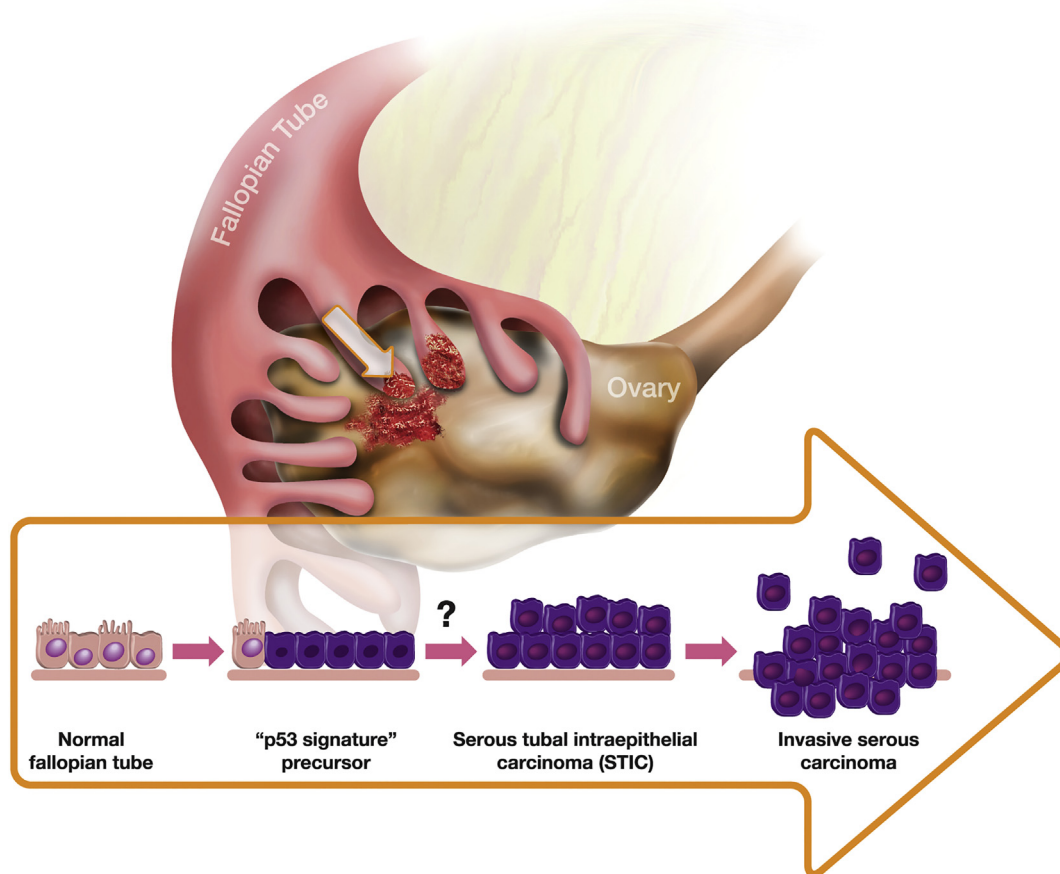

Fig. 2. Schematic for evolution of HGSC from malignant FTE precursor cells. Mutations in TP53 are a very early event in the pathogenesis of HGSC, occurring in benign-appearing secretory cells. These preneoplastic lesions are referred to as 'p53 signatures.' Acquisition of a neoplastic phenotype and proliferative capacity results in the development of serous tubal intraepithelial carcinoma (STIC). The question mark represents the unknown molecular changes associated with this transition. Invasion of the basement membrane and localized dissemination to the ovary and/or peritoneal cavity heralds the development of invasive HGSC and the associated clinical scenario.

and normal fallopian tube, normal ovary and normal peritoneum through molecular barcodes, and found that the most likely site of origin for all cases is indeed the distal fallopian tube. This is further supported by DNA methylome analyses showing that the methylomes of HGSC and FTE are significantly and consistently more highly conserved than are HGSC and OSE (Klinkebiel, Zhang, Akers, Odunsi, & Karpf, 2016).

Next generation DNA sequencing studies have provided further support for the tubal hypothesis. Using either macrodissection or laser-capture microdissection to precisely isolate the epithelial compartment of tubal precursor lesions, HGSC, and metastases to the ovary, omentum, and other structures in the peritoneum, whole-exome sequencing was combined with analyses for copy number variation and phylogeny to show that STICs are the precursor to HGSCs (Eckert et al., 2016; Labidi-Galy et al., 2017; McDaniel et al., 2015; R. C. Wu et al., 2019). Two studies included p53 signatures in their analyses and showed that mutations in *TP53* are the earliest and only recurring event in this disease (Labidi-Galy et al., 2017; Wu et al., 2019). However, each patient also exhibited distinct mutations within the p53 signatures and STIC lesions. Loss of heterozygosity and copy number alterations, the hallmark of HGSC, were also detected in precursor lesions but to a lesser degree than the associated HGSC, indicating their earlier occurrence in the tumor evolutionary timeline. Importantly, both of these studies used mathematical modeling to estimate the time between the development of the earliest neoplastic clones in the FT and the development of ovarian and other metastatic lesions. They similarly concluded that the average time between a STIC and an ovarian tumor is 6–7 years. However, in patients with metastatic lesions, the time between the ovarian carcinoma and development of metastases is rapid, typically less than 2 years. Therefore, the window of opportunity for future studies aimed at prevention or early detection is between the emergence of a STIC and its colonization of the ovary.

Spontaneous HGSC induction in genetically engineered mouse models (GEMMs) has provided experimental support for tubal transformation in HGSC. With a *Cre*-recombinase system, it has become possible to knockout TSGs under the control of tissue specific promoters like *Pax8* or *Ovgp1*. While mice lack the fallopian tube structures of the human female reproductive system, knockout of TSGs such as *TP53, BRCA1/2,* and/or *PTEN* in the most similar structure, the oviduct, gives rise to both STICs and serous ovarian carcinomas (J. Kim et al., 2012; Morin & Weeraratna, 2016; Perets et al., 2013; Sherman-Baust et al., 2014; Stuckelberger & Drapkin, 2018; R. Wu et al., 2016; Zhai et al., 2017; Zhang et al., 2019). Importantly, these GEMMs recapitulate the copy number alterations seen in human HGSCs (McCool et al., 2020; Perets et al., 2013).

3. Fallopian tube epithelial (FTE) precursors and the epigenome

In addition to recent studies defining the genetic evolution of HGSC from the FTE, mounting data suggests that epigenomic dysregulation also contributes to HGSC pathogenesis (Fig. 3). Alterations in DNA methylation (particularly changes in CpG island methylation upstream of promoters), histone modifications (primarily methylation and acetylation marks previously associated with repression or activation of nearby transcription, respectively), and chromatin remodeling genes (mutations in writers, readers, and erasers of the post-translational modifications) ultimately regulate transcriptional activity and chromatin accessibility of the genome, and have thus garnered interest in understanding how epigenetic aberrations drive tumorigenesis (Bennett & Licht, 2018; Jones, Issa, & Baylin, 2016; Sen, Shah, Nativio, & Berger, 2016; Valencia & Kadoch, 2019).

While particularly interesting in the setting of HGSC development from early FT precursors, this paradigm of epigenetic dysregulation driving tumorigenesis has been previously established in other disease states (Tsai & Baylin, 2011). For example, mutations in genes regulating DNA methylation, histone methylation, and histone acetylation are common in prostate tumors (Yegnasubramanian, De Marzo, & Nelson,

2019). These regulatory genes include epigenetic 'writers' that deposit the respective post-translational modification (PTM), 'readers' that catalyze downstream signaling in the presence of the PTM, and 'erasers' that return the site to its unmodified state. The crosstalk between epigenetic regulatory components ultimately allows for dynamic regulation of cellular signaling by manipulating transcription and localized condensation and relaxation of the chromatin. In the scope of prostate cancer, one of the most well-studied epigenetic phenomena is DNA hypermethylation where the balance between DNA methyltransferases and demethyltransferases becomes disrupted. This disruption leads to an increased frequency of methyl marks near promoter regions of important TSGs such as *GSTP1, RASSF1A, and APC* (Yegnasubramanian et al., 2019). Accumulation of methyl marks at promoter CpG islands blocks transcription factor binding and has been associated with transcriptional repression of TSGs. Loss of tumor suppression enables the outgrowth of cancer precursor cells and prompts tumorigenesis. Understanding when these events occur during tumorigenesis and which key regulators become dysfunctional provides researchers with a new approach to therapeutics.

3.1. DNA methylation

The rise of detection methods for covalent DNA modification patterns has encouraged investigation of epigenomic similarities between FTE precursors and HGSC. DNA methylome analysis of patient samples from tumor tissue, FTE, and ovarian surface epithelium revealed that dominant changes in the HGSC epigenome most directly correspond to methylation patterns in the matched FTE (Klinkebiel et al., 2016). In a larger-scale analysis of DNA methylation signatures, Pisanic et al. showed that there are indeed detectable hypermethylation loci at *TUBB6, IRX2, and c17orf64* promoters that reliably differentiate malignant FTE from benign tissue (Pisanic 2nd et al., 2018). Further analysis of the HGSC methylome has begun to identify how early FT epigenetic reprogramming ultimately drives changes that initiate characteristic genomic instability in *BRCA1/2* mutant tumors (Bartlett et al., 2016). Whether these altered or aberrant patterns harbor clinical utility for early detection or as biomarkers to strengthen risk-assessment panels is under investigation.

In addition to *BRCA1/2* mutations, HR deficiency in HGSC patients has also been associated with *BRCA1* promoter region hypermethylation in approximately 10% of cases (Baldwin et al., 2000; Esteller et al., 2000; Swisher et al., 2017). In support of this idea, it has been shown that *BRCA1* promoter hypermethylation can be detected in serum DNA of stage I HGSC patients (Ibanez de Caceres et al., 2004). Much like *BRCA1/2* mutant tumors, those with *BRCA1* promoter hypermethylation are targetable with PARP inhibitor treatment. Conversely, *BRCA1* promoter hypomethylation has been identified as a mechanism of resistance in PARPi-treated patients (Ter Brugge et al., 2016). To support this idea, a number of studies have shown that DNA hypomethylation is a predominant feature in cancers, including HGSC (Ehrlich, 2002; Widschwendter et al., 2004; Woloszynska-Read, Mhawech-Fauceglia, Yu, Odunsi, & Karpf, 2008). Significantly, a 2018 study has provided strong evidence that DNA hypomethylation, or more specifically loss of 5-hmC, may serve as a diagnostic and prognostic biomarker for HGSC (Tucker et al., 2018). These findings of early detectable alterations in the epigenome are important not only for diagnostic and therapeutic purposes, but also to aid in understanding the preliminary events affecting downstream gene expression patterns seen in HGSC. While these studies identify readily detectable epigenetic alterations specific to HGSC, the search for the molecular mechanisms that are driving these early DNA methylation changes is still ongoing.

Based on the notion that promoter methylation transcriptionally inactivates TSGs early in tumorigenesis, DNA methyltransferases (DNMTs) have been targeted in the clinic. Originally approved for use in myelodysplastic syndrome, 5-azacytidine (AZA) and 5-aza-2′-deoxycytidine (decitabine) are DNMT inhibitors (DNMTi) that have
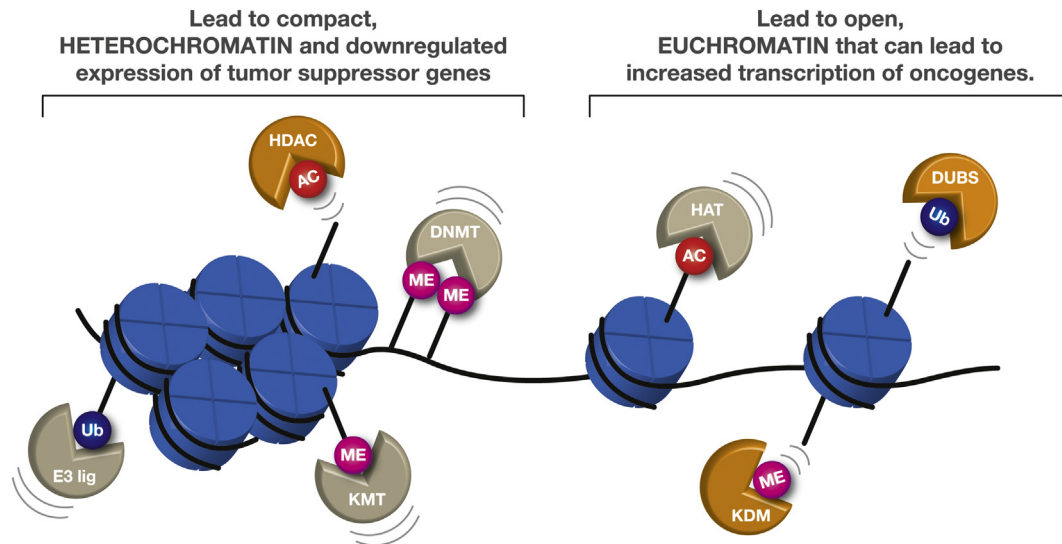
Fig. 3. Regulation of chromatin dynamics by epigenetic remodelers and the effects of chromatin accessibility on tumorigenic gene transcription. On the left, chromatin compaction can be regulated by removal of histone acetyl marks by deacetylating enzymes (HDACs), deposition of histone methyl marks by histone methyltransferases (KMTs), E3 ligase-mediated histone monoubiquitination, or direct methylation of DNA by DNA methyltransferases (DNMTs). Negative regulation of these processes, shown on the right, leads to chromatin relaxation, mediated by addition of acetyl marks by histone acetyltransferases (HATs), removal of monoubiquitin by deubiquitinases (DUBs), or removal of histone methyl marks by lysine demethylases (KDMs).

shown promise in resensitization of platinum resistant ovarian cancers (Matei et al., 2012). It is believed that this reduced promoter methylation corresponds to re-expression of genes controlling DNA damage repair and immune response and therefore inhibits the cancer cells ability to evade death in the presence of standard chemotherapy (Fang et al., 2018; Stone et al., 2017; Travers et al., 2019; Wang et al., 2015). Given that epigenetic remodeling events like DNA demethylation can enhance anti-tumor response to chemotherapy along with the fact that these events precede tumorigenesis, it is clear that epigenetic instability is a crucial vulnerability in ovarian cancer. However, due to the global nature of DNA methylation and the few catalytically active DNMTs in the human proteome, there is a concern that inhibition of these enzymes may be harmful in a monotherapy setting. While hypomethylation upstream of TSGs may inhibit the rise of cancer cells, consequent hypomethylation upstream of oncogenes may alternatively advance tumorigenesis.

3.2. Histone modifications

The human genome is composed of DNA wrapped tightly around octameric histone proteins to form nucleosomes of compact chromatin; changes in addition or removal of covalent PTMs on histones can change the polarity of neighboring chromatin and cause compaction or relaxation to regulate gene transcription. By this logic, understanding early epigenetic histone modifications that precede genetic aberrations but stimulate the development of HGSC is critical. These epigenetic modifications encompass a myriad of alterations including but not limited to, methylation, acetylation, phosphorylation, lactylation, and ubiquitination (Audia & Campbell, 2016; Dawson & Kouzarides, 2012; Zhao & Shilatifard, 2019).

In the context of HGSC development, we recently reported that there is a marked decrease in histone H2B monoubiquitination (H2Bub1) between wild-type FTE and associated STIC lesions, followed by its eventual absence in the primary HGSC (Hooda et al., 2019). A mechanism for loss of H2Bub1 was originally reported in HeLa cells where it was demonstrated that knockdown of the E3 ubiquitin ligase, RNF20, is sufficient to suppress H2B monoubiquitination and selectively attenuate expression of TSGs such as TP53 (Shema et al., 2008). Knocking down RNF20 in FT secretory cells by RNA interference leads to increased migration, clonogenicity, and anchorage-independent growth of the cells

(Hooda et al., 2019). These malignant phenotypes are in part caused by an increase in immune cytokine secretion, including interleukin-6, and an increase in cell adhesion programs. Given that loss of H2Bub1 occurs in over 70% of HGSC cases, these findings emphasize the importance of epigenetic maintenance, especially H2Bub1, in ovarian cancer progression (Dickson et al., 2016; Hooda et al., 2019).

While RNF20 is believed to be the canonical E3 ligase that deposits ubiquitin on H2B at lysine 120, recent studies have proposed that BRCA1 may also be an important non-canonical E3 ligase responsible for the regulation of H2Bub1 (Dickson et al., 2016). Identifying epigenetic regulators that are commonly altered in HGSC provides new therapeutic opportunities to overcome the challenges of treating advanced disease. Alternatively, targeting ubiquitin-ligase/de-ubiquitinase pathways involved in early epigenetic reprogramming of FTE may provide a new avenue for therapeutic intervention. For example, approximately 53% of HGSC patients exhibit copy number loss of the E3 ligase RNF20 (Cerami et al., 2012; Gao et al., 2013; Hooda et al., 2019). While, it may be difficult to reverse the heterozygous loss of the E3 ligase, a more tangible therapeutic goal may instead be targeting of deubiquitinating enzymes (DUBs) whose amplification in HGSC may be further driving H2Bub1 loss during tumorigenesis. While the DUBs predominantly responsible for this phenomenon in FTE to HGSC remain unidentified, the prospect of DUB inhibition shows promise in other cancer contexts (Cheng et al., 2019; Harrigan, Jacq, Martin, & Jackson, 2018; Poondla, Chandrasekaran, Kim, & Ramakrishna, 2019).

The rationale for targeting ubiquitin-specific histone modifications in HGSC stems from the recent success in targeting other chromatin remodelers. In ovarian clear cell carcinoma (OCCC), common changes in chromatin remodelers such as the overexpression of the histone methyltransferase, EZH2 exist. EZH2 has been most thoroughly investigated as a target in large B-cell lymphomas, and the success of small molecule inhibitors against EZH2 in this context has spurred interest in the ovarian cancer field. In addition to EZH2 overexpression, mutations in the ARID1A component of the SWI/SNF chromatin remodeling complex have been reported in almost 60% of OCCC patients (Bitler et al., 2015). A screen of ARID1A-mutant OCCCs revealed significant single agent sensitivity to EZH2 inhibitors and these compounds can be exploited for in vitro and in vivo synthetic lethality in this setting. With such a large percentage of OCCC patients exhibiting mutations in chromatin remodeling proteins, strategic targeting of EZH2 can inhibit

histone methylation events that silence TSGs which are important for OCCC progression. Similarly, the potential for use of EZH2 inhibitors has been investigated in high-grade tumors. Karakashev et al. investigated markers of EZH2 inhibitor sensitivity in HGSC and found that a subset of tumors marked by CARM1 overexpression also benefit from EZH2i monotherapy (Karakashev et al., 2018). CARM1 actively silences EZH2 target genes and the introduction of EZH2 inhibition in these settings synergizes to suppress pro-tumorigenic effectors. Altogether, these results are striking in that they identify subpopulations of patients that may show particular benefit from epigenetic therapies.

As previously mentioned, one of the main concerns with the use of DNA methylation and histone modification-targeted therapeutics is "broad reprogramming" (Jones et al., 2016). For example, most small molecule inhibitors targeting acetylation pathways have been aimed at histone deacetylating enzymes (HDACs); the goal has been to maintain anti-tumorigenic gene expression by preventing removal of chromatin-relaxing acetyl marks (H. J. Kim & Bae, 2011). However, due to the genome-wide nature of histone acetylation, monotherapy with HDAC inhibitors has seen little success. For this reason, focused efforts to identify drug combinations and specific patient populations that may benefit from HDAC inhibition continue (Batty, Malouf, & Issa, 2009). Bitler et al. found that ARID1A-mutant OCCC patients not only benefit from EZH2 inhibition but also HDAC6 inhibition (Bitler et al., 2017). While these small molecule inhibitors have been heavily investigated in the clinic, it remains unclear how to best use them in the setting of HGSC. By identifying biomarkers for epigenetic vulnerabilities and honing in on localized regulation of the epigenome, off-target effects can be minimized, which will ultimately improve patient quality of life.

While the traditional approach for development of targeted therapies in HGSC has revolved around genetic defects, the emergence of early epigenetic changes in precursor cells provides an opportunity for new investigation. Early changes in chromatin dynamics suggest that prophylactic intervention could prevent high-risk precursors from progressing, and inhibit the milieu of pro-tumorigenic pathways regulated by this epigenetic reprogramming.

4. Role of the early epigenome in sculpting the tumor microenvironment

With an established role for epigenetic reprogramming in epithelial precursor cells, there is an effort to identify how these changes may be functionally promoting tumorigenesis. It is known that there are discrete differences in DNA methylation and histone ubiquitination patterns in HGSC development, and it is of interest to identify other relevant alterations that nurture a pro-tumorigenic environment. Epigenetic changes that affect chromatin structure ultimately dictate accessibility of the genome necessary to preserve cellular homeostasis. When chromatin conformation becomes more euchromatic in regions rich in oncogenes, or more heterochromatic in regions rich in tumor suppressors, cell intrinsic and extrinsic properties are altered in favor of a pro-tumor microenvironment. A 2018 pan-cancer study reported a striking correlation between accessible chromatin and RNA expression, showing that euchromatic regions surrounding transcriptional start sites for oncogenes such as *BCL2* and *SRC* are associated with increased expression levels in a variety of tissue types (Corces et al., 2018). Selective preparation or 'poising' of chromatin through epigenetic dysregulation may affect downstream gene expression that can promote hallmarks of cancer including, but not limited to, migration and tumor-promoting inflammation (Hanahan & Weinberg, 2011).

The epithelial-to-mesenchymal transition (EMT) describes the shift of epithelial precursor cells to a more migratory and invasive phenotype. Post-translational histone modifications have recently been implicated in this pro-tumorigenic change from the 'default' epithelial program (Tam & Weinberg, 2013). EZH2 and SUZ12 are two components of the polycomb repressive complex 2 (PRC2) that have been identified as important players in promoting H3K27me3 methylation
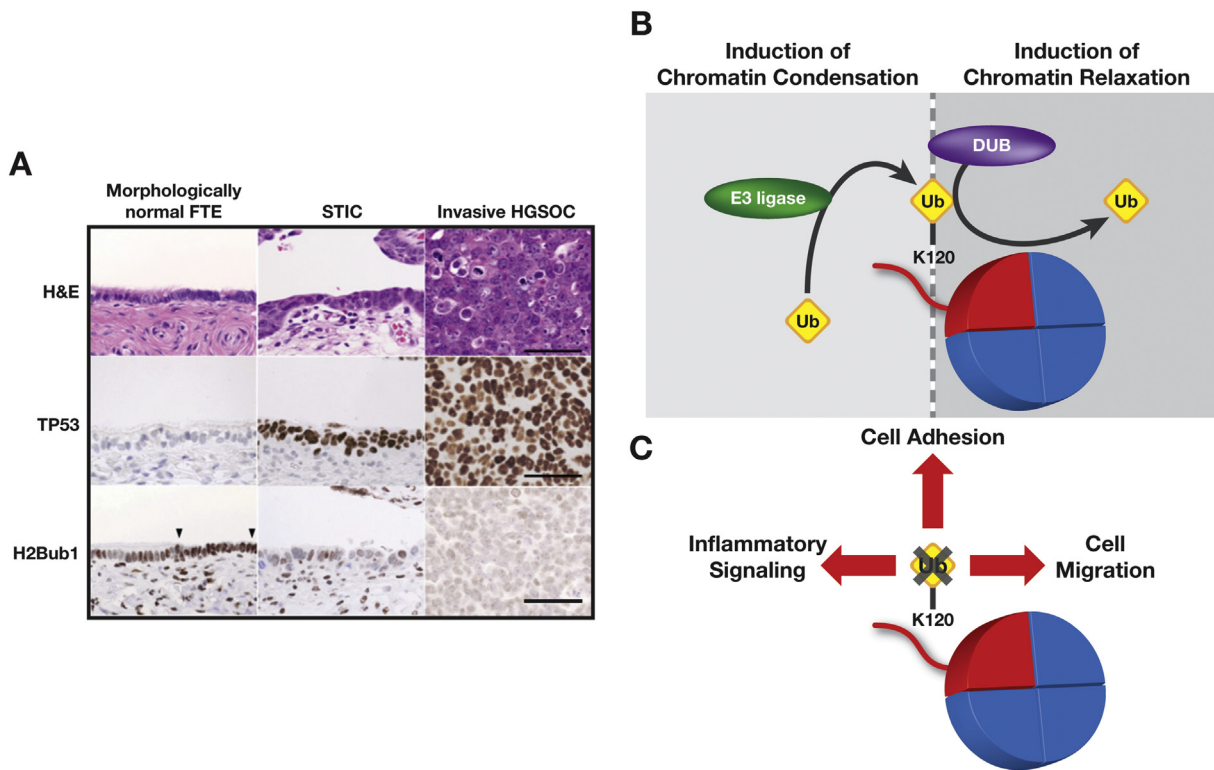

Fig. 4. Morphology and immunohistochemical staining demonstrating gradual loss of H2Bub1 throughout HGSC development (A). H2Bub1 is dynamically regulated by positive effectors like the E3 ligase RNF20 and negative deubiquitinating effectors (B). Loss of H2Bub1 can have profound effects on tumor cell biology leading to increased migration, cell adhesion and inflammatory signals dominating in the absence of H2Bub1 (C).

of promoters upstream of classic epithelial program genes including E-cadherin. Enhanced H3K27me3 methylation at these promoter sites effectively represses transcription of epithelial programs and may therefore be promoting the EMT seen in epithelial ovarian carcinomas. While H3K27me3 is considered to be a transcriptionally repressive post-translational modification, other methyl marks like H3K4me3 are considered to be transcriptionally activating. Silencing of epithelial gene programs may indeed drive the EMT in epithelial carcinomas, but enhanced transcriptional activity upstream of mesenchymal genes may bolster this effect. In a 2013 study, Chaffer et al. showed that the introduction of growth factors to poised chromatin containing both H3K27me3 and H3K4me3 marks at ZEB1 promoters shift transcription of this pro-mesenchymal gene toward a more active state by promoting preferential presence of the euchromatic H3K4me3 mark in breast cancer cells (Chaffer et al., 2013). Altogether these data suggest an important role for epigenetic reprogramming and its modulation of tumorigenic properties.

Several recent studies have also begun to highlight the role of epigenetic alterations in cell autonomous promotion of an inflammatory microenvironment. As previously mentioned, HGSC development has been linked to the gradual loss of H2Bub1 (Fig. 4). While the loss of this mark in early precursor cells affects several pro-tumorigenic pathways, one of particular interest includes the effect on chronic inflammation. Heterozygous knockout of the *Rnf20* E3 ligase in a mouse model led to decreased H2Bub1 levels and secretion of proinflammatory cytokines, including interleukin-6 (IL-6), by nuclear factor κB (NF-κB). Concordantly, RNF20$^{+/-}$ mice are predisposed to acute and chronic colonic inflammation and inflammation-associated colorectal cancer, with excessive myeloid-derived suppressor cells (MDSCs) that may quench anti-tumoral T cell activity (Tarcic et al., 2016). Similarly, *in vitro* analysis of FT cell lines with reduced H2Bub1 revealed increased IL-6 that enhanced migration of FT cells lacking H2Bub1. Increased secretion of this cytokine may favorably prime the fallopian tube microenvironment to give rise to both STIC lesions as well as eventual HGSCs (Hooda et al., 2019). IL-6 mediates pancreatic cancer metastasis to the liver by encouraging pro-metastatic niche formation (J. W. Lee et al., 2019). Therefore, it is likely that IL-6 secretion by transformed FTE cells similarly prepares the ovarian microenvironment for invasion (Reavis & Drapkin, 2019). Together, these findings highlight the importance of H2Bub1 in HGSC and other early epigenetic reprogramming events in pro-metastatic niche formation and warrant translational investigation for prophylactic inhibition of these events.

5. Summary

While The Cancer Genome Atlas and other genomic studies have cataloged the landscape of ovarian cancer, there is an emerging appreciation that alterations in the epigenome also play a role in tumor development and progress. Matched DNA methylation signatures between HGSC patient FTE and the primary tumor have confirmed not only that FT cells are likely HGSC progenitors, but also that detrimental epigenetic changes occur early during tumorigenesis. DNA methylation can impair DNA damage repair by suppressing *BRCA1* transcription, and the loss of H2Bub1 can trigger both cell migration and a proinflammatory tumor microenvironment. Identifying the key enzymes involved in these pathogenic processes can aid in the development of novel biomarkers for early detection as well as potential therapeutics that would help prevent ovarian cancer before it begins.

Declaration of Competing Interest

R. Drapkin is a consultant/advisory board member for Repare Therapeutics, Mersana Therapeutics, and Siamab Therapeutics. No other authors declare potential conflicts of interest.

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