Published in final edited form as:

Curr Opin Genet Dev. 2015 February ; 30: 25–31. doi:10.1016/j.gde.2014.12.004.

The Mutational Landscape of Endometrial Cancer

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Abstract

Globally, endometrial carcinoma causes about 74,000 deaths annually. Endometrial carcinomas can be classified into several histological subtypes including endometrioid and serous histologies. Over the course of the past two years, a number of studies have decoded the exomes of endometrioid and serous endometrial carcinomas revealing novel somatically mutated genes that are likely to drive their development. Moreover, an integrated genomic analysis of these two histological subtypes by The Cancer Genome Atlas has led to their molecular reclassification into four discrete molecular subgroups. Collectively, these genomic advances set the stage for future biological and clinical studies to determine their relevance for patient care.

Introduction

The American Cancer Society predicts that in 2014 approximately 52,630 American women will be newly diagnosed with uterine cancer, and approximately 8,590 woman will die from this disease [1]. Although the majority of endometrial cancers arise sporadically, a small fraction of cases are attributable to inherited genetic susceptibility.

Most diagnosed uterine cancers are endometrial carcinomas (ECs) of which ~80–90% are endometrioid tumors, and 2%–10% are serous tumors [2]. Patients with low-risk or early-stage endometrioid ECs have a good prognosis, but the outcome associated with high-risk or advanced-stage endometrioid EC is less favorable [3]. Serous ECs are much more clinically aggressive than endometrioid ECs and have a relatively poor overall outcome [4,5]. The clinical impact of endometrial carcinoma has motivated molecular genetic studies to understand their genetic etiology (reviewed in [6]). Here, we review recent new insights into the genomic landscapes of serous and endometrioid ECs stemming from large-scale genome sequencing studies reported during the past two years (2012–2014) [7–12]. The major focus of our discussion will be on genes that are somatically mutated at statistically significantly higher rates than the background mutation rate of endometrial carcinomas. As such, these so-called significantly mutated genes (SMGs) represent candidate pathogenic driver genes

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for endometrial cancer. We also highlight recent novel insights into inherited genetic susceptibility to endometrial cancer.

The genomic landscape of serous endometrial carcinomas

As a result of early and recent studies that employed candidate gene sequencing, it was established that serous ECs exhibit frequent somatic mutations in *TP53*, in the PI3-kinase pathway, and in the *PPP2R1A* protein phosphatase (reviewed in [6]). The first unbiased glimpse into the genomic landscape of serous ECs came in 2012 with the publication of two studies, one by Kuhn et al. [7], and one by ourselves [8]. Using massively parallel sequencing to decode the exomes of primary serous ECs (10–13 cases per study), both laboratories uncovered frequent somatic mutations in *FBXW7*, a *bona fide* cancer gene [7]. *FBXW7* is a subunit of the SCF^{FBXW7} ubiquitin ligase complex that regulates the levels of oncogenic protein substrates including cyclin E1 (*CCNE1*). *CCNE1* is itself amplified in serous ECs, and in a mutually exclusive manner with *FBXW7* mutations suggesting functional redundancy [7]. Our own exome sequencing of serous ECs identified frequent somatic mutations not only in *FBXW7* but also in the *SPOP* ubiquitin ligase complex gene, and in the *CHD4*, *ARID1A*, and *EP300* chromatin-remodeling genes thus providing compelling genetic evidence that these two biological processes are frequently perturbed in serous endometrial tumorigenesis [8]. By extension, we also showed that *FBXW7* and *CHD4* are somatically mutated in endometrioid ECs.

The frequent occurrence of mutations in *FBXW7*, *SPOP*, and *CHD4* among serous ECs was confirmed by Zhao et al., [9], based on the exome sequencing of 30 cases. Furthermore, Zhao et al nominated *TAF1*, *HCFC1R1*, *CDKN1A*, *YIPF3*, and *FAM132A* as candidate driver genes. Of these five genes *TAF1*, which encodes a subunit of the TFIID basal transcription factor, exhibited the highest degree of statistical significance and was also notable because the mutations it sustained tended to occur at highly conserved sites with the histone acetyltransferase domain [9].

The integrated genomic analysis of serous ECs by The Cancer Genome Atlas (TCGA) is the most comprehensive assessment of the genomic landscape of serous ECs reported thus far [12]. Eight SMGs (*TP53*, *PIK3CA*, *FBXW7*, *PPP2R1A*, *CHD4*, *CSMD3*, *SLC9A11*, *PTEN*) were nominated by TCGA from an analysis of 43 serous ECs. Another six genes (*COL11A1*, *PRPF18*, *SPOP*, *CDH19*, *HIST1H2AM*, *CELP*) approached the definition of an SMG. In light of the newly described paradigm that most human tumors are typified by mutation (“M” class tumors) or by copy number alteration (“C” class tumors) but generally not by both [13], the relatively low mutational load of serous ECs might reflect the observation that they are frequently copy number altered, as evidenced by widespread genomic gains and losses [7,9,12]. Thus far, more than 30 amplified regions and 30 deleted regions have been annotated as statistically significant copy number altered regions in serous EC [7,9,12]. Some of these regions encompass only a single gene whereas others encompass more than 1,000 genes [12]. Uncovering the driver gene(s) targeted by these changes will be challenging. Nonetheless it is noteworthy that the *MYC* and *CCNE1* oncogenes [7,9], which encode oncoproteins regulated by the SCF^{FBXW7} ubiquitin ligase, as well as *CHD4* and *MBD3* which encode two subunits of the NuRD chromatin remodeling complex [9], and

PIK3CA, which encodes the catalytic subunit of PI3-kinase [9] reside with statistically significant regions of copy number alteration. Because mutations in *FBXW7*, *CHD4*, and the *PIK3CA* have been implicated in serous EC [8,14–16], copy number alterations affecting components of the associated pathways or processes may also point to driver events.

The genomic landscape of endometrioid endometrial carcinomas

Longstanding observations have firmly established that endometrioid ECs are frequently driven by mismatch repair defects resulting in microsatellite instability (MSI), and by somatic mutations in the PI3K-PTEN-AKT and RAS-RAF-MEK-ERK pathways, in the *FGFR2* receptor tyrosine kinase, in *CTNNB1* (β -catenin) within the canonical WNT signaling pathway, and in *ARID1A* which encodes a subunit (BAF250A) of the SWI-SNF chromatin remodeling complex (reviewed in [6]). Recent work from our own laboratory has also implicated the mutational disruption of *FBXW7* and *CHD4* in endometrioid EC [8].

The first whole exome sequencing studies of endometrioid ECs collectively interrogated 33 cases [10,11]. Among the most highly mutated genes identified in these studies were *PTEN*, *PIK3CA*, *ARID1A*, *CTNNB1*, *MLL2*, *FBXW7*, *RNF43*, *APC*, *FGFR2*, *EGFR*, and *KRAS* [11]. In addition to *ARID1A*, other candidate driver genes that were mutated in endometrioid ECs and had an effect on the viability of BaF3 cells either upon shRNA depletion or overexpression of the wildtype counterpart, were *INHBA*, *KMO*, *TLL5*, *GRM8*, *IGFBP3*, *AKTIP*, *PHKA2*, *TRPS1*, and *WNT11* [10]. In addition, Kinde et al., [11], noted that endometrioid EC exomes were genomically heterogeneous and could be classified into two groups, one that was highly mutated and MSI+ (median 674 mutations per tumor; range 164 to 4629) and another that was non-highly mutated and microsatellite stable (median 32 mutations per tumor; range 7 to 50).

Classification of serous and endometrioid ECs into four distinct genomic subgroups

The extent of genomic heterogeneity both among endometrioid ECs, and between endometrioid and serous ECs, was further clarified by the integrated genomic analysis of endometrioid and serous ECs reported by TCGA [12], which led to their stratification into four distinct molecular subgroups: ultramutated/POLE-mutant, hypermutated/MSI+, copy number low/microsatellite stable (MSS), and copy number high/serous-like. The genomic landscapes of the four subgroups differ one from another, resulting in a molecular classification that is distinct from the overlaying histological classification.

Defining features of the ultramutated EC subgroup are somatic mutations within the exonuclease domain of *POLE* in conjunction with a mutation rate of 232 mutations per Mb [12]. Therefore, sequencing of *POLE* alone is insufficient to classify a tumor as ultramutated, since the mutation rate also needs to be considered. This is an important consideration in the design of studies aimed at testing the proposal that ultramutated ECs exhibit a more favorable progression-free survival than the other molecular subgroups [12]. Ultramutated ECs are also characterized by a specific mutation signature, which represents one of six mutational signatures observed in endometrial cancer, with mismatch repair

defects, spontaneous deamination of 5-methyl-cytosine, and upregulation of APOBEC activity attributed to three of the remaining signatures [12,17,18]. Overall, 190 SMGs have been annotated among the 17 ultramutated endometrial tumors described thus far (Figure 1) [12].

The genomic landscape of hypermutated/MSI+ tumors is typified by a high mutation rate and a microsatellite instability phenotype that is indicative of defective mismatch repair resulting from MLH1 silencing [12,19,20]. Although MSI-positivity has been recognized in endometrial cancers for many years [20], whole exome sequencing has provided the first comprehensive insights into the genes that frequently accumulate somatic mutations resulting from mismatch repair defects [11,12,19]. Moreover, in terms of interpreting MSI-positivity as -high, -low, or -stable, using the Bethesda panel of microsatellite markers, Kim et al., have shown that MSI-high endometrial carcinomas have elevated (hypermutated) mutation rates, whereas MSI-low endometrial carcinomas have mutation rates that are not significantly different to MSI-stable tumors [19]. Overall, 21 SMGs have been described in hypermutated/MSI+ ECs (Figure 1), including ten genes with no previously known role in EC (Figure 2). Among the protein-encoding genes that are newly recognized as frequently undergoing MSI-associated frameshift mutations are *RPL22* [12,21], *CTCF* [22], *JAK1* [19] and *TFAM* [19] (Figure 2). Other SMGs that are mutated in at least 20% of hypermutated-MSI tumors are *PTEN*, *PIK3CA* and *PIK3R1* within the PI3K kinase pathway, *KRAS* within the MAPK pathway, *ARID1A* and *ARID5B* which encode ARID family members that remodel chromatin and regulate transcription, and *CTNNB1* (β -catenin) which functions within the canonical WNT signaling pathway [12] (Figure 1). These genes are noteworthy because of their potential therapeutic relevance. Therapeutic agents that target the PI3K and/or MAPK pathways are already in clinical trials for endometrial cancer [2]. There is also considerable interest in drugging the WNT pathway in cancer [23]. Moreover, recent preclinical studies suggest that ARID1A-mutated cancer cells may be dependent on the activity of ARID1B for their survival, thus implicating ARID1B as a potential therapeutic target in this genomic context [24].

Copy number low/MSS tumors are typified by genomic stability and microsatellite stability, and a mutation rate of 2.3 mutations per Mb [12]. In the TCGA study, the copy number low/MSS subgroup was predominated by grade 1 and grade 2 endometrioid ECs. Overall, sixteen SMGs have been defined among copy number low/MSS ECs. Among these genes are six novel candidate driver genes that exhibit non-random mutation patterns, indicative of deleterious mutations (Figure 3). Approximately half of all tumors within this subgroup have sustained a somatic mutation in *CTNNB1* (β -catenin) (Figure 1), and more than 90% exhibit a genomic alteration within the *PIK3CA-PIK3R1-PTEN* axis [12].

Copy number high/serous like tumors are genomically unstable and are almost all *TP53* mutant [12]. In addition to *TP53*, the other major driver genes nominated in serous-like ECs are *PIK3CA*, *FBXW7*, *PTEN*, *PPP2R1A*, *PIK3R1*, *CHD4*, as was also observed for serous tumors [7–9,12] (Figure 1). In terms of histology, the serous-like subgroup is the most heterogeneous of the four molecular subgroups of EC, being composed of endometrioid, serous, and mixed histology cases [12]. Moreover, 19% of high-grade endometrioid ECs in the TCGA study were classified as copy number high/serous like, leading to the proposition

that it might be prudent to consider treating patients with endometrioid tumors that are copy number high/serous-like at the molecular level with therapeutic strategies typically used to treat serous EC [12].

The full catalogue of SMGs within each of the four molecular subgroups of endometrial cancer has been reported in detail in the original TCGA study [12]. Moreover, pan-cancer analyses [25], which systematically interrogated genomic datasets generated from tumors occurring at disparate anatomical sites, including endometrial cancer, have nominated additional SMGs that were not revealed by the analysis of a single tumor type [26–30].

Inherent limitations in the annotation of significantly mutated genes

It is important to acknowledge several caveats related to the nomination of SMGs [29]. First, the annotation of SMGs can differ depending on the algorithms used to call variants, the filtering strategy used to parse variant calls, and the statistical approaches, assumptions, and thresholds used to designate SMGs. Second, mutated genes that do not meet the definition of an SMG can nonetheless be pathogenic driver genes. Third, catalogues of SMGs reflect the cohorts of tumors from which they were generated; any bias in tumor selection may bias gene annotation. Finally, the number of tumors sequenced influences the statistical power to reliably detect genes that are only infrequently mutated within a given type of cancer. This is particularly relevant for common cancers, such as endometrial cancer, for which a small percent of cases can represent a large number of patients. Indeed, Lawrence et al., recently predicted that ~1,500 cases of endometrial cancer would need to be sequenced in order to attain 90% power to detect mutations in 90% of genes with a mutation frequency of 2% [27], making it likely that continued sequencing of endometrial tumors will uncover additional candidate driver genes.

Germline susceptibility to endometrial cancer

Most inherited ECs occur within the context of hereditary nonpolyposis colorectal cancer (HNPCC), a highly penetrant cancer syndrome in which carriers of mutations in mismatch repair genes are at increased risk of developing specific cancers including colorectal and endometrial cancer (Reviewed in [31]). An increased incidence of endometrial cancer has also been noted in Cowdens syndrome, which is linked to germline mutations in *PTEN*, but the risk of EC in this clinical context is still under debate [32–34].

Recently, whole genome sequencing of constitutional DNAs from individuals and families with early-onset, multiple colorectal adenomas revealed that germline mutations in the replicative polymerases *POLD1* (*POLD1*^{Ser478Asn}) and *POLE* (*POLE*^{Leu424Val}) increase the risk of carriers to develop colorectal adenomas and colorectal carcinomas, [35], in what is now coined “polymerase proofreading associated polyposis (PPAP)”. Notably, within this context, *POLD1* mutation carriers are also predisposed to endometrial cancer [35]. In contrast, the vast majority of *POLE*^{L424V} mutation carriers do not appear to be predisposed to endometrial cancer [35]. However, a recent analysis of a large multi-cancer family indicated that carriers of the *POLE*^{Asn363Lys} variant exhibit predisposition to colorectal cancer, endometrial cancer, and other cancers, suggesting possible genotype-phenotype correlations [36].

Concluding remarks

The past two years have witnessed remarkable advances in our understanding of the mutations acquired by endometrial cancer genomes. The challenge now will be to decipher the biological and clinical relevance of these findings, which is an arduous task but one that is motivated by the need to develop biomarkers that identify patients at high risk of disease recurrence, and to identify alternative therapeutic approaches to treat patients with clinically aggressive forms of the disease.

Acknowledgments

All authors are funded by the Intramural Research Program of the National Human Genome Research Institute at NIH.

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Ultramutated/POLE-mutant	Hypermutated/MSI	Copy number low/ MSS	Serous-like/ copy number high		
232 mutations/Mb	18 mutations/Mb	2.9 mutations/Mb	2.3 mutations/Mb		
190 SMGs, including:	21 SMGs:	16 SMGs:	8 SMGs:		
PTEN (94.1%) MUC5B (94.1%) CSMD3 (94.1%) MUC16 (94.1%) RGPD3 (94.1%) GPR112 (94.1%) CCNB3 (94.1%) SLITRK3 (94.1%) DNAH5 (94.1%) APOB (94.1%) LAMA2 (94.1%) NBEA (94.1%) LRP2 (88.2%) ZDBF2 (88.2%) KIF20B (88.2%) ASPM (88.2%) TEX15 (88.2%) APC (88.2%) PTPRZ1 (88.2%) DYNC2H1 (88.2%) ABCA5 (88.2%) SAMD9 (88.2%)	SI (82.3%) MSH4 (82.3%) BAI3 (82.3%) ANKRD30A (82.3%) DNAH7 (82.3%) NCAM2 (82.3%) CDH10 (82.3%) UNC13C (82.3%) MYO3A (82.3%) LRRIQ1 (82.3%) RYP2 (82.3%) XIRP2 (82.3%) EYS (82.3%) LYST (82.3%) PPFIA2 (82.3%) ZNF836 (82.3%) HMCN1 (82.3%) FBXW7 (82.3%) GK2 (82.3%) F8 (82.3%) ABCB5 (82.3%) DNAH8 (76.5%)	L1TD1 (76.5%) SYCP2 (76.5%) ANOS (76.5%) USH2A (76.5%) WDR87 (76.5%) LIFR (76.5%) GPR158 (76.5%) BRCA2 (76.5%) BRWD3 (76.5%) F5 (76.5%) CNTN6 (76.5%) RP1 (76.5%) MORC1 (76.5%) FANCB (76.5%) ABCA12 (76.5%) PRDM9 (76.5%) ZNF334 (76.5%) ZNF268 (76.5%) PPP1R3A (76.5%) GNPTAB (76.5%) LRCH2 (76.5%) MIR1277 (6.2%)	PTEN (87.7%) PIK3CA (53.8%) PIK3R1 (41.5%) ARID1A (36.9%) RPL22 (36.9%) KRAS (35.4%) ARID5B (23.1%) CTCF (23.1%) CTNNB1 (20.0%) ATR (18.5%) GIGYF2 (16.9%) CSDE1 (15.4%) FGFR2 (13.8%) CCND1 (12.3%) LIMCH1 (12.3%) RBMX (12.3%) NKAP (10.8%) HIST1H2DB (7.7%) TNFAIP6 (7.7%) MIR1277 (6.2%)	PTEN (76.7%) PIK3CA (53.3%) CTNNB1 (52.2%) ARID1A (42.2%) PIK3R1 (33.3%) CTCF (21.1%) KRAS (15.6%) FGFR2 (13.3%) CHD4 (12.2%) SPOP (10.0%) CSMD3 (10.0%) SOX17 (7.8%) SGK1 (6.7%) BCOR (6.7%) MECOM (4.4%) METTL14 (3.3%)	TP53 (91.7%) PIK3CA (46.7%) FBXW7 (21.7%) PPP2R1A (21.7%) PIK3R1 (13.3%) CHD4 (13.3%) PTEN (10.0%) CSMD3 (10.0%)

Figure 1. Graphic summary of the statistically significantly mutated genes (SMGs) identified by TCGA within the four molecular subgroups of endometrioid and serous endometrial carcinomas Genes that are mutated at statistically significantly higher rates than the background mutation rate for endometrial cancer are shown. For each of the four molecular subgroups of endometrial cancer (ultramutated/POLE-mutant, hypermutated/MSI+, copy number low/MSS, serous-like/copy number high), the mutation frequency (% of mutated tumors) for individual genes is displayed. For the ultramutated subgroup, the genes displayed represent the 66 most frequently mutated of the 190 SMGs in this subgroup.

Figure 2. Ten novel statistically significantly mutated genes (SMGs) identified among hypermutated/MSI+ endometrial carcinomas

The position of mutations is displayed relative to known functional domains of the encoded proteins. Each mutation is represented by a square: missense mutations (red squares); frameshift and nonsense mutations (yellow squares); insertions-deletions (green squares); splice site mutations (white squares). Mutation data were generated by TCGA and obtained via the cBioPortal for Cancer Genomics (URL: <http://www.cbioportal.org/public-portal/>). Abbreviations: ARID (AT-rich interaction domain), CH (Calponin homology), GYF (glycine-tyrosine-phenylalanine), HMG (High Mobility Group), LIM (Lin-11, Isl-1 and Mec-3 domains), Pkinase (Protein kinase), RRM (RNA recognition motif), zf (zinc finger).

Figure 3. Six novel statistically significantly mutated genes (SMGs) identified among copy number-low/MSS endometrial carcinomas

The position of mutations is displayed relative to known functional domains of the encoded proteins. Each mutation is represented by a square: missense mutations (red squares); frameshift and nonsense mutations (yellow squares); insertions-deletions (green squares); splice site mutations (white squares). Mutation data were generated by TCGA and obtained via the cBioPortal for Cancer Genomics (URL: <http://www.cbioportal.org/public-portal/>). Abbreviations: Ank (Ankryin), HMG (High Mobility Group), Pkinase (Protein kinase), TAD (Transactivation Domain).