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RNASeq analysis reveals biological processes governing the clinical behavior of endometrioid and serous endometrial cancers

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Abstract

Background—Endometrial carcinoma comprises a group of tumors with distinct histologic and molecular features, and clinical behavior. Here we sought to define the biological processes that govern the clinical behavior of endometrial cancers.

Methods—Sixteen prototype genes representative of different biological processes that would likely play a role in endometrial and other hormone-driven cancers were defined. RNA-sequencing gene expression data from 323 endometrial cancers from The Cancer Genome Atlas were used to determine the transcription module of each prototype gene. The expression of prototype genes and modules and their association with outcome was assessed in univariate and multivariate survival analyses. The association of MSH6 expression with outcome was validated in an independent cohort of 243 primary endometrial cancers using immunohistochemistry.

Results—We observed that the clinical behavior of endometrial carcinomas as a group was associated with hormone receptor signaling, PI3K pathway signaling and DNA mismatch repair processes. When analyzed separately, in endometrioid carcinomas, hormone receptor, PI3K and

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Conflict of interest statement

The authors have no conflicts of interest to declare.

Appendix A. Supplementary data

DNA mismatch repair modules were significantly associated with outcome in univariate analysis, whereas the clinical behavior of serous cancers was likely governed by apoptosis and Wnt signaling. Multivariate survival analysis revealed that *MSH6* expression was associated with outcome of endometrial cancer patients independently from traditional prognostic clinicopathologic parameters, which was confirmed in an independent cohort at the protein level.

Conclusion—Endometrioid and serous endometrial cancers are underpinned by distinct molecular pathways. MSH6 expression levels may be associated with outcome in endometrial cancers as a group.

Keywords

endometrial cancer; gene expression; biological process; outcome

1. INTRODUCTION

Endometrial cancer (EC), the most common gynecologic malignancy in the USA, comprises a heterogeneous group of tumors with distinct histologic features, biological behavior and treatment response. Endometrioid and serous carcinomas account for the majority of ECs. Treatment decisions for patients with EC are primarily determined by surgical stage at presentation, histologic type and grade[1].

There is evidence to suggest that some molecular alterations are preferentially found in endometrioid endometrial carcinomas (EECs), including mutations in *PTEN* and *CTNNB1*, whereas others are more prevalent in serous endometrial carcinomas (SECs), such as *TP53* mutations[2,3]. These observations have been corroborated and expanded by the transcriptomic and genomic analyses of a large set of EECs and SECs carried out by The Cancer Genome Atlas (TCGA)[4]. The analyses performed by TCGA have led to an integrated genomic classification of EECs and SECs and the identification of the POLE (ultramutated), microsatellite instability (MSI) (hypermutated), copy-number low (endometrioid) and copy-number high (serous-like) subtypes, which have been shown to be underpinned by distinct combinations of genomic and epigenetic alterations[4].

Based on the molecular heterogeneity observed in ECs, we posited that the biological processes and pathways associated with outcome may be distinct between ECs of different histologic types, grades or integrated genomic subtypes, and that additional markers predictive of clinical behavior may be present in different subsets of the disease.

The aims of this study were i) to determine genes or gene expression modules representative of biological processes known to play a role in EC or in other hormone-driven cancers, and ii) to define the association of these genes and/or gene expression modules with outcome in ECs as a group, and in subgroups stratified according to histologic type, grade or integrated genomic types using RNA-sequencing data from the TCGA study[4].

2. METHODS

2.1. Transcription modules and univariate analysis

RNA-sequencing gene expression and clinicopathologic data including outcome from 323 ECs were retrieved from the TCGA data portal (https://tcga-data.nci.nih.gov/docs/publications/ucec_2013/; files ‘RNASeq’ and ‘Key Clinical Data’; accessed December 2015)[4](Supplementary Table 1). The RSEM normalized gene-level expression data were obtained. As described by TCGA[4], genes lacking HGNC annotation or with small expression values in at least one-fourth of the samples were removed. The expression values of the final set of 20,502 genes were log₂ transformed. We selected 16 ‘prototype’ genes representative of biological processes that have been shown to play a role in EC or other hormone-driven cancers. The details of the gene selection are described in the Supplementary Methods[3-5]. The transcription modules of each prototype gene, which comprise the genes specifically co-expressed with each prototype gene (Supplementary Tables 2 and 3), were defined essentially as described by Desmedt et al.[5] and are described in the Supplementary Methods. The R script for the univariate models, transcription module development and module score calculations is available in the Supplement.

2.2. Gene ontology enrichment analysis

Gene ontology enrichment analyses of the 16 transcription modules were performed using Cytoscape v.2.8.3 with the BinGO plugin (v.2.44)[6], and a hypergeometric test, with the False Discovery Rate controlled using the Benjamini and Hochberg procedure. Biological processes with a corrected $P < 0.05$ were deemed significant.

2.3. Tissue microarrays and immunohistochemistry

Tissue microarrays (TMAs) from the University Hospital Basel, Basel, Switzerland and University Hospital Virgen del Rocío, Seville, Spain containing two replicate 0.6 mm and 1.0 mm cores, respectively, from 276 ECs were constructed as previously described[7] and the expression levels of MSH6 protein were assessed by immunohistochemistry (Supplementary Methods). For the purpose of this study, only ECs of endometrioid (n=228) and serous (n=15) types based on review of the diagnostic histologic slides were included.

2.4. Statistics

A detailed description of the statistical methods employed is available in the Supplementary Methods. For the analysis of the RNA-sequencing gene expression data, univariate and multivariate analyses for overall survival were performed using the Cox proportional hazards regression model, with the survival data censored at 5 years (Supplementary Methods). Forest plots were generated using ggplot2 (<http://ggplot2.org/>) in R (<http://www.r-project.org/>; v 3.0.1). For the identification of the optimal cut-offs for survival analysis, the X-tile software[8] was employed, dividing the cohort into training and validation subsets (split sample approach; see Supplementary Methods). For the MSH6 protein expression analysis, disease-free survival was expressed as the number of months from diagnosis to the occurrence of distant or local relapse, as overall survival was not available for this cohort, using Kaplan–Meier method and log-rank test (Supplementary Methods).

3. RESULTS

3.1 Biological processes associated with outcome in EC

We selected 16 ‘prototype genes’ that are either recurrently (over-) expressed and/or amplified or targeted by mutations in ECs, or have been shown to be associated with outcome in breast cancer, which akin to EC, is a hormone-dependent disease (Supplementary Methods). Transcription modules representative of biological processes of these 16 prototype genes were defined (Supplementary Tables 2 and 3), and a module score[5] was determined for each of the transcription modules and used for univariate and multivariate survival analyses.

On univariate analysis, the clinicopathologic parameters International Federation of Gynecology and Obstetrics (FIGO) stage, age, histologic type and grade were significantly associated with progression-free and overall survival in this set of 323 ECs (Table 1; Supplementary Table 4). For the remaining analyses of this study, we focused on overall survival as the end point. In this cohort, in addition to the clinicopathologic parameters, the expression of both the prototype genes *CTNNB1*, *PIK3CA*, *ERBB2*, *ESR1*, *PGR*, and *MSH6* and their respective Wnt signaling, PI3K pathway, HER2 signaling, estrogen receptor (ER) signaling, progesterone (PR) signaling and *MSH6* DNA mismatch repair modules were associated significantly with outcome in univariate analysis, as was the chromatin organization *ARID1A* module ($P < 0.05$, Table 1, Supplementary Fig. 1). Prototype genes and transcription modules whose continuous values were significantly associated with overall survival were subsequently converted into categorical variables using X-tile. These survival analyses revealed that in ECs not only the prototype genes *ESR1*, *PGR*, *PIK3CA* and *MSH6* were significantly associated with overall survival ($P < 0.05$), but also the hormone receptor modules *ESR1* and *PGR*, whose expression is highly correlated (Supplementary Table 5), and the PI3K pathway (as defined by the *PIK3CA* module) and DNA mismatch repair (*MSH6*) modules (Fig. 1). Furthermore, the *ERBB2* prototype gene and the *ARID1A* module were found to be significantly associated with outcome in this EC dataset using a categorical analysis (Supplementary Fig. 2a). These data provide evidence to suggest that ECs with high gene expression levels of *ESR1* or *PGR*, and low gene expression levels of *PIK3CA* or *MSH6* have a more favorable outcome. Multivariate survival analysis including the clinicopathologic parameters revealed that the DNA *MSH6* prototype gene remained an independent predictor of overall survival in EC (Table 2). The *MSH6* module was of borderline significance ($P = 0.05$; Table 2); however, in a model omitting the *PIK3CA* module, which showed a strong correlation with the *MSH6* module, a statistically significant association between the *MSH6* DNA mismatch repair module and overall survival was observed ($P = 0.031$; Supplementary Tables 5 and 6).

3.2. Biological processes associated with outcome are distinct between EECs and SECs

Next we sought to define whether outcome of EECs ($n = 271$) and SECs ($n = 52$) would be governed by distinct biological processes. While in EECs the prototype genes *ESR1*, *PGR*, *PIK3CA*, and *PTEN*, the hormone receptor modules (i.e. *ESR1* and *PGR*) and the PI3K pathway signaling modules (i.e. *PIK3CA* and *PTEN*), and the prototype gene *MSH6* and the DNA mismatch repair module were associated significantly with outcome in univariate

analysis, in SECs only the apoptosis-related *CASP3* and *CTNNB1* prototype genes and the Wnt signaling module were significantly associated with overall survival ($P<0.05$; Table 1, Supplementary Fig. 1). Survival analysis using the X-tile threshold values of the prototype genes and transcription modules revealed that in EECs the prototype genes *ESR1* and *PGR* and their respective hormone receptor modules, as well as the prototype genes *PTEN* and *MSH6*, and the DNA mismatch repair module were significantly associated with overall survival ($P<0.05$; Fig. 2). None of the biological processes or prototype genes identified to be governing clinical behavior of SECs in univariate analysis were found to be significant when used as categorical variables as defined by the X-tile software (data not shown). Multivariate analysis correcting for age, grade and stage revealed that the prototype gene and PI3K pathway module *PTEN* and the *MSH6* prototype gene were independent predictors of outcome in EECs (Table 2).

3.3. Biological processes associated with outcome in ECs of distinct grades and genomic subtypes

The stratification of all ECs according to grade revealed that, in univariate survival analysis, the tumor invasion and metastasis module and the prototype gene *PLAU* were significantly associated with outcome in grade 3 ECs, the prototype gene and module *ESR1* and the prototype gene *CTNNB1* were significantly associated with outcome in grade 2 ECs, however no prototype genes or transcription modules governing overall survival in grade 1 ECs were identified (Supplementary Table 7, Supplementary Fig. 2b). Likewise, stratification into the genomic subtypes as described by TCGA[4] showed that the chromatin organization module and prototype gene *ARID1A* and the Wnt signaling module *CTNNB1* were significantly associated with the overall survival in ECs of copy-number high (serous-like) subtype (Supplementary Table 7, Supplementary Fig. 2c) but no associations with other genomics subtypes were found. It should be noted, however, that with the exception of grade in the copy-number low cancers none of the clinicopathologic parameters were significantly associated with overall survival in the EC genomic subgroups (Supplementary Table 7).

3.4. MSH6 protein expression is associated with outcome in EC

To independently validate our RNA-sequencing-based findings, we selected the only prototype gene found to be an independent predictor of outcome in ECs in multivariate analysis, namely the prototype gene *MSH6*. To assess whether high levels of MSH6 protein expression were also associated with outcome in EC, we performed MSH6 immunohistochemical analysis in an independent series of ECs (n=228 EECs, n=15 SECs; Supplementary Table 8). Akin to the associations with outcome observed in the TCGA series, the clinicopathologic parameters FIGO stage, age, histologic type and grade were significantly associated with disease-free survival in this set of 243 ECs (Supplementary Fig. 3). We further observed that high MSH6 protein expression levels were significantly associated with disease-free survival in univariate and multivariate analyses in ECs (Fig. 3; Table 3), confirming our observations made based on *MSH6* mRNA analysis. Finally, MSH6 protein levels were found to be significantly associated with disease-free survival in the 228 EECs of the TMA cohort on univariate analysis, but not on multivariate analysis (Supplementary Fig. 4, Supplementary Table 9).

4. DISCUSSION

Here we demonstrate through the analysis of prototype genes and gene expression modules that low levels of *ESR1* and *PGR* gene expression and their respective gene expression modules are associated with less favorable outcome in ECs in general as well as in EECs. Our findings corroborate and expand on observations demonstrating that PR-negativity as determined by immunohistochemistry is an independent prognostic factor for disease-free survival in patients with EEC[9,10]. Prospective assessment of ER and PR expression using pre-determined cut-offs would be required, however, to ascertain their value as prognostic biomarkers[11].

Our findings expand on the observations that EECs and SECs are distinct. Here we observed that PI3K signaling and DNA mismatch repair processes may play a role in the clinical behavior of EECs, as opposed to Wnt signaling (*CTNNB1*) and apoptosis (*CASP3*)-related processes in SECs. In a subset of ER-negative breast cancers, increased expression of the Wnt/ β -catenin signaling transcriptome has been observed[12], although activation of the canonical Wnt pathway through mutations seems to be rare in breast cancer. A similar phenomenon may be observed in SECs as these rarely display β -catenin nuclear expression (<3%) and only up to 3% harbor *CTNNB1* mutations[4,13-15]. Of note, high levels of caspase-3 protein expression as determined by immunohistochemistry have been shown to be associated with favorable prognosis in high-grade serous ovarian cancer[16,17].

Here we provide transcriptomic evidence to demonstrate that the *PIK3CA* prototype gene and pathway module are associated with overall survival in EC. While the *PTEN* prototype gene and module expression were found to be independent prognostic factors of overall survival in patients with EEC, neither *PTEN* nor *PIK3CA* prototype genes or modules were found to be associated with the outcome of SECs, despite the presence of *PIK3CA* mutations in approximately 40% of cases[4,18]. The prognostic value of loss of PTEN protein expression in EC has proven controversial and conflicting results have been reported[19], which may in part be due to the challenges posed by PTEN immunohistochemical analysis[20] and the lack of standardized thresholds for its interpretation. It should be noted that expression levels of the *PIK3CA* prototype gene and module were found not to correlate with the *PIK3CA* gene mutation status (Supplementary Fig. 5), which is in agreement with previous reports based on the analysis of breast cancers[21]. Our results support previous reports suggesting that co-occurring mutations and epigenetic aberrations affecting different components of the PI3K pathway may converge on regulation of PTEN expression levels and function in ECs[22].

The gene expression module and prototype gene *MSH6* were found to be an independent predictor of outcome in this large dataset of ECs, where cases with low *MSH6* expression have a more favorable clinical behavior. This observation was confirmed at the protein level in an independent cohort of ECs. Whilst germline mutations in *MSH6* are associated with increased risk for Lynch syndrome-associated EC[23], the role of MSH6 in sporadic EC is less well understood. *MSH6* promoter hypermethylation seems to be rare in EC[24] and *MSH6* somatic mutations were found in 7% of cases included in the TCGA study (cBioPortal, <http://www.cbioportal.org/>, accessed May 2015)[4,25], however these did not

correlate with *MSH6* prototype or module expression (Supplementary Fig. 5). Of note, the *MSH6* expression module included *MCM3* and *MCM6*, and *MCM2*, *MCM3* and *MCM7* have been suggested to act as proliferation markers in the endometrium and in EC[26,27]. This is of interest given that, in contrast to ER-positive breast cancer where the expression of proliferation-related genes is an important determinant of outcome[5], no significant association of the proliferation module and survival in ECs was found. These data provide evidence to suggest that the impact of proliferation on the outcome of these two hormone-driven cancers is likely distinct. In addition, further studies are warranted to determine the biological basis of the high expression of the *MSH6* prototype gene in sporadic ECs with a poor outcome.

The limitations of our study include the analysis of a retrospective cohort, of which a subset received adjuvant chemotherapy with or without radiation therapy; however the therapies could not be included in the survival model as the adjuvant treatment information is not available for a large subset of patients in the TCGA study[4]. In addition, our analysis was performed assessing mRNA expression levels, which do not always translate into protein expression changes; however, similar analyses of breast cancers have yielded important insights for the identification of biological processes and transcriptional modules that define the outcome of hormone receptor disease[5] and the observations related to the *MSH6* prototype gene expression were validated at the protein level. Regrettably, validation of the transcriptomic results derived from the analysis of the TCGA dataset could not be performed, given the lack of sufficiently-sized publicly-available independent EC gene expression datasets with follow-up information. Moreover, the composition of ECs in terms of grade was different between our validation cohort and the TCGA dataset, as the latter was enriched for grade 3 ECs[4]. Finally, our study was limited to EECs and SECs; additional studies investigating the biological processes that govern the outcome of clear cell carcinomas and carcinosarcomas are warranted.

Despite these limitations, here we demonstrate that the expression of the DNA mismatch repair *MSH6* gene and its expression module are independent predictors of outcome in EC, and that the clinical behavior of EECs and SECs is governed by distinct processes. Given the molecular diversity of ECs, the development of prognostic models taking into account molecular features in addition to the current anatomical prognostic factors is warranted, as is the stratification of ECs not only according to histologic type, but also molecular characteristics in studies aiming to define novel markers of prognosis and therapy response in EC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Abu-Rustum NR, Zhou Q, Gomez JD, Alektiar KM, Hensley ML, Soslow RA, et al. A nomogram for predicting overall survival of women with endometrial cancer following primary therapy: toward improving individualized cancer care. *Gynecol Oncol.* 2010; 116(3):399–403. [PubMed: 20022094]
2. Matias-Guiu X, Prat J. Molecular pathology of endometrial carcinoma. *Histopathology.* 2013; 62(1): 111–23. [PubMed: 23240673]
3. Murali R, Soslow RA, Weigelt B. Classification of endometrial carcinoma: more than two types. *Lancet Oncol.* 2014; 15(7):e268–78. [PubMed: 24872110]
4. Cancer Genome Atlas Research Network. Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated genomic characterization of endometrial carcinoma. *Nature.* 2013; 497(7447):67–73. [PubMed: 23636398]
5. Desmedt C, Haibe-Kains B, Wirapati P, Buyse M, Larsimont D, Bontempi G, et al. Biological processes associated with breast cancer clinical outcome depend on the molecular subtypes. *Clin Cancer Res.* 2008; 14(16):5158–65. [PubMed: 18698033]
6. Smoot ME, Ono K, Ruscheinski J, Wang PL, Ideker T. Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics.* 2011; 27(3):431–2. [PubMed: 21149340]
7. Biscuola M, Van de Vijver K, Castilla MA, Romero-Perez L, Lopez-Garcia MA, Diaz-Martin J, et al. Oncogene alterations in endometrial carcinosarcomas. *Hum Pathol.* 2013; 44(5):852–9. [PubMed: 23199529]
8. Camp RL, Dolled-Filhart M, Rimm DL. X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. *Clin Cancer Res.* 2004; 10(21):7252–9. [PubMed: 15534099]
9. Huvila J, Talve L, Carpen O, Edqvist PH, Ponten F, Grenman S, et al. Progesterone receptor negativity is an independent risk factor for relapse in patients with early stage endometrioid endometrial adenocarcinoma. *Gynecol Oncol.* 2013; 130(3):463–9. [PubMed: 23777659]
10. Jongen V, Briet J, de Jong R, ten Hoor K, Boezen M, van der Zee A, et al. Expression of estrogen receptor-alpha and -beta and progesterone receptor-A and -B in a large cohort of patients with endometrioid endometrial cancer. *Gynecol Oncol.* 2009; 112(3):537–42. [PubMed: 19108875]
11. Salvesen HB, Haldorsen IS, Trovik J. Markers for individualised therapy in endometrial carcinoma. *Lancet Oncol.* 2012; 13(8):e353–61. [PubMed: 22846840]
12. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest.* 2011; 121(7):2750–67. [PubMed: 21633166]
13. McConechy MK, Ding J, Cheang MC, Wiegand KC, Senz J, Tone AA, et al. Use of mutation profiles to refine the classification of endometrial carcinomas. *J Pathol.* 2012; 228(1):20–30. [PubMed: 22653804]
14. Moreno-Bueno G, Hardisson D, Sanchez C, Sarrio D, Cassia R, Garcia-Rostan G, et al. Abnormalities of the APC/beta-catenin pathway in endometrial cancer. *Oncogene.* 2002; 21(52): 7981–90. [PubMed: 12439748]
15. Dedes KJ, Watterskog D, Ashworth A, Kaye SB, Reis-Filho JS. Emerging therapeutic targets in endometrial cancer. *Nat Rev Clin Oncol.* 2011; 8(5):261–71. [PubMed: 21221135]
16. Materna V, Surowiak P, Markwitz E, Spaczynski M, Drag-Zalesinska M, Zabel M, et al. Expression of factors involved in regulation of DNA mismatch repair- and apoptosis pathways in ovarian cancer patients. *Oncol Rep.* 2007; 17(3):505–16. [PubMed: 17273726]

17. Espinosa I, Catusas L, Canet B, D'Angelo E, Munoz J, Prat J. Gene expression analysis identifies two groups of ovarian high-grade serous carcinomas with different prognosis. *Mod Pathol*. 2011; 24(6):846–54. [PubMed: 21317880]
18. Urick ME, Rudd ML, Godwin AK, Sgroi D, Merino M, Bell DW. PIK3R1 (p85alpha) is somatically mutated at high frequency in primary endometrial cancer. *Cancer Res*. 2011; 71(12):4061–7. [PubMed: 21478295]
19. Mackay HJ, Gallinger S, Tsao MS, McLachlin CM, Tu D, Keiser K, et al. Prognostic value of microsatellite instability (MSI) and PTEN expression in women with endometrial cancer: results from studies of the NCIC Clinical Trials Group (NCIC CTG). *Eur J Cancer*. 2010; 46(8):1365–73. [PubMed: 20304627]
20. Pallares J, Bussaglia E, Martinez-Guitarte JL, Dolcet X, Llobet D, Rue M, et al. Immunohistochemical analysis of PTEN in endometrial carcinoma: a tissue microarray study with a comparison of four commercial antibodies in correlation with molecular abnormalities. *Mod Pathol*. 2005; 18(5):719–27. [PubMed: 15578076]
21. Palimaru I, Bruggmann A, Wium-Andersen MK, Nexø E, Sørensen BS. Expression of PIK3CA, PTEN mRNA and PIK3CA mutations in primary breast cancer: association with lymph node metastases. *Springerplus*. 2013; 2:464. [PubMed: 24083111]
22. Cheung LW, Hennessy BT, Li J, Yu S, Myers AP, Djordjevic B, et al. High frequency of PIK3R1 and PIK3R2 mutations in endometrial cancer elucidates a novel mechanism for regulation of PTEN protein stability. *Cancer Discov*. 2011; 1(2):170–85. [PubMed: 21984976]
23. Baglietto L, Lindor NM, Dowty JG, White DM, Wagner A, Gomez Garcia EB, et al. Risks of Lynch syndrome cancers for MSH6 mutation carriers. *J Natl Cancer Inst*. 2010; 102(3):193–201. [PubMed: 20028993]
24. Goodfellow PJ, Buttin BM, Herzog TJ, Rader JS, Gibb RK, Swisher E, et al. Prevalence of defective DNA mismatch repair and MSH6 mutation in an unselected series of endometrial cancers. *Proc Natl Acad Sci U S A*. 2003; 100(10):5908–13. [PubMed: 12732731]
25. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*. 2013; 6(269):p11. [PubMed: 23550210]
26. Kato K, Toki T, Shimizu M, Shiozawa T, Fujii S, Nikaido T, et al. Expression of replication-licensing factors MCM2 and MCM3 in normal, hyperplastic, and carcinomatous endometrium: correlation with expression of Ki-67 and estrogen and progesterone receptors. *Int J Gynecol Pathol*. 2003; 22(4):334–40. [PubMed: 14501812]
27. Li SS, Xue WC, Khoo US, Ngan HY, Chan KY, Tam IY, et al. Replicative MCM7 protein as a proliferation marker in endometrial carcinoma: a tissue microarray and clinicopathological analysis. *Histopathology*. 2005; 46(3):307–13. [PubMed: 15720416]

HIGHLIGHTS

- Endometrioid and serous carcinomas are underpinned by distinct molecular pathways.
- PI3K and DNA mismatch repair modules govern the outcome of endometrioid cancers.
- The clinical behavior of serous cancers is defined by apoptosis and Wnt signaling.
- MSH6 expression is associated with outcome of patients with endometrial carcinomas.

Fig. 1. Overall survival curves of module scores and prototype genes in endometrial carcinomas
 The optimal cut-point for stratification was determined using X-tile [8] for module scores and prototype genes of a, *PGR*, b, *ESR1*, c, *PIK3CA*, and d, *MSH6*. Monte Carlo-corrected P -values of the log-rank test are shown. Categorical values obtained with the split-sample approach of X-tile (i.e. low- and high-levels) of prototype genes and module scores were employed in the Kaplan-Meier curves.

Fig. 2. Overall survival curves of module scores and prototype genes in endometrial cancers of endometrioid histology

The optimal cut-point for stratification was determined using X-tile [8] for module scores and prototype genes of a, *PGR*, b, *ESR1*, c, *PTEN*, and d, *MSH6*. Monte Carlo-corrected P -values of the log-rank test are shown. Categorical values obtained with the split-sample approach of X-tile (i.e. low- and high-levels) of prototype genes and module scores were employed in the Kaplan-Meier curves.

Fig. 3. MSH6 protein expression and association with outcome in endometrial cancers
 a, Representative micrographs of endometrial cancers with low/absent levels of MSH6 expression and high levels of MSH6 expression as assessed by immunohistochemistry on tissue microarrays. b, Disease-free survival curves of MSH6 protein expression in an independent series of 243 endometrial cancers. *P*-value of the log-rank test is shown. Categorical values for MSH6 (i.e. MSH6 high expression (Allred score ≥ 6) and MSH6 low expression (Allred score of <6)) were employed in the Kaplan-Meier curves.

Table 1

Univariate Cox's regression analysis of 5-year overall survival in endometrial cancer patients (TCGA) including standard clinicopathologic parameters, prototype genes and module scores.

	All cases (n=323)		Endometrioid cancers (n=271)		Serous cancers (n=52)	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Age	1.047 (1.011-1.083)	0.009	1.041 (1.001-1.082)	0.044	1.034 (0.94-1.137)	0.493
Grade	2.935 (1.586-5.433)	0.001	2.866 (1.496-5.493)	0.002		
Histology	2.191 (1.042-4.606)	0.039				
FIGO stage	2.072 (1.553-2.763)	<0.001	1.966 (1.401-2.759)	<0.001	2.324 (1.117-4.837)	0.024
Prototype ESR1	0.830 (0.729-0.945)	0.005	0.788 (0.664-0.935)	0.006	1.056 (0.743-1.5)	0.763
Prototype PGR	0.888 (0.804-0.982)	0.021	0.836 (0.733-0.955)	0.008	1.223 (0.93-1.608)	0.150
Prototype PTEN	0.722 (0.501-1.039)	0.079	0.662 (0.446-0.984)	0.041	0.968 (0.293-3.194)	0.958
Prototype PIK3CA	1.477 (1.04-2.097)	0.029	2.015 (1.089-3.729)	0.026	0.787 (0.348-1.78)	0.565
Prototype CTNNB1	1.942 (1.206-3.128)	0.006	1.748 (0.991-3.084)	0.054	2.632 (1.048-6.612)	0.039
Prototype ARID1A	1.433 (0.886-2.319)	0.143	1.329 (0.744-2.374)	0.336	1.356 (0.553-3.323)	0.506
Prototype MLH1	1.061 (0.845-1.332)	0.611	0.982 (0.771-1.25)	0.881	1.581 (0.431-5.803)	0.490
Prototype MSH6	2.119 (1.297-3.461)	0.003	2.072 (1.113-3.859)	0.022	1.731 (0.64-4.678)	0.279
Prototype POLE	1.178 (0.741-1.874)	0.488	1.114 (0.646-1.921)	0.699	1.311 (0.481-3.576)	0.597
Prototype TP53	1.041 (0.685-1.583)	0.851	0.774 (0.441-1.358)	0.371	1.506 (0.819-2.769)	0.187
Prototype ERBB2	1.283 (1.024-1.608)	0.030	1.262 (0.741-2.147)	0.392	1.146 (0.832-1.577)	0.404
Prototype AURKA	1.286 (0.902-1.833)	0.165	1.332 (0.859-2.066)	0.200	0.550 (0.209-1.447)	0.226
Prototype PLAU	0.809 (0.598-1.095)	0.170	0.804 (0.563-1.149)	0.231	0.805 (0.445-1.455)	0.473
Prototype STAT1	1.058 (0.781-1.432)	0.718	0.987 (0.653-1.493)	0.952	0.765 (0.448-1.307)	0.328
Prototype VEGF	1.143 (0.832-1.571)	0.410	1.198 (0.814-1.764)	0.359	0.971 (0.554-1.703)	0.919
Prototype CASP3	0.728 (0.359-1.476)	0.379	1.536 (0.61-3.87)	0.362	0.282 (0.096-0.825)	0.021
Module ESR1	0.991 (0.985-0.998)	0.007	0.989 (0.981-0.997)	0.006	1.019 (0.995-1.044)	0.118
Module PGR	0.993 (0.988-0.998)	0.003	0.991 (0.984-0.997)	0.004	1.016 (0.995-1.038)	0.134
Module PTEN	0.840 (0.703-1.003)	0.054	0.806 (0.664-0.978)	0.029	0.918 (0.518-1.626)	0.769
Module PIK3CA	1.010 (1.004-1.017)	0.002	1.011 (1.003-1.02)	0.011	1.005 (0.992-1.018)	0.469
Module CTNNB1	1.006 (1.001-1.011)	0.015	1.004 (0.999-1.01)	0.136	1.013 (1-1.026)	0.046
Module ARID1A	1.024 (1.003-1.045)	0.024	1.016 (0.992-1.04)	0.191	1.050 (0.996-1.107)	0.069
Module MLH1	1.017 (0.942-1.097)	0.675	0.976 (0.899-1.059)	0.557	1.429 (0.795-2.566)	0.232
Module MSH6	1.117 (1.041-1.198)	0.002	1.103 (1.015-1.2)	0.021	1.115 (0.928-1.338)	0.245
Module POLE	1.005 (0.998-1.012)	0.156	1.005 (0.997-1.013)	0.249	0.996 (0.977-1.015)	0.688
Module TP53	1.041 (0.685-1.583)	0.851	0.774 (0.441-1.358)	0.371	1.506 (0.819-2.769)	0.187
Module ERBB2	1.113 (1.031-1.201)	0.006	1.175 (0.98-1.409)	0.081	1.052 (0.937-1.182)	0.390
Module AURKA	1.002 (1-1.004)	0.058	1.002 (1-1.005)	0.109	0.999 (0.991-1.006)	0.709
Module PLAU	0.945 (0.815-1.097)	0.461	0.914 (0.765-1.092)	0.322	0.973 (0.727-1.301)	0.852
Module STAT1	0.999 (0.991-1.007)	0.835	0.999 (0.988-1.01)	0.812	0.990 (0.976-1.004)	0.144
Module VEGF	1.040 (0.904-1.196)	0.581	1.062 (0.895-1.259)	0.490	1.014 (0.802-1.282)	0.909

	All cases (n=323)		Endometrioid cancers (n=271)		Serous cancers (n=52)	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
Module CASP3	0.851 (0.623-1.164)	0.313	1.062 (0.684-1.648)	0.789	0.738 (0.475-1.147)	0.177

All module scores and prototype genes are considered as continuous variables. Statistically significant *P*-values are highlighted in bold. CI, confidence interval; HR, hazard ratio; TCGA, The Cancer Genome Atlas.

Table 2

Multivariate Cox's regression analysis of 5-year overall survival in endometrial cancer patients (TCGA) including standard clinicopathologic parameters, prototype genes and module scores.

All cases (n=323) – Prototype genes			All cases (n=323) - Modules		
	HR (95% CI)	<i>P</i>		HR (95% CI)	<i>P</i>
Age	1.070 (1.027-1.114)	0.001	Age	1.059 (1.019-1.101)	0.004
Grade	1.787 (0.886-3.602)	0.105	Grade	1.904 (0.939-3.863)	0.074
Histology	0.366 (0.146-0.920)	0.032	Histology	0.346 (0.126-0.954)	0.040
FIGO stage	1.951 (1.383-2.751)	<0.001	FIGO stage	1.918 (1.366-2.693)	<0.001
Prototype ESR1	0.629 (0.231-1.714)	0.364	Module ESR1	1.864 (0.452-7.689)	0.389
Prototype PGR	1.291 (0.479-3.478)	0.614	Module PGR	0.447 (0.112-1.786)	0.254
Prototype PIK3CA	1.030 (0.443-2.393)	0.945	Module PIK3CA	1.076 (0.455-2.544)	0.868
Prototype MSH6	3.542 (1.504-8.339)	0.004	Module MSH6	2.406 (0.999-5.794)	0.050

Endometrioid cancers (n=271) – Prototype genes			Endometrioid cancers (n=271) - Modules		
	HR (95% CI)	<i>P</i>		HR (95% CI)	<i>P</i>
Age	1.074 (1.029-1.120)	0.001	Age	1.073 (1.027-1.121)	0.002
Grade	1.612 (0.772-3.365)	0.203	Grade	1.891 (0.919-3.891)	0.084
FIGO stage	2.167 (1.411-3.328)	<0.001	FIGO stage	2.104 (1.365-3.242)	0.001
Prototype ESR1	0.842 (0.281-2.525)	0.759	Module ESR1	1.547 (0.250-9.558)	0.639
Prototype PGR	1.1164 (0.398-3.406)	0.782	Module PGR	0.528 (0.085-3.282)	0.493
Prototype PTEN	0.370 (0.148-0.923)	0.033	Module PTEN	0.240 (0.095-0.605)	0.002
Prototype MSH6	3.705 (1.265-10.854)	0.017	Module MSH6	1.689 (0.637-4.480)	0.293

All module scores and prototype genes are considered as categorical variables. Statistically significant *P*-values are highlighted in bold. CI, confidence interval; HR, hazard ratio; TCGA, The Cancer Genome Atlas.

Table 3

Multivariate Cox's regression analysis of disease-free survival in endometrial cancer patients of the validation series including standard clinicopathologic parameters and MSH6 protein expression assessed by immunohistochemical analysis.

All endometrial cancers validation series (n=243)		
	HR (95% CI)	P
Age	2.552 (1.359-4.793)	0.004
Grade	1.834 (1.225-2.747)	0.003
Histology	1.092 (0.445-2.680)	0.848
FIGO stage	2.025 (1.387-2.956)	<0.001
MSH6 protein expression (high vs low)	2.150 (1.104-4.190)	0.024

Statistically significant *P*-values are highlighted in bold. CI, confidence interval; HR, hazard ratio.