Integrating Pathology, Chromosomal Instability and Mutations for Risk Stratification in Early-stage Endometrioid Endometrial Carcinoma

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Research

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Abstract

**Background:** Risk stratifications for endometrial carcinoma (EC) depend on histopathology and molecular pathology. Histopathological risk stratification lacks reproducibility, neglects heterogeneity and contributes little to surgical procedures. Existing molecular stratification is useless in patient with specific pathological or molecular characteristics, and cannot guide postoperative adjuvant radiotherapies. Chromosomal instability (CIN), the numerical and structural alterations of chromosome resulting from ongoing errors of chromosome segregation, is an intrinsic biological mechanism for the evolution of different prognostic factors of histopathology and molecular pathology, which may be applicable to the risk stratification of EC.

**Results:** By analysis of CIN25 and CIN70, two reliable gene expression signatures for CIN, we found that EC with unfavorable prognostic factors of histopathology or molecular pathology had serious CIN. However, POLE-mutant, as a favorable prognostic factor, had elevated CIN signatures, and CTNNB1-mutant, as an unfavorable prognostic factor, had decreased CIN signatures. Only if these two mutations were excluded were CIN signatures strongly prognostic for outcomes in different adjuvant radiotherapy subgroups. Integrating pathology, CIN signatures and POLE / CTNNB1 mutation stratified stageendometrioid EC into four groups with improved risk prognostication and treatment recommendations.

**Conclusions:** We revealed the possibility of integrating histopathology and molecular pathology by CIN for risk stratification in early-stage EC. Our integrated risk model deserves for further improvement and validation.

**Background**

Endometrial carcinoma (EC) is the sixth most common malignant tumor in females worldwide and the second most common in the female reproductive system (1). The risk stratification of EC is the prerequisite for the accurate evaluation of prognosis, and its ultimate goal is to improve the outcome of patients through the optimization of treatment guidelines. There are currently two kinds of stratification systems, conventional pathology assignment in the guidelines and emerging molecular classification proposed by The Cancer Genome Atlas (TCGA) (2, 3).

In the former system, prognostic factors of histopathology, such as histopathological type, grade, stage, myometrial invasion (MI) and lymphovascular space invasion (LVSI), constitute indications for risk assessment and adjuvant radiotherapy (2). Numerous retro- and prospective clinical studies have demonstrated that the number and severity of prognostic factors of histopathology positively correlate with the risk of recurrence and the extent of adjuvant therapy in EC (2). Nevertheless, the lack of consensus among pathologists on the histopathological type and tumor grade assignment has resulted in the same woman with different classifications, treatments, and clinical outcomes (4). Except for this poor reproducibility of prognostic factors, tremendous diversity in clinical outcomes of patients with the
same clinicopathological features suggests that the heterogeneity of EC is ignored in this traditional system (5). Since most of the prognostic factors of histopathology used for risk stratification are only available after surgery, such as MI and LVSI, this risk model contributes little to decisions regarding surgical procedures.

Existing molecular prognostic factors, such as POLE mutation, copy number variation (CNV) and abnormal expression of mismatch repair proteins, classified EC into four molecular subtypes including POLE-mutant, microsatellite instability (MSI), low copy number variation (CNV-L) and high copy number variation (CNV-H) (3). In addition, CTNNB1 mutation and L1CAM expression are two independent unfavorable prognostic factors (5–7). The accurate and objective detection of all these molecular features makes up for the defects of histopathology mentioned above and improves the risk assessment of EC (5, 7). However, this prognostic refinement, which only exists in patients categorized as “high-intermediate-risk” by the guidelines (5), is not conclusive in “high-risk” EC and utterly ineffective in “low-risk” disease (8, 9). Besides being very expensive and complicated, multi-platform and multi-molecular detections also generate some “multiple classifiers” who cannot be stratified accurately and reasonably due to the multiple molecular features the same patient has (9, 10). More importantly, adjuvant radiotherapy recommendation for patient with specific molecular abnormality still comes from guidelines based on histopathology and no targeted indication can be used as a reference (2). Therefore, both histopathological and existing molecular stratifications have their advantages and disadvantages. We envisioned whether there were more suitable biomarkers and strategies to integrate histopathology and molecular pathology in clinical practice.

Chromosomal instability (CIN), which originates from ongoing errors of chromosome segregation and eventually manifests as both the numerical and structural aberrations of chromosomes including aneuploidy, polyploidy, CNV and so on (11, 12), exists in about 60% – 80% of tumors (13). On the one hand, CIN contributes to adverse phenotypes of tumor, including malignant transformation, poor differentiation, invasion, metastasis, immune evasion and treatment resistance (14–18). On the other hand, it is the end result of a number of molecular processes, such as mutations in DNA checkpoint genes, microtubule spindle defects, telomere dysfunction and even MSI (19–21). As a common hallmark and mechanism underlying different phenotypes and molecular features of tumor, CIN may be a common entry point to explore different prognostic factors of histopathology and molecular pathology in EC. Although the respective role of chromosomal content and chromatin structure in EC has been associated with histopathology and molecular pathology (22–25), the overall impact of the numerical and structural aberrations of chromosomes, which is the significance of CIN, is unclear. Since there is no CIN-specific biomarker for EC, we selected the CIN25 and CIN70 signatures from a pan-cancer genomic instability study to measure the CIN status (26). By the top 25 and 70 genes which have correlations with the “total functional aneuploidy” in solid tumors, CIN25 and CIN70 signatures have been proven to fully reflect the numerical and structural complexity of chromosomes and successfully used in a broad variety of cancer types and research fields (14, 15, 26–28). In the present study, our aims were, first, to investigate the interrelationships between CIN signature and prognostic factors of histopathology or molecular pathology in EC. Second, relying on the integration of CIN signature and existing stratification systems, a
novel risk stratification model was designed for improved prognostic refinement and better management of EC.

Results

Relationships between CIN and prognostic factors of histopathology in EC

In order to investigate the CIN reflected by CIN signatures in EC, to begin, we confirmed the difference in CIN signatures between benign and malignant endometria. In TCGA Uterine Corpus Endometrial Carcinoma (UCEC) cohort, 23 cancer samples had notably increased CIN25 and CIN70 expressions compared to matched adjacent normal tissues (CIN25: p < 0.001; CIN70: p < 0.001; Figure S1A). Analysis in GSE63678 dataset which contained endometrioid EC (EEC) and four rare pathological types (mixed carcinoma with villoglandular, squamous differentiation, clear cell or papillary serous) gave a similar result (CIN25: p = 0.003; CIN70: p = 0.003; Figure S1B). Apart from that, in GSE17025 dataset, ECs had significantly increased CIN25 and CIN70 compared with benign lesions of endometrium including polyps and atrophic, inactive or cystic endometria (CIN25: p < 0.001; CIN70: p < 0.001; Figure S1C).

The nearly identical outcomes of these detections indicated that abnormal chromosomal stability represented by elevated CIN signatures was a dominant feature of EC. For further exploration of CIN in EC, we then compared CIN signatures among prognostic factors of histopathology.

Firstly, in TCGA UCEC cohort, the highest, intermediate and lowest CIN25 and CIN70 were found in Grade 3, Grade 2 and Grade 1 patients respectively (CIN25: p < 0.001; CIN70: p < 0.001; Fig. 1A). Meta-analysis including 483 Grade 1 & 2 and 478 Grade 3 patients from 9 EC datasets confirmed the aggravated CIN in grade 3 (CIN25 SMD: 0.985, 95% confidence interval (CI): 0.85 to 1.13, p = 0.000; CIN70 SMD: 1.009, 95% CI: 0.87 to 1.15, p = 0.000; Fig. 1B and Figure S1D). This suggested that the more serious the CIN, the poorer the tumor differentiation. Secondly, we observed low-level expression of CIN signatures in EEC and high-level expression in non-EEC from TCGA (CIN25: p < 0.001; CIN70: p < 0.001; Fig. 1C). That unfavorable histopathological type of EC tended to have severe CIN was further demonstrated by meta-analysis comprising 710 EEC and 253 non-EEC patients from 9 datasets (CIN25 SMD: 0.69, 95% CI: 0.54 to 0.84, p = 0.000; CIN70 SMD: 0.63, 95% CI: 0.48 to 0.78, p = 0.000; Fig. 1D and Figure S1E). Thirdly, meta-analysis of 447 Stage I & II patients versus 184 Stage III & IV patients from 4 EC datasets shown that Stage I & II patients had obviously increased CIN signatures (CIN25 SMD: 0.602, 95% CI: 0.43 to 0.78, p = 0.000; CIN70 SMD: 0.592, 95% CI: 0.42 to 0.77, p = 0.000; Fig. 1E and Figure S1F). Further, patients with longer distance of lymph node metastasis (aortic) or deeper MI (MI > 50%) in TCGA had much higher CIN25 and CIN70 (CIN25: p < 0.05; CIN70: p < 0.05; Fig. 1F and 1G). Thus, the variation on CIN was also an important characteristic of EC progression. Finally, we detected significantly positive correlations between diagnosis age and CIN signatures in EEC patients with Stage II, Grade 1 & 2 and MI < 50% from TCGA (CIN25: R² = 0.05, p = 0.010; CIN70: R² = 0.05, p = 0.011; Fig. 1H). Analysis in GSE17025 dataset gave
similar results (CIN25: $R^2 = 0.20$, $p = 0.050$; CIN70: $R^2 = 0.25$, $p = 0.025$; Fig. 1I). Patients older than 60 tended to have elevated CIN25 and CIN70 compared with younger patients in TCGA (CIN25: $p = 0.0050$; CIN70: $p = 0.0054$; Fig. 2J left). This trend between the two age subgroups did not reach a level of statistical significance in GSE17025, possibly due to insufficient samples (Fig. 2J left).

**Relationships between CIN and prognostic factors of molecular pathology in EC**

As all unfavorable prognostic factors of histopathology tightly associated with aggravated CIN, we speculated whether CIN signatures could be used to conduct risk assessments for different patients in the same adjuvant radiotherapy subgroup classified by the guidelines (observation (OB) subgroup, vaginal brachytherapy (VBT) subgroup and external beam radiation therapy (EBRT) subgroup; Materials and Methods and Table 1), thus providing some opportunities to further optimize indications for postoperative adjuvant therapy. Although patients with high risk of recurrence or progression tended to have high CIN signatures, area under the curve (AUC) for 5-year disease-free survival (DFS) of OB, VBT, EBRT and EBRT EEC subgroups were not more than 0.67 (Fig. 2A), which was unsatisfactory and prompted us to investigate possible factors for weakening the predictive power of CIN signatures.
Table 1
Clinicopathologic parameters according to adjuvant radiotherapy classification in Stage II patients of TCGA UCEC cohort.

| Prognostic factors | Total  | OB      | VBT     | EBRT    | P       |
|--------------------|--------|---------|---------|---------|---------|
| Age                | n = 294 (100%) | n = 123 (42%) | n = 92 (31%) | n = 79 (27%) |       |
| Mean (range)       | 64 (31 ~ 90) | 60 (31 ~ 89) | 66 (35 ~ 90) | 68 (35 ~ 87) | 0.000 ANOVA |
|                    | < 60    | 97 (33%) | 65 (53%) | 20 (22%) | 12 (15%) | 0.000 Pearson Chi² |
| ≥ 60               | 195 (67%) | 58 (47%) | 71 (78%) | 66 (85%) |       |

| Histologic Type    |        |        |        |        | 0.000 Pearson Chi² |
|--------------------|--------|---------|---------|---------|-------------------|
| Type I, EEC        | 250 (85%) | 123 (100%) | 92 (100%) | 35 (44%) |       |
| Type II, non-EEC   | 44 (15%) | 0       | 0       | 44 (56%) |       |

| Grade              |        |        |        |        | 0.000 Pearson Chi² |
|--------------------|--------|---------|---------|---------|-------------------|
| 1                  | 76 (26%) | 66 (54%) | 10 (11%) | 0       |       |
| 2                  | 76 (26%) | 57 (46%) | 20 (22%) | 1 (1%)  |       |
| 3                  | 140 (48%) | 0       | 62 (67%) | 78 (99%) |       |

| Stage              |        |        |        |        | 0.000 Pearson Chi² |
|--------------------|--------|---------|---------|---------|-------------------|
| IA, MI < 50%       | 199 (68%) | 105 (87%) | 62 (67%) | 32 (41%) |       |
| IB, MI > 50%       | 92 (32%) | 16 (13%) | 30 (33%) | 46 (59%) |       |

| CIN expression     |        |        |        |        |                   |
|--------------------|--------|---------|---------|---------|-------------------|
| CIN25 Mean (range) | -0.03 (-1.45 ~ 3.24) | -0.41 (-1.45 ~ 2.38) | 0.18 (-1.20 ~ 3.24) | 0.54 (-1.29 ~ 2.23) | 0.000 ANOVA |
| CIN70 Mean (range) | -0.03 (-1.53 ~ 3.07) | -0.38 (-1.53 ~ 1.95) | 0.17 (-1.09 ~ 3.07) | 0.50 (-1.14 ~ 1.86) | 0.000 ANOVA |
| Aneuploidy Score   | 4.82 (0 ~ 31) | 2.35 (0 ~ 20) | 4.38 (0 ~ 27) | 9.18 (0 ~ 31) | 0.000 ANOVA |
|       | Total  | OB    | VBT   | EBRT  |        |
|-------|--------|-------|-------|-------|--------|
| FGA   | 0.15 (0 ~ 0.95) | 0.08 (0 ~ 0.95) | 0.15 (0 ~ 0.81) | 0.25 (0 ~ 0.69) | 0.000  | ANOVA  |

Continued
Table 1
Clinicopathologic parameters according to adjuvant radiotherapy classification in Stage II patients of TCGA UCEC cohort (continued).

| Prognostic factors | Total | OB   | VBT  | EBRT  | P          |
|--------------------|-------|------|------|-------|------------|
|                    | n = 294 (100%) | n = 123 (42%) | n = 92 (31%) | n = 79 (27%) | 0.000 Pearson Chi² |
| Guidelines risk group |       |      |      |       |            |
| Low                | 105 (36%) | 105 (87%) | 0   | 0   |            |
| Intermediate       | 46 (16%) | 16 (13%) | 30 (33%) | 0   |            |
| High-intermediate  | 62 (21%) | 0   | 62 (67%) | 0   |            |
| High               | 79 (27%) | 0   | 0   | 79 (100%) |            |
| TCGA Subtype       |       |      |      |       | 0.000 Pearson Chi² |
| POLE-mutant        | 29 (10%) | 12 (10%) | 10 (11%) | 7 (9%) |            |
| MSI                | 99 (34%) | 39 (32%) | 37 (40%) | 23 (29%) |            |
| CNV Low            | 101 (34%) | 67 (54%) | 25 (27%) | 9 (11%) |            |
| CNV High           | 65 (22%) | 5 (4%) | 20 (22%) | 40 (51%) |            |
| Mutation           |       |      |      |       |            |
| PTEN               | 223 (76%) | 111 (90%) | 73 (79%) | 39 (49%) | 0.000 Pearson Chi² |
| FGFR2              | 59 (20%) | 23 (19%) | 22 (24%) | 14 (18%) | n.s. Pearson Chi² |
| CTNNB1             | 72 (24%) | 44 (36%) | 22 (24%) | 6 (8%) | 0.000 Pearson Chi² |
| PIK3CA             | 147 (50%) | 67 (54%) | 47 (51%) | 33 (42%) | n.s. Pearson Chi² |
| PPP2R1A            | 40 (14%) | 5 (4%) | 12 (13%) | 23 (29%) | 0.000 Pearson Chi² |

a For the two cases without age, one was in VBT group, another was in EBRT group; b One-way analysis of variance; c For the three cases without accurate MI, two were in OB group, one was in EBRT group; d There were two cases in OB group without complete clinicopathological information for guidelines risk assessment.
Prognostic factors of molecular pathology became the focus of our suspicion. Among the TCGA molecular subtypes of EC except POLE-mutant, CNV-L, MSI and CNV-H respectively had the lowest, intermediate and highest risk of recurrence and correspondingly had the lowest, intermediate and highest CIN25 and CIN70 (CIN25: p < 0.001; CIN70: p < 0.001; Fig. 2B and 2C) (5, 29, 30), which once again implied that CIN might positively correlate with the risk of recurrence in EC. The only exceptional subtype was POLE-mutant, whose prognosis was the best among the four TCGA molecular subtypes but CIN signature expressions were comparable to CNV-H which had the worst outcome (CIN25: p > 0.05; CIN70: p > 0.05; Fig. 2B and 2C) (5, 29, 30). This phenomenon inspired us to explore whether other mutations with prognostic value also had special CIN signatures, and which adjuvant radiotherapy subgroup these special CIN signatures existed in. To this end, we compared CIN signatures in wild types with those in POLE, CTNNB1, PTEN, PIK3CA, FGFR2 and PPP2R1A mutant patients from subgroups of OB, VBT, EBRT and ICGC PanCancer Analysis of Whole Genomes (PCAWG) respectively (Fig. 2D and Figure S2). POLE mutant patients in OB and VBT subgroups did not relapse or die (Fig. 2E), but had higher expressions of CIN25 and CIN70 compared with wild types (CIN25: p < 0.05; CIN70: p < 0.05; Fig. 2D), which might interfere the risk assessment of CIN signatures. In OB and EBRT subgroups, CTNNB1 mutation was another special mutation, which had much lower CIN signatures (CIN25: p < 0.05; CIN70: p < 0.05; Fig. 2D and Figure S2E) but had much worse prognosis than wide types (Fig. 2F) (5, 31). Multivariable Cox models further demonstrated that CTNNB1 mutation was an unfavorable prognostic factor independent of CIN signatures in OB and EBRT subgroups (Table 2 and Table 3). But this conclusion did not hold in VBT subgroup whose CIN signature expressions were exactly similar between CTNNB1 mutant and wild types (Fig. 2D and Table 2).
Table 2
Multivariable analysis on the prognosis role of CIN signatures and CTNNB1 mutation in OB and VBT subgroups without POLE mutation.

| Disease-free survival | Overall survival |
|-----------------------|-----------------|
| **OB**                |                 |
| n                     | HR (95% CI)     | P  | n                     | HR (95% CI)     | P  |
| CIN25                 |                 |
| Low                   | 61 1            |    | 33 1                   |                |
| High                  | 50 2.295 (0.749–7.029) | 0.146 | 78 -                   |                |
| CTNNB1                |                 |
| Wild type             | 67 1            |    | 67 1                   |                |
| Mutation              | 44 1.400 (0.473–4.138) | 0.543 | 44 12.393 (1.325–99.433) | 0.022 |
| CIN70                 |                 |
| Low                   | 24 1            |    | 44 1                   |                |
| High                  | 87 -            | 0.958 | 67 -                   | 0.966 |
| CTNNB1                |                 |
| Wild type             | 67 1            |    | 67 1                   |                |
| Mutation              | 44 1.576 (0.545–4.554) | 0.401 | 44 12.289 (1.431–105.564) | 0.022 |
| VBT                   |                 |
| n                     | HR (95% CI)     | P  | n                     | HR (95% CI)     | P  |
| CIN25                 |                 |
| Low                   | 62 1            |    | 63 1                   |                |
| High                  | 20 6.183 (1.416–26.991) | 0.015 | 19 2.644 (0.372–18.807) | 0.331 |
| CTNNB1                |                 |
| Wild type             | 60 1            |    | 60 1                   |                |
| Mutation              | 22 0.562 (0.065–4.838) | 0.6 | 22 -                   | 0.978 |
| CIN70                 |                 |
| Low                   | 50 1            |    | 55 1                   |                |
| High                  | 32 6.032 (1.215–29.949) | 0.028 | 27 2.311 (0.325–16.422) | 0.403 |
| CTNNB1                |                 |
| Wild type             | 60 1            |    | 60 1                   |                |
| Mutation              | 22 0.307 (0.038-2.500) | 0.27 | 22 -                   | 0.976 |
Table 3
Multivariable analysis on the prognosis role of CTNNB1 and POLE mutations and CIN signatures in EBRT subgroup.

|                  | Disease-free survival | Overall survival |
|------------------|-----------------------|-----------------|
|                  | n  | HR (95% CI) | P   | n  | HR (95% CI) | P   |
| EBRT             |    |            |     |    |            |     |
| CIN25            |    |            |     |    |            |     |
| Low              | 30 | 1          | 0.132 | 11 | 1          | 0.977 |
| High             | 49 | 2.772 (0.735–10.459) |     | 68 | -          | 0.977 |
| CTNNB1           |    |            |     |    |            |     |
| Wild type        | 73 | 1          |     | 73 | 1          |     |
| Mutation         | 6  | 4.907 (1.008–23.880) | 0.049 | 6  | 6.654 (1.280-34.586) | 0.024 |
| POLE             |    |            |     |    |            |     |
| Wild type        | 72 | 1          |     | 72 | 1          |     |
| Mutation         | 7  | 0.740 (0.093–5.872) | 0.776 | 7  | -          | 0.983 |
| CIN70            |    |            |     |    |            |     |
| Low              | 33 | 1          |     | 12 | 1          |     |
| High             | 46 | 3.039 (0.812–11.369) | 0.092 | 67 | -          | 0.976 |
| CTNNB1           |    |            |     |    |            |     |
| Wild type        | 73 | 1          |     | 73 | 1          |     |
| Mutation         | 6  | 4.889 (1.013–23.602) | 0.048 | 6  | 6.494 (1.249–33.759) | 0.026 |
| POLE             |    |            |     |    |            |     |
| Wild type        | 72 | 1          |     | 72 | 1          |     |
| Mutation         | 7  | 0.727 (0.092–5.755) | 0.763 | 7  | -          | 0.983 |

CIN signatures were prognostic in different adjuvant radiotherapy subgroups of EC

Consequently, we tested the prognostic value of CIN signatures in different adjuvant radiotherapy subgroups excluding different special mutations. For OB subgroup without POLE and CTNNB1 mutations, the AUC based on CIN70 was 0.76 (Fig. 3A), and CIN70 High group predicted worse DFS than CIN70 Low group (Fig. 3B). For POLE wild types from VBT subgroup, the AUC based on CIN25 was 0.71 (Fig. 3A), and CIN25 High group had much lower 5-year DFS rate compared with CIN25 Low group (Fig. 3C). For CTNNB1 wild types from EBRT and EBRT EEC patients, the AUC based on CIN25 was 0.62
and 0.72 (Fig. 3A), and the outcomes of CIN25 High group were much worse than CIN25 Low group (Fig. 3D and 3E). The predictive power of Fraction Genome Altered (FGA) and Aneuploidy Score, two signatures that only evaluated chromosomal content, was far inferior to that of CIN signatures (Fig. 3A). Recurrent patients belonged to different histopathological types or TCGA molecular subtypes can be effectively evaluated by CIN signatures in different adjuvant radiotherapy subgroups (Fig. 3F).

Since the AUCs based on CIN70 for DFS and OS of CTNNB1-mutant patients from OB subgroup were 0.71 and 0.72 (Fig. 3G), we were curious whether CIN could also play a role in the risk assessment of these patients. Although no statistically significant association between the CIN70 Low group and the CIN70 High group was observed, patients with sufficiently long follow-up in CIN70 High group exhibited a trend toward worse 5-year DFS (Fig. 3H left). We extended our analysis to 10-year OS and found that the outcome of CIN70 High group was much worse than that of CIN70 Low group (Fig. 3H right). We therefore reasoned that CIN signature could and should be used to stratify the CTNNB1-mutant patients from OB subgroup.

**Integrated risk assessment for Stage II EEC from TCGA**

According to different effects of CIN signatures, mutations and pathology, a risk assessment model integrated all these factors was proposed in Fig. 4A for Stage II EEC. In this model, four risk profiles (Low, Intermediate, High and Ultrahigh Risk) with different prognosis were respectively considered suitable to receive OB, VBT, EBRT and radiotherapy in combination with systemic therapy after surgery. Among different existing risk stratification systems, our integrated risk model had the highest AUCs for both DFS and OS (AUC for DFS = 0.75, AUC for OS = 0.76; Fig. 4B), and was the only system that had significant prognostic value for both DFS and OS (Fig. 4C and 4D; Figure S3).

**Discussion**

Through the comparison and meta-analysis of CIN signatures in multiple EC datasets, our study demonstrated that unfavorable prognostic factors of histopathology and molecular pathology, including poor differentiation, non-EEC, advanced disease, deep MI, advanced age, MSI and CNV-H, usually had aggravated CIN. A favorable prognostic factor, POLE mutation, and an unfavorable prognostic factor, CTNNB1 mutation, did not follow the above trend. The prognostic value of CIN signatures were well established in different adjuvant radiotherapy subgroups without POLE / CTNNB1 mutation and in CTNNB1 mutant patients from OB subgroup. An integrated risk model that combines pathology, CIN signatures and mutations was defined for improved prognostic refinement and better management of Stage II EEC.

Most non-EECs are serous and high-grade cancers, which exactly have complex aneuploidies and polyploidy (32), hence CIN showed a consistent change in fields of histopathological type and tumor differentiation of EC (Fig. 1A ~ 1D). At least three potential mechanisms generated by CIN, including the induction of mesenchymal transition, the activation of STING pathway and immune evasion, may contribute to invasion and metastasis (11), which may explain the high CIN25 and CIN70 in Stage II & II
patients and in patients with deep MI or aortic lymph node metastasis (Fig. 1E ~ 1G). Although we cannot verify the CIN status in LVSI-positive patients due to a lack of sufficient pathological information, we speculate that CIN may also increase in LVSI-positive cases since aneuploidy has been correlated with the LVSI of EC (25). Given the propensity for aging somatic cells to generate unstable chromosomes resulting from gene misexpression, telomeric attrition and senescence failure (33–35), the older EC patients were more prone to CIN enrichment (Fig. 1H ~ 1J).

Several well recognized molecular features of EC also had characteristic CIN. One of the final results of CIN is CNV (11). That is why we observed the lowest CIN signature expression in CNV-L and the highest expression in CNV-H (Fig. 2C). As for the moderate exacerbation of CIN in MSI patients, the fact that MSI causes some degree of genomic instability and the tendency for MSI to have aggressive phenotypes are two possible reasons (18–20, 29, 30). From CNV-L to MSI and then to CNV-H, as the CIN gradually becomes serious, the risk of recurrence gradually increases (Fig. 2B). In terms of MSI subtype itself, high CIN signatures were unfavorable prognostic factors (22). These two evidences, combined with the fact that CIN signatures did identify recurrent patients who belonged to different TCGA molecular subtypes in each adjuvant radiotherapy subgroup (Fig. 3B ~ 3F), implies that CNV-L, MSI, and CNV-H may be pooled together for prognosis evaluation by CIN.

Mutation of POLE causes impaired proofreading activity and DNA repair ability, followed by the poor fidelity of DNA replication and severe genomic instability (36, 37). This makes the CIN of POLE-mutant subtype was roughly the same as that of CNV-H (Fig. 2C). Unlike POLE mutation, however, why the mutation of CTNNB1 associated with a more stable chromosome status is not clear (Fig. 2D). Aberrant WNT/CTNNB1 pathway in colon cancer always induces CIN (38, 39), so the complete opposite relationship between CTNNB1 mutation and CIN in EC is confusing and interesting. Considering that patients with unstable chromosomes usually have poor clinical outcomes (26), how the aggravated CIN produces the excellent prognosis of POLE-mutant patients and the alleviated CIN leads to the poor outcomes of CTNNB1-mutant patients is another important issue worthy for further research (Fig. 2D ~ 2F; Tables 2 and 3). Serious CIN allows tumor to have different clonal selections in response to various biological stimuli and environmental stresses. However, this selective advantage also has a fitness cost of CIN, because the extremely excessive instability of chromosomes is not conducive to the stable survival of tumor cell itself (11, 14, 27, 40, 41). For this reason, besides the immune activation triggered by POLE-related mutations (42), severe CIN may contribute to the excellent prognosis of POLE-mutant cases. Similarly, CTNNB1-mutant cases, which benefit from the progression and proliferation caused by the activation of WNT/CTNNB1 signaling (6, 43), may protect cells from the adverse effects of this pathway activation with the help of the alleviated CIN. Although this conjecture is still to be confirmed by molecular biology, it may provide CIN-targeted therapeutic strategies for mutation-specific EC.

From these data and references, the inherent biological connections between CIN and different prognostic factors of EC suggest that CIN may be a common hallmark in the evolution of different clinicopathological and molecular features, which is the root cause for the success of our integrated risk model (Fig. 3 and Fig. 4). From the perspective of risk assessment, CIN signature, on the one hand,
properly addressed the problems of heterogeneity and reproducibility in the conventional pathology system by the precise quantification of CIN status, thereby achieving prognostic refinement. "Multiple classifiers" who cannot be stratified by TCGA subtypes also be able to get accurate and reasonable risk assessments. On the other hand, the prognostic refinement achieved by CIN signatures existed in all adjuvant radiotherapy subgroups in the guidelines, which means that CIN may have more universal applications compared to other risk stratification systems such as TCGA subtypes, FGA and Aneuploidy Score. From a therapeutic point of view, high concordance of molecular alterations between curettage samples and hysterectomy specimens from EC suggested the potential for CIN signatures to guide surgical management (24, 44). More importantly, because the accurate risk stratification accomplished by CIN signature presupposed the adjuvant radiotherapy classification based on the guidelines, the treatment recommendations obtained from our integrated risk model may be an intact inheritance of and effective supplement to the indications for postoperative radiotherapy in the guidelines. In summary, the intrinsic relationships between CIN and clinicopathological or molecular features make CIN a bridge that comprehensively integrates histopathology and molecular pathology, which is difficult for other biomarkers to achieve.

To further refine our integrated risk model, we face two outstanding challenges. Firstly, the CIN signature should be optimized on the basis of CIN25 and CIN70. Different adjuvant radiotherapy subgroups in the guidelines have different clinicopathological and molecular features (Table 1), which leads to that the same CIN signature had different capabilities of risk assessment in different adjuvant radiotherapy subgroups. For the same adjuvant radiotherapy subgroup, different CIN signatures also have different risk assessment capabilities. Consequently, we believe that it is necessary to improve respective CIN signatures for the OB, OB & CTNNB1 mutation, VBT and EBRT EEC patients classified by the guidelines to realize the full potential of CIN. Secondly, the relationships between CIN and LVSI or several EC prognostic factors assessed by immunohistochemistry (45), such as L1CAM, ER and PR, remain to be explored. It is unclear whether these features are still independent prognostic factors in our integrated model. We look forward to high-quality retrospective studies with mature long-term follow-up data and large sample size that will meet these two challenges and provide a solid foundation for future clinical applications.

**Conclusions**

Overall, except POLE and CTNNB1 mutations, serious CIN represented by increased CIN25 and CIN70 are characteristic of unfavorable prognostic factors in EC. Integration of pathology, CIN signatures and mutation of POLE / CTNNB1 in Stage Ⅲ EEC leads to improved prognostic refinement with potential clinical utility. Our integrated risk model holds promise to reduce both overtreatment and undertreatment and deserves for further validation and improvement.

**Materials And Methods**

**Data collection**
Clinical information, gene expression (Z-score), mutation profiles, FGA and Aneuploidy Score of TCGA UCEC cohort were available at cBioPortal for Cancer Genomics (46). RNAseq data (FPKM-UQ) of EC samples and matched adjacent normal tissues were downloaded from the TCGA data portal (47). Mutation profiles and RNAseq data (FPKM-UQ) of EEC samples in ICGC PCAWG project were downloaded from ICGC data portal (48, 49). E-MTAB-1358 and E-MTAB-5018 were downloaded from ArrayExpress database (50–52). GSE2109, GSE23518, GSE56026, GSE33723, GSE32507, GSE17025, GSE24537, GSE21882 and GSE63678 were downloaded from Gene Expression Omnibus (GEO) database (53–61). Each dataset downloaded from ArrayExpress and GEO databases was standardized using the Z-score transformation before calculations of CIN signatures. CIN25 and CIN70 signatures for each sample were the average expressions of the 25 and 70 genes identified by Cart et al. (Table S1) (26), and were compared among prognostic factors of histopathology or molecular pathology. The clinicopathological information for each dataset was shown in meta-analysis.

**Adjuvant radiotherapy classification for stage EC patients in TCGA UCEC cohort**

There were three adjuvant therapeutic strategies after surgery for stage EC patients, namely observation (OB), vaginal brachytherapy (VBT), and external beam radiation therapy (EBRT). Indications for the three adjuvant radiotherapies in the guidelines of ESMO-ESGO-ESTRO were based on six established clinicopathological risk factors including age, histologic type, grade, stage, MI, and LVSI (2). LVSI was missing in the TCGA UCEC cohort, so we had to conduct the classification with the other five risk factors. OB subgroup in the guidelines was defined as a) stage IA EEC with grade 1 & 2; b) stage IB EEC with grade 1 & 2 and less than 60 years old. Patients in the EBRT subgroup followed criteria: a) stage IB EEC with grade 3; b) stage non-EEC. VBT subgroup was consisted of the remaining patients, including a) stage IB EEC with grade 1 & 2 and age > 60; b) stage IA EEC with grade 3. Patients who did not have complete or accurate information for classification and survival analysis, who had other malignancy or who had positive surgical margin were excluded. Finally, there were 123, 92 and 79 patients in OB, VBT and EBRT subgroups, respectively. Detailed information was presented in Table 1.

**Survival analysis for different adjuvant radiotherapy subgroups**

In OB, VBT, EBRT and EBRT EEC subgroups, AUC and optimal cutoff values based on CIN25 and CIN70 signatures, FGA and Aneuploidy Score were determined by the time-dependent receiver operating curve using “survivalROC” package on the R platform. Kaplan-Meier curves and log-rank tests were carried out to predict 5-year DFS and 10-year OS based on the optimal cutoff values or mutation status of different subgroups. Cox proportional hazards models were used to evaluate the prognostic value of mutations and CIN signatures. Covariates violating the proportional hazards assumption were added as time-dependent covariates in the Cox regression models.

**Statistical analysis**

If not specified otherwise, comparisons among clinicopathological features and among molecular alterations were tested using the chi-square test for categorical variables and using the Mann-Whitney
test or one-way analysis of variance (ANOVA) in case of continuous. SPSS 21.0 and GraphPad Prism 8 software were used to perform statistical and survival analysis and to plot graphs. Meta-analysis was done using STATA 12.0. Data in this article were presented as mean ± S.D and P values were based on two-sided tests with < 0.05 considered statistically significant.

List Of Abbreviations

CIN, chromosomal instability; EC, endometrial carcinoma; TCGA, The Cancer Genome Atlas; MI, myometrial invasion; LVSI, lymphovascular space invasion; CNV, copy number variation; MSI, microsatellite instability; CNV-L, low copy number variation; CNV-H, high copy number variation; UCEC, Uterine Corpus Endometrial Carcinoma; EEC, endometrioid endometrial carcinoma; OB, observation; VBT, vaginal brachytherapy; EBRT, external beam radiation therapy; AUC, area under the curve; DFS, disease-free survival; OS, overall survival

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: All authors consent for publication.

Availability of data and materials: All data generated or analysed during this study are included in this published article and its supplementary information files.

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**Figures**
Figure 1

Comparison of CIN signatures among prognostic factors of histopathology. (A) Boxplot of CIN25 and CIN70 expressions in Grade 1, Grade 2 and Grade 3 patients from TCGA. If Levene test for homogeneity demonstrates unequal variances among these three groups, p values are calculations of Welch-corrected ANOVA with Games-Howell post-hoc-tests. (B) Forest plot comparing CIN25 expression in Grade 1 & 2 versus Grade 3 patients. (C) Boxplot of CIN25 and CIN70 expressions in EEC and non-EEC patients from TCGA. (D) Forest plot comparing CIN25 expression in EEC versus non-EEC samples. (E) Forest plot comparing CIN25 expression in Stage I & II versus Stage III & IV patients. In (B), (D) and (E), an Inverse Variance (IV) fixed effects method is used to meta-analyze the data; squares (blue) represent standardized mean difference (SMD); square size is proportional to weights used in the analysis; bars (grey) represent 95% confidence intervals (CI); diamond (yellow) represent overall SMD with associated 95% CI (lateral tips). (F) Boxplot of CIN25 and CIN70 expressions in Stage IIIC samples with positive pelvic lymph node and positive aortic lymph node in TCGA dataset. (G) Boxplot of CIN25 and CIN70 expressions in Stage II and Grade 1 & 2 EEC samples with MI < 50% and MI > 50% from TCGA dataset. (H) Pearson correlation between age and CIN25 or CIN70 expression in Stage II EEC patients with Grade 1 & 2 and MI <
50% from TCGA dataset. (I) Same as (H) but utilizing samples in GSE17025 dataset. (J) Boxplot of CIN25 and CIN70 expressions in patients from (G) and (H) with age < 60 and > 60. P values presented in (C), (F), (G) and (J) are Mann-Whitney test calculations. P values: *p < 0.05, **p < 0.01, ***p < 0.001, n.s. not significantly different.

Figure 2

Relationships between CIN and molecular prognostic factors. (A) AUC for 5-year DFS based on CIN25 and CIN70 in OB, VBT, EBRT and EBRT EEC subgroups. (B) Kaplan-Meier plot for 5-year DFS based on TCGA molecular subtypes. (C) Boxplot of CIN25 and CIN70 expressions in patients belonged to four TCGA molecular subtypes. If Levene test for homogeneity demonstrates unequal variances among these four groups. P values are calculations of Welch-corrected ANOVA with Games-Howell post-hoc-tests. (D) Boxplot of CIN25 and CIN70 expressions in POLE or CTNNB1 wild-types versus mutant patients from OB, VBT and EBRT subgroups. P values are Mann-Whitney test calculations. (E) Kaplan-Meier plot for 5-year DFS and 10-year OS based on POLE mutation status in OB and VBT subgroups. (F) Kaplan-Meier plot for 5-year DFS and 10-year OS based on CTNNB1 mutation status in OB and EBRT subgroups. P values in
(B), (E) and (F) are calculations of log-rank test. P values: *p < 0.05, **p < 0.01, ***p < 0.001, n.s. not significantly different.

Figure 3

Prognostic significance of CIN signatures in different adjuvant radiotherapy subgroups. (A) AUC for 5-year DFS based on CIN signatures, FGA and Aneuploidy Score in OB subgroup excluding mutations of POLE and CTNNB1, in VBT subgroup excluding POLE-mutant patients and in EBRT and EBRT EEC subgroups without CTNNB1 mutations. (B) Kaplan-Meier plot for 5-year DFS based on CIN70 in OB subgroup excluding mutations of POLE and CTNNB1. (C) Kaplan-Meier plot for 5-year DFS based on CIN25 in VBT subgroup excluding POLE-mutant patients. (D) Kaplan-Meier plot for 5-year DFS based on CIN25 in CTNNB1 wild-types from EBRT subgroup. (E) Kaplan-Meier plot for 5-year DFS based on CIN25 in CTNNB1 wild-types from EBRT EEC patients. P values in (B) ~ (E) are calculations of log-rank test. (F) Characteristics of recurrent patients in (B) ~ (E). Cumulative bar chart represents the proportion of TCGA molecular subtypes, the proportion of CIN subgroups and the proportion of histopathological types. (G) AUC for 5-year DFS and 10-year OS based on CIN signatures, FGA and Aneuploidy Score in CTNNB1-mutant patients from OB subgroup. (H) Kaplan-Meier plot for 5-year DFS and 10-year overall survival (OS) based on CIN70 in CTNNB1-mutant patients from OB subgroup. P values are calculations of log-rank test.
Figure 4

Integrated risk assessment for stage EEC. (A) Flow chart of our integrated risk model. (B) AUC for DFS and OS in Stage EEC from TCGA based on Guidelines, FGA, Aneuploidy Score, TCGA subtypes and our integrated risk model. (C) Kaplan-Meier plot for 5-year DFS based on integrated risk model in Stage EEC from TCGA. (D) Kaplan-Meier plot for 10-year OS based on integrated risk model in Stage EEC from TCGA. P values in (C) and (D) are calculations of log-rank test.

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