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Age-related copy number variations and expression levels of F-box protein *FBXL20* predict ovarian cancer prognosis

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ABSTRACT

About 70% of ovarian cancer (OvCa) cases are diagnosed at advanced stages (stage III/IV) with only 20–40% of them survive over 5 years after diagnosis. A reliably screening marker could enable a paradigm shift in OvCa early diagnosis and risk stratification. Age is one of the most significant risk factors for OvCa. Older women have much higher rates of OvCa diagnosis and poorer clinical outcomes. In this article, we studied the correlation between aging and genetic alterations in The Cancer Genome Atlas Ovarian Cancer dataset. We demonstrated that copy number variations (CNVs) and expression levels of the F-Box and Leucine-Rich Repeat Protein 20 (*FBXL20*), a substrate recognizing protein in the SKP1-Cullin1-F-box-protein E3 ligase, can predict OvCa overall survival, disease-free survival and progression-free survival. More importantly, *FBXL20* copy number loss predicts the diagnosis of OvCa at a younger age, with over 60% of patients in that subgroup have OvCa diagnosed at age less than 60 years. Clinicopathological studies further demonstrated malignant histological and radiographical features associated with elevated *FBXL20* expression levels. This study has thus identified a potential biomarker for OvCa prognosis.

Introduction

Despite intensive treatment with surgical cytoreduction and platinum-based chemotherapy, ovarian cancer (OvCa) remains the most lethal gynecologic malignancy worldwide with relapses developed in the majority of advanced-stage cases (stage III/IV) [1,2]. About 70% of cases are diagnosed at advanced stages, with only 20–40% of them survive over 5 years after diagnosis [3]. A reliably screening and diagnostic marker can enable early OvCa diagnosis, risk stratification, and treatment planning.

Studies of age-specific incidence rates identified that the mean ages at OvCa diagnosis for *BRCA1* and *BRCA2* mutant patients were 51.3 and 61.4 years, respectively [4]. Therefore, prophylactic bilateral salpingo-oophorectomy (BSO) before age 40 for *BRCA1* mutated and age 45 for *BRCA2* mutated patients was recommended [4]. However, only about 10% of the general population carry germline *BRCA* mutations [5,6]. Indeed, less than 21% of OvCa patients carry *BRCA1* mutation and 8% have *BRCA2* mutation [5,6]. The mutation rates of other OvCa risk-conferring genes such as *RAD51C*, *RAD51D*, *BRIP1*, *FANCM*, and the mismatch repair genes *MLH1*, *MSH2*, *MSH6*, and *PMS2* mutations are less than 1% in the general population [7]. Therefore, mutations account for only a small fraction of OvCa cases, and the majority of the population will not benefit from the screening of these genetic alterations.

Age is one of the biggest independent risk factors for the disease diagnosis. Older OvCa patients often have poorer clinical outcomes [8]. Aging is associated with an increased prevalence of frailty, comorbidities, progressive decrease of organ function, as well as adverse drug reactions due to decreasing therapeutic window and distribution volume [9]. However, clinical studies identified that older patients (over 70 years) experienced the same percentage of morbidity with no significant difference in survival when compared with younger (under 70 years) women who were equally debulked [10]. Further studies demonstrated that elderly (65–75 years) and very elderly (>75 years) patients could tolerate radical surgery without an increase of morbidity rates when compared with those reported in younger patients, indicating older age is not a risk factor for aggressive surgical cytoreduction [11]. Clinical trials of chemotherapy with carboplatin/paclitaxel versus cisplatin/paclitaxel following cytoreductive surgery also demonstrated that OvCa patients over the age of 70 could tolerate the combinational treatment regimens [12]. Therefore, the alteration of treatment plans is not solely responsible for the older-age OvCa patient's unfavorable prognosis. Aging-related genetic changes, including accumulation of mutations, epigenetic modifications, and copy number variations (CNVs), may offer new clues in identifying OvCa prognostic and screening biomarkers.

In this article, the correlation of aging with genetic alterations in The Cancer Genome Atlas Ovarian Cancer (TCGA-OV) dataset was studied. We found copy numbers loss of the F-Box protein *FBXL20* predicts OvCa diagnosis at a younger age (<60 years). Moreover, decreased *FBXL20*

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expression as the result of copy number loss predicts favorable overall survival (OS), disease-free survival (DFS), and progression-free survival (PFS).

Methods and materials

Public datasets

The clinical and genetic information of OvCa samples were derived from The Cancer Genome Atlas Ovarian Cancer (TCGA-OV) dataset. Analysis of mutations was conducted based on the Genomic Data Commons (GDC) Data Portal (https://portal.gdc.cancer.gov/). Specifically, 608 OvCa patients were included in TCGA-OV dataset. The Cancer Imaging Archive (TCIA) provides radiological data of 143 OvCa patients (https://www.cancerimagingarchive.net/). Expression levels of genes in the normal tissue were based on the Genotype-Tissue Expression (GTEx) project (https://www.gtexportal.org/home/). The data regarding the 'Longest Dimension' of TCGA-OV cases was downloaded from the UCSC Xena (https://xena.ucsc.edu/). All these clinical samples were collected with informed consent.

Clinicopathological study

The immunohistochemistry (IHC) data was derived from The Human Protein Atlas, in which staining for FBXL20 was conducted using anti-FBXL20 antibody HPA050397 (Sigma-Aldrich, MO, USA) (https://www.proteinatlas.org/) [13]. The samples with differential FBXL20 staining intensity ('weak' and 'moderate') for serous, mucinous, and endometrioid subtypes of OvCa were collected. The magnification scale is 50 μm. Hematoxylin and eosin (H&E) staining and computed tomography (CT) scan images of serous OvCa were derived from TCGA-OV dataset. Patients were grouped into '*FBXL20* Low' and '*FBXL20* High' cohorts based on the median *FBXL20* copy numbers or expression levels.

Copy number variations (CNVs) of OvCa patients

OvCa patients in TCGA-OV dataset were grouped into different age groups of 26–45, 46–59, 60–75, and 76–89 years based on the age at initial OvCa diagnosis. Prevalence and frequency of each type of mutations and CNVs were analyzed using the Firebrowse (http://firebrowse.org/). CNVs of genes *KSR1*, *TNFAIP1*, *TRAF4*, *SLC6A4*, *NF1*, *SUZ12* and *RAD51D*, *CCL5*, *FBXO47*, *FBXL20*, *ERBB2*, *MIEN1* located on chromosome bands 17q11.2 and 17q12, respectively, were identified. Those samples were aligned with patients' age at initial OvCa diagnosis using the UCSC Xena platform. These genes were selected to represent the CNVs of chromosome bands 17q11.2 and 17q12. Their involvement in carcinogenesis is indicated in the Atlas of Genetics and Cytogenetics in Oncology and Hematology.

Demographic analysis of OvCa patients with differential FBXL20 copy numbers

Patients from TCGA-OV dataset were grouped into higher-than-median copy numbers of *FBXL20* ('*FBXL20* High'; $n = 227$) and lower-than-median copy numbers of *FBXL20* ('*FBXL20* Low'; n = 227). The demographics of the two groups, including ethnicity, race, and age at initial OvCa diagnosis, were derived from TCGA-OV. Patients grouped into these groups were further categorized based on age at initial OvCa diagnosis ($\leq$49, 50–59, and $\geq$60 years).

FBXL20 expression levels and OvCa staging

Violin plot analysis for OvCa staging and *FBXL20* expression levels was carried out on GEPIA (http://gepia2.cancer-pku.cn/#index). Briefly, *FBXL20* expression levels were transformed with the equation of $\log_2$(Transcript Count Per Million (TPM) + 1). Ovarian serous cystadenocarcinoma subtype derived from TCGA-OV dataset was used for the Violin plot analysis. The method for differential gene expression analysis is one-way ANOVA. Proteomic data used in this publication was generated by the National Cancer Institute Clinical Proteomic Tumor Analysis Consortium (https://cptac-dataportal.georgetown.edu/) ($n = 95$) [14]. Mass spectrometry analysis was conducted using the 10-plexed isobaric tandem mass tags (TMT-10). Protein abundance was presented as log2-ratio of the expression of the sample to a normal control. Samples were aligned based on OvCa stages. The protein levels of these aligned cases were then color-coded. Proteins involved in CRL, including FBX20, cullin 1, 2, 3, 4A/B, 5, and 7, were studied. Actin was used as a reference control.

Kaplan-Meier(K-M) survival analysis

K-M analyses of overall survival (OS), disease-free survival (DFS), and progression-free survival (PFS) of OvCa patients were carried out based on TCGA-OV dataset. The survival data was downloaded from the UCSC Xena. Quartiles of genes' CNVs were used as cutoff values in grouping patients into 'Upper quartile', 'Second quartile', 'Third quartile', and 'Lower quartile' cohorts. Survival analyses comparing 'Upper quartile' and 'Lower quartile' cohorts were presented. The survival analysis of the cohorts of 'Second quartile' and 'Third quartile' was not shown due to the lack of statistical significance (Supplementary 1). The OS analyses of patients with mutated (MT) *ERBB2*, *TP53*, *BRCA1*, and *BRCA2* and wildtype (WT) cases were carried out based on the GDC TCGA-OV dataset. The Log-rank test was used for calculations of *P*-values.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6.0 software. All statistical tests were 2-sided, and *P*-values smaller than 0.05 were considered statistically significant.

Results

Copy number losses on 17q11.2-q12 and OvCa

To identify potential age-related genetic alterations, we grouped OvCa patients from TCGA-OV dataset into different age groups: 26–45, 46–59, 60–75, and 76–89 years (Fig. 1A). We found even distribution for both the prevalence and types of mutations across different age groups (Supplementary 2). The mutation status of key genes, including *TP53*, *NF1*, *BRCA1/2*, *CDK12*, *RB1*, *EFEMP1*, *HNF1B*, *KRAS*, *PTEN*, *ERCC6*, *LARP1*, *MTA2*, and *NRAS*, was also not affected by patients' ages at initial OvCa diagnosis (Supplementary 1). Copy number variations (CNVs) represent a significant source of genetic variation in the human genome [15]. Therefore, we further investigated CNVs between different age groups with OvCa.

We found that most OvCa patients at 26–59 years old (y/o) age group have copy number losses on chromosome band 17q11.2 (Fig. 1A, Supplementary 2). Interestingly, 17q11.2 and its flanking region 17q12 (17q11.2-q12) have been proposed by the Ovarian Cancer Association Consortium (OCAC) as susceptibility loci bearing common germline genetic variations for polygenic risk prediction for OvCa [7]. CNVs of genes *KSR1*, *TNFAIP1*, *TRAF4*, *SLC6A4*, *NF1*, *SUZ12* and *RAD51D*, *CCL5*, *FBXO47*, *FBXL20*, *ERBB2*, *MIEN1* located on 17q11.2 and 17q12, respectively, were selected and aligned with patients' age at initial OvCa diagnosis (Fig. 1B, C). These genes were selected based on their locations on the genome and their involvement in carcinogenesis. Consistent with Fig. 1A, loss of copy numbers of individual genes on 17q11.2-q12 correlates with OvCa diagnosis at a younger age (Fig. 1B, C).

CNVs of genes on 17q11.2-q12 and OvCa prognosis

CNVs of each oncology-related gene on 17q11.2-q12 were studied. We found copy numbers of 4 genes, including F-box only protein 47 (*FBXO47*), F-box/LRR-repeat protein 20 (*FBXL20*), erb-b2 receptor tyrosine kinase 2 (*ERBB2*), and migration and invasion enhancer 1 (*MIEN1*), can predict OvCa survival rates. The *P*-values between 'Upper quartile' and 'Lower

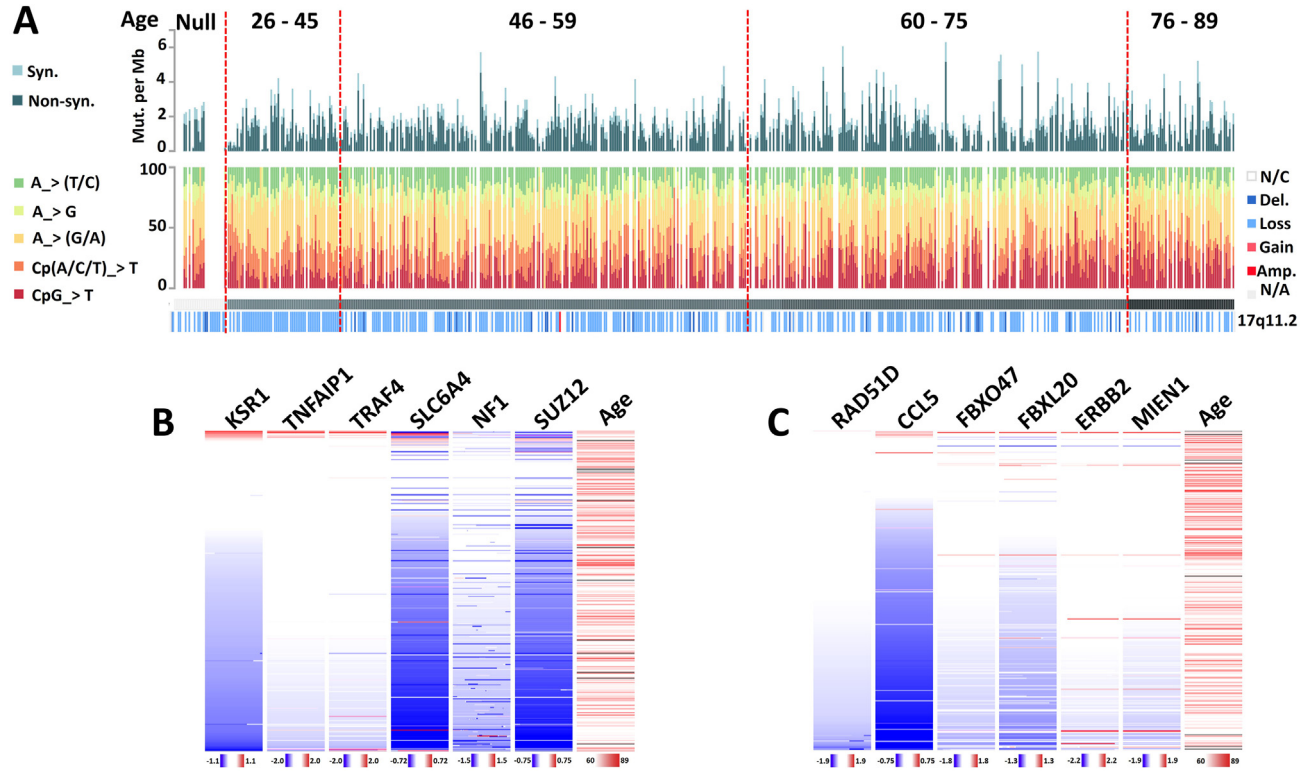

Fig. 1. Genetic variations of OvCa patients diagnosed at different ages A) OvCa patients were grouped into different age groups based on the age at initial diagnosis of the disease. Prevalence (top panel) and frequency of each types of mutations (middle panel) and copy number variations (CNVs) on chromosome band 17q11.2 were plotted in accordance with patient's age B) CNVs of indicated genes on 17q11.2 were aligned with patients' age at initial OvCa diagnosis (n = 606). C) CNVs of indicated genes on 17q12 were aligned with patients' age at initial OvCa diagnosis (n = 606).

quartile' cohorts of these four genes are 0.0024, 0.0076, 0.0012, and 0.0011, respectively (Fig. 2). FBXO47 and FBXL20 are F-box proteins that function as substrate recognizing proteins in the SKP1-Cullin1-F-box (SCF) E3 complex for ubiquitin (Ub) conjugations [16] (Fig. 7, Table 1). ErbB2 (Neu/Her2) was proposed in numerous studies as a potential OvCa prognostic marker, and MIEN1 was involved in cancer progression and metastasis [17]. Screening of CNVs based on patients' age groups has thus successfully identified genes on 17q11.2-q12 that can potentially predict the OvCa prognosis.

CNVs and gene expression of FBXL20 predict OvCa prognosis

CNVs regulate differential gene expression levels via gene dosage effects [15]. We found expression levels of *FBXO47*, *FBXL20*, *ERBB2*, and *MIEN1* are in accordance with their CNVs (Fig. 3A, B, Supplementary 3). However, only *FBXL20* expression levels can reliably predict the prognosis of OS, disease-free survival (DFS), and progression-free survival (PFS) with *P*-values of 0.0073, 0.0045, and 0.00475, respectively (Fig. 3B, from left to right panels). *ERBB2* is the most frequently studied putative molecular prognostic factor in OvCa [18]. Therefore, we also studied the potential prognostic value of *ERBB2* expression level and mutation status to rule out the possibility that the prognostic role of *FBXL20* is due to its proximity to *ERBB2* in the chromosome. We found neither *ERBB2* expression nor its mutation status can predict OvCa prognosis (Fig. 3A). Furthermore, we found *FBXL20* outperformed other potential OvCa prognostic molecular markers, including mutation status of *BRAC1/2* [19] and *TP53* [20], and expression levels of *WTAP* (Wilms' tumor 1-associating protein) [21] and *EGFR* [22] (Supplementary 4). We also found that *FBXL20* expression levels and cellular protein levels do not correlate with OvCa staging, indicating FBXL20 can be an independent OvCa prognostic biomarker (Supplementary 5). The expression of *FBXO47* also failed to predict OvCa prognosis (Supplementary 3). All these data indicate *FBXL20* CNVs and expression levels can predict OvCa prognosis. The prognostic role of *MIEN1* expression was not studied due to the lack of RNA-Seq data in TCGA-OV.

FBXL20 copy number loss predicts OvCa diagnosis at a younger age

To study other demographic features of patients with differential *FBXL20* expression, OvCa patients in TCGA-OV dataset were grouped into '*FBXL20* High' and '*FBXL20* Low' cohorts (n = 227 vs. n = 227) using the median CNV value as the cutoff. No significant difference in ethnicity and race exists between the cohorts (data not shown). In the cohort of lower-than-median *FBXL20* copy numbers, 26.87% (n = 61), 33.92% (n = 77) and 36.56% (n = 83) of the patients were diagnosed with OvCa at the age of ≤49, 50–59 and ≥60 years, respectively (Fig. 3C). In the cohort with higher-than-median *FBXL20* copy numbers, 11.77% (n = 29), 28.63% (n = 65) and 54.19% (n = 123) of the patients were diagnosed with OvCa at the age of ≤49, and 50–59 and ≥60 years, respectively (P < 0.001) (Fig. 3C).

Elevated FBXL20 protein levels correlate with malignant histological features of OvCa

The function of FBXL20 in OvCa progression is barely studied. We further studied the clinicopathological features of OvCa subtypes with different cellular levels of FBXL20. Three subtypes of OvCa, including serous, mucinous, and endometrioid, were selected from the Human Protein Atlas. Patients were grouped into 'FBXL20 Low' and 'FBXL20 High' cohorts based on FBXL20 staining intensity. The serous subtype of OvCa with high FBXL20 staining intensity showed malignant histological phenotypes with micropapillary, trabecular structures, detached tumor cells, and glandular complexity (Fig. 4). The mucinous OvCa subtype with high FBXL20 expression showed malignant features of infiltrative patterns with small glands, nests, and small clusters of floating cells (Fig. 4). The endometrioid OvCa

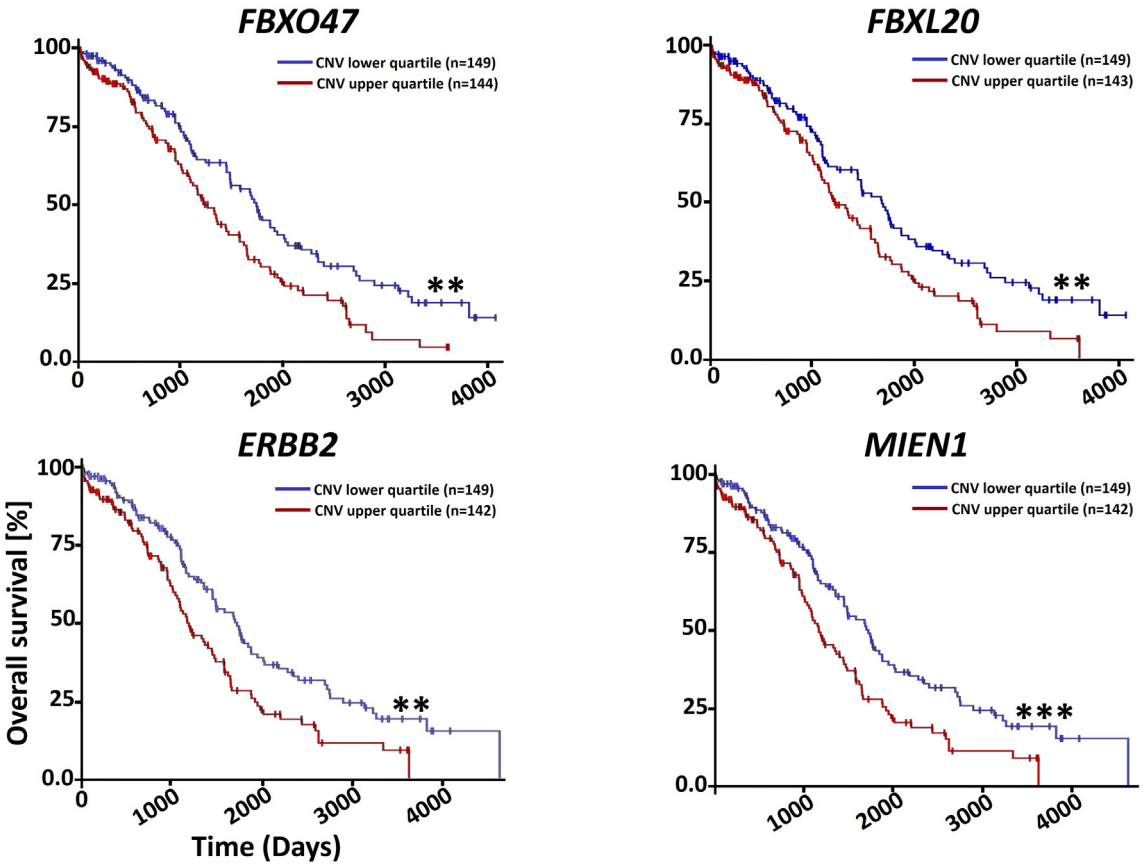

Fig. 2. Survival analysis based on copy number variations (CNVs) of individual genes. The upper and lower quartiles were used as cutoff values for classification of patients into groups with 'high' or 'low' copy numbers of the indicated genes. Hazard ratio for *FBXO47*, *FBXL20*, *ERBB2*, and *MIEN1* is: 9.193, 7.115, 10.5, and 10.68, respectively. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

subtype with high FBXL20 showed abundant proliferative cells and nuclear atypia (Fig. 4, arrows).

Elevated FBXL20 expression correlates malignant histological features in serous OvCa

Serous subtype is the most common and lethal form of OvCa. Therefore, we further studied histological features of serous OvCa samples with differential *FBXL20* expression levels using hematoxylin and eosin (H&E) staining. Serous subtype OvCa patients were grouped into '*FBXL20* Low' and '*FBXL20* High' cohorts based on expression levels of *FBXL20*. We found samples with low *FBXL20* expression levels (TCGA-23-1111, TCGA-61-2003, TCGA-61-1910) generally have benign features such as psammoma bodies (circles) and less frequent and less extensive necrosis when compared with samples with higher expression levels of *FBXL20* (TCGA-13-1511, TCGA-61-1743, TCGA-29-1691) (Fig. 5). These data

Table 1
The involvement of F-box proteins in ovarian cancer. The F-box proteins are categorized into sub-families, FBXL, FBXW, and FBXO based on their substrate-binding motifs, including Leucine Rich Repeats, WD40 motifs, and other domains, respectively [35]. Relatively well studied F-box proteins that are related with OvCa progression including FBXW1/11 [27,36–40], FBXW7 [41–46], FBXW8 [47–49], FBXL1 (SKP2) [50–52], FBXL3 [53,54], FBXL10 [55,56], FBXL20 [26], FBXO4 [57,58], FBXO7 [59–61] and FBXO22 [62–64]. Abbreviations: AEBP2, AE binding protein 2; Cdc25A, cell division cycle 25 A; cIAP1, cellular inhibitor of apoptosis protein 1; DEPTOR, DEP domain containing MTOR interacting protein; FMRP, fragile X mental retardation protein; KDM4B, lysine demethylase 4B; LKB1, liver kinase B1; Mdm2, murine double minute 2 homolog; TLK1/2, tousled-like kinases 1/2; TRAF1/2, TNF receptor associated factor 1/2; TRF1, telomeric repeat factor 1; Vps34, vacuolar protein sorting 34.

Class	F-box protein	Key substrate	Pathway involved	Ovarian cancer
FBXWs	FBXW1/11 (β-TrCP1/2)	Mdm2, Cdc25A, Wee 1, DEPTOR, β-catenin, IkB, AEBP2	DNA damage response; mTOR pathway; Wnt pathway; NFkB pathway; epigenetic modifications	Cisplatin and Platinum resistance; metastasis; invasion; EMT; cancer stem cell [20,29–33].
	FBXW7	c-Myc, Cyclin E, Notch1, Mcl-1, mTOR, HIFα, c-Jun	Cell cycle; Notch pathway, apoptosis, mTOR pathway, HIFα pathway	PARPi resistance; cancer stem cell; chemo-resistance; angiogenesis [34–39]
	FBXW8	Cyclin D1, IRS1	Cell cycle; PI3K pathway	Tumorigenesis, progression [40–42]
FBXLs	FBXL1 (SKP2)	P47, P21, P27, P16, FOXO3, BRCA1, Akt	Cell cycle; apoptosis; FOXO pathway, Akt pathway	Cisplatin resistance; invasion; growth and proliferation [43–45]
	FBXL3	c-Myc, TLK2	Cell cycle; DNA replication; checkpoint signaling	PARPi resistance; PARPi sensitization [46,47]
	FBXL10	c-Fos	c-Fos pathway;	Early dissemination [48,49]
	FBXL20	Vps34	Phosphatidylinositol production; autophagy	Cisplatin resistance; metastasis [21]
FBXOs	FBXO4	Cyclin D1, TRF1, FMRP	Cell cycle; telomeres maintenance; RNA binding	Cell cycle; genomic instability; OvCa risk [50,51]
	FBXO7	TRAF1/2, cIAP1, Gsk3β	Apoptosis; Wnt pathway	Chemo-resistance; proliferation [52–54]
	FBXO22	Mdm2, LKB1, KDM4B	P53 pathway; DNA damage response; LKB1-AMPK pathway; methylation	Chemo-resistance; metastasis; early dissemination [55–57]

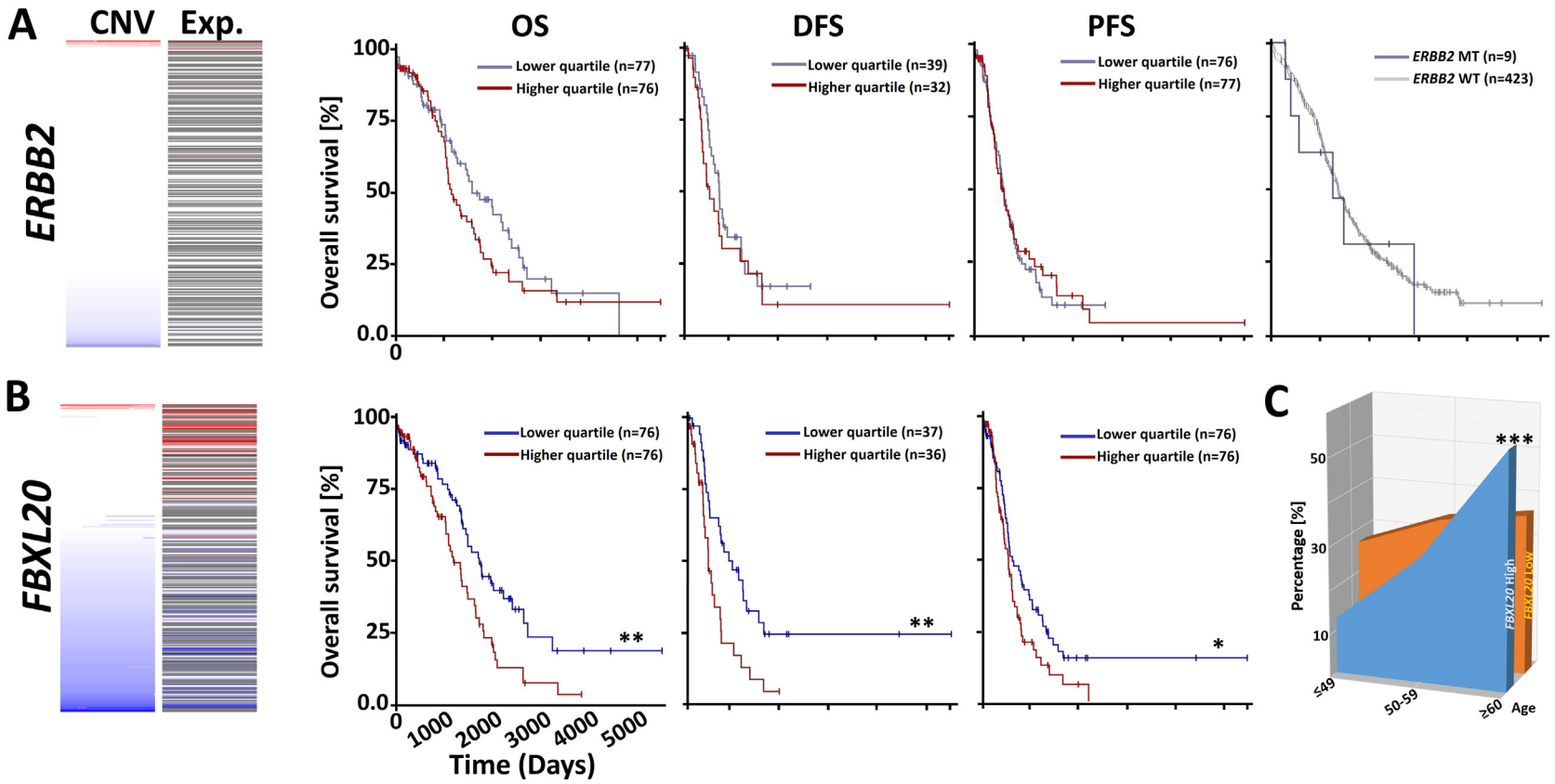

Fig. 3. The effect of *FBXL20* expression levels on OvCa prognosis A) *ERBB2* expression is aligned with its copy numbers using the UCSC Xena platform based on TCGA-OV dataset ($n = 308$). Overall survival (OS), disease-free survival (DFS) and progression-free survival (PFS) with differential *ERBB2* expression levels were compared (from the left to the right panel). The survival of patients with mutated *ERBB2* was also plotted against those wildtypes. Hazard ratio for OS, DFS, and PFS is: 1.67, 0.951, and 0.045, respectively. B) The expression of *FBXL20* is aligned with its copy numbers (n = 308). OS, DFS and PFS were plotted with differential *FBXL20* expression levels as cutoffs. Hazard ratio for OS, DFS, and PFS is: 7.207, 8.09, and 3.929, respectively. C) Demographic of OvCa patients with differential FBXL20 CNVs diagnosed at indicated age groups. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

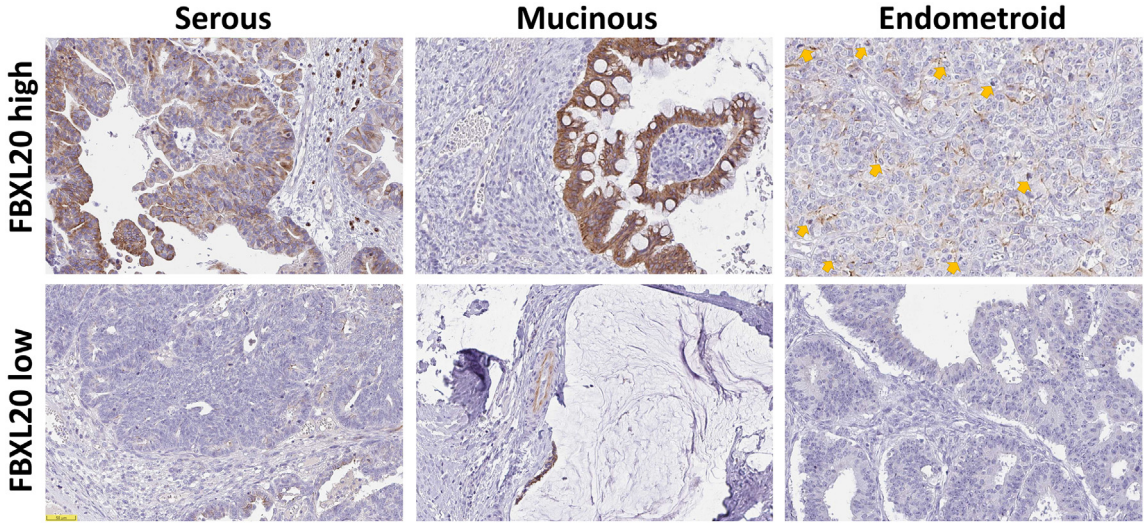

Fig. 4. Clinicopathological features related with different FBXL20 protein levels. Immunohistochemistry staining of different subtypes of OvCa with differential staining intensities of FBXL20. Arrows indicate cells with active replication and/or nuclear atypia. Image credit: Human Protein Atlas. Images available from v19.proteinatlas.org.

indicate that elevated *FBXL20* expression level is associated with malignant histological phenotypes.

Radiogenomics of OvCa samples with differential FBXL20 expression

We further evaluated whether the histological features of clinical samples with *FBXL20* differential expression correlated with radiographic phenotypes. Computerized tomography (CT) images from The Cancer Imaging Archive (TCIA) were employed. Patients were grouped into '*FBXL20* Low' and '*FBXL20* High' cohorts based on the median expression level of *FBXL20*. Samples with low *FBXL20* expression levels (TCGA-13-0799, TCGA-61-2003, TCGA-61-1910) generally have benign features such as thick encapsulation (arrows) and calcification (red circles) (Fig. 6A). Samples with high *FBXL20* expression levels (TCGA-13-1511, TCGA-10-0936, TCGA-09-2044) frequently have features of invasive spreading and extensive necrosis (Fig. 6A). Tumor volumetry is closely related with OvCa staging and prognosis [23]. To further investigate the correlation between radiographical features with differential *FBXL20* expression levels, we analyzed the tumor sizes of '*FBXL20* Low' and '*FBXL20* High' cohorts. We found patients with higher-than-median (n = 191) *FBXL20* expression levels have larger tumor size as reflected by the 'Longest Dimension' than those with lower-than-median (n = 190) expression levels (P = 0.002) (Fig. 6B). These data suggest that elevated *FBXL20* expression is associated with more malignant radiographical features and larger tumor sizes, highlighting the potential of integrating *FBXL20* expression levels in radiogenomic studies for a more informative diagnostic workup of OvCa cases.

Discussion

The prognosis for advanced-stage OvCa remains dismal despite intensive chemotherapy and cytoreduction [24]. Based on 2018 data, approximately 22,000 new OvCa cases were diagnosed with over 14,000 deaths in the United States, making OvCa the most lethal and the second most common gynecologic malignancy in western countries [24]. The early dissemination of OvCa cells complicated surgical debulking. Meanwhile, chemoresistance develops with recurrent regimens, leading to frequent relapses and high mortality rates [24]. Based on the study of age-specific genetic information, we identified CNVs and expression levels of *FBXL20* as valuable markers in predicting OvCa patients' overall survival (OS), disease-free survival (DFS), and progression-free survival (PFS). We

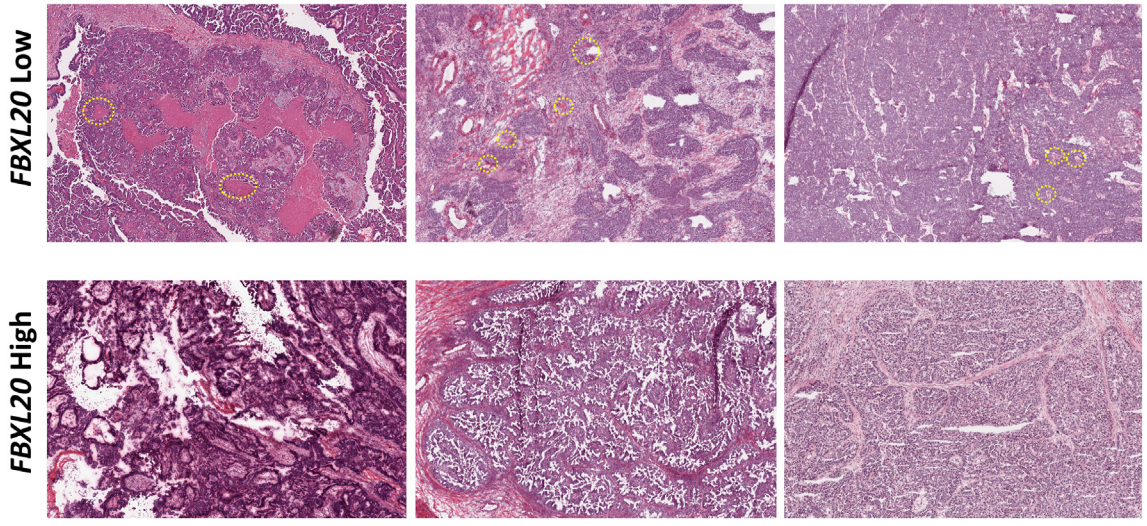

Fig. 5. Histological study of serous subtype OvCa with differential *FBXL20* expression levels. Hematoxylin and eosin (H&E) staining of samples differential expression levels of *FBXL20*. Psammoma bodies (circles) were indicated.

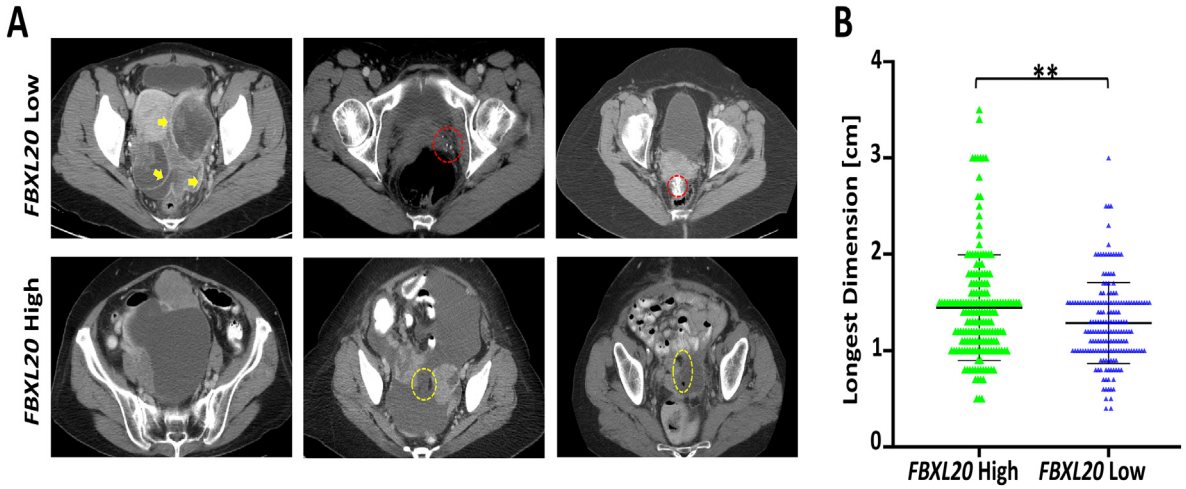

Fig. 6. Computerized tomography (CT) scan of OvCa cases with differential *FBXL20* expression levels. A) OvCa patients was grouped into 'FBXL20 Low' and 'FBXL20 High' cohorts based on the median expression level of *FBXL20*. CT images of OvCa patients with differential *FBXL20* expression levels. Features of encapsulation (arrows) and calcification (red circles) and necrosis (yellow circles) were indicated. B) The longest dimensions of OvCa cases in the 'FBXL20 Low' (n = 190) and 'FBXL20 High' (n = 191) cohorts were presented. The dimensions were measured in centimeters (cm). **: $P < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

identified a unique group of OvCa patients with *FBXL20* copy number loss who will have a much higher percentage of OvCa diagnosis at a younger age (over 60% diagnosed at age >60 years) (Fig. 3C). These patients generally have better clinical outcomes than patients with *FBXL20* copy number amplification. Therefore, screening of *FBXL20* copy numbers may help identify patients who might benefit from clinical workup at a younger age for the purpose of OvCa early detection.

The human genome contains 69 of F-box proteins that recognize substrates for Ub conjugations via the Skp1-Cullin1-F-box (SCF) E3 ubiquitin ligase [16]. Table 1 provides a non-exhaustive list of relatively well-studied F-box proteins involved in OvCa progression. Pathways regulated by those F-box proteins play pivotal roles in OvCa early dissemination, progression, chemoresistance, and metastasis (Table 1). One well-studied substrate of FBXL20 is the vacuolar protein-sorting 34 (Vps34), also known as phosphatidylinositol 3-kinase catalytic subunit type 3 (PI3KC3), which mainly functions in the initiation of autophagy [25]. DNA damage response (DDR) triggers Vps34 phosphorylation, and subsequent FBXL20-mediated polyubiquitination and degradation can dampen the induction of autophagy [26].

The exact roles of FBXL20 in OvCa progression remains unclear. Nevertheless, our data indicate that the downregulation of FBXL20 activities may improve OvCa clinical outcomes. Besides Vps34, other key proteins involved in DDR, including Wee1, checkpoint kinase 1 (CHK1), p21, and cell division cycle 25 A (CDC25A), are SCF E3 ligase substrates [27–31] (Fig. 7, Table 1). Fully activation of CRLs requires conjugation of an ubiquitin (Ub)-like protein called neural precursor cell-expressed developmentally downregulated 8 (NEDD8) to near the C-terminus of cullin1 in the SCF complex [32]. Conjugation of NEDD8 to cullins is carried out in three enzymatic steps involving NEDD8-activating enzyme (NAE; E1), UBC12 and Ube2F (E2s) and E3s (Fig. 7). The NEDD8 conjugation can be inhibited

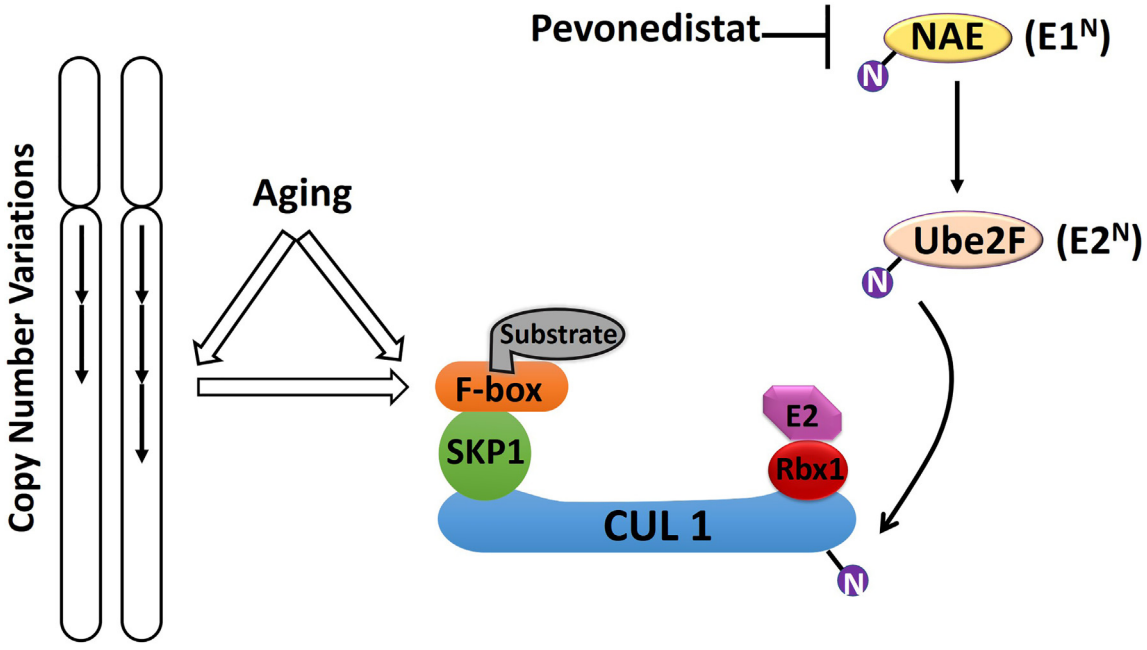

Fig. 7. FBXL20 as a substrate recognizing protein in the Skp1-Cullin1-F-box (SCF) E3 ligase. Schematic overview of how Age-related CNVs of *FBXL20* can affect its expression and thus regulate SCF-mediated degradation of substrates recognized by FBXL20.

by its first-in-class inhibitor pevonedistat and, by doing so, shutting down the Ub-conjugation activities of the SCF complex. Indeed, pre-clinical data showed that pevonedistat treatment induced significant DDR activation in OvCa, with no overlapping sensitivity profile with cisplatin/platinum [33,34]. As such, the therapeutic eradication of FBXL20 activities via pevonedistat-mediated inhibition of NEDD8 conjugation may offer similar survival advantage as those with less *FBXL20* expression.

CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

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