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From Cell Biology to Tissue Engineering

APOBEC3B protein expression and mRNA analyses in patients with high-grade serous ovarian carcinoma

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Summary. APOBEC3 enzymes are part of the innate immune system and they are important in retroviral defense. The number of mutations in ovarian cancer increases with rising levels of APOBEC3B mRNA. We could confirm that APOBEC3B mRNA is upregulated in ovarian cancer cell lines and in ovarian cancer tissue. We evaluated APOBEC3B expression in histologically defined subtypes of ovarian cancer to identify its influence on overall survival (OS) and progression-free survival (PFS).

Tissue microarrays from 219 patients with high-grade serous (HGSC), 61 with low-grade serous (LGSC), 62 with endometrioid (EC) and 55 with clear cell (CCC) ovarian carcinoma were stained using an antibody against APOBEC3B. Real-time quantitative PCR was performed to detect APOBEC3B mRNA levels in 274 cases of HGSC, in 11 cases of LGSC, in 47 cases of EC and in 29 cases of CCC. Tumor-infiltrating lymphocytes (TILs) have been evaluated in a previous project.

APOBEC3B staining was cytoplasmic as well as nuclear and both were positively correlated ($P<0.001$). In HGSC a trend was detectable for positive cytoplasmic staining as favorable regarding OS ($P=0.283$) and PFS ($P=0.137$). High levels of APOBEC3B mRNA were associated with prolonged PFS in HGSC in univariate analyses ($P=0.043$) and multivariate analyses (HR 0.55; 95% CI 0.35-0.88; $P=0.012$). APOBEC3B cytoplasmic staining and APOBEC3B mRNA were positively correlated with TILs.

APOBEC3B in HGSC is related to an active immune infiltrate. However, there is no evidence for APOBEC3B as a clinically relevant prognostic biomarker.

Key words: APOBEC3B, High-grade serous carcinoma, Ovarian, Prognosis

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Introduction

Despite different advances in detecting oncological diseases in earlier stages and in administering targeted therapies, women suffering from ovarian carcinoma still have a poor prognosis with a 5-year-relative survival rate from 45.5% (Howlader et al., 2015). Thus, ovarian cancer belongs to the gynecological malignancies with a high mortality rate accounting for 14 200 estimated deaths (approximately 5%) among American women in 2016 (Siegel et al., 2016).

Due to the unfavorable survival, new strategies against ovarian cancer are constantly emerging. In the past years, many studies focused on the effects of the immune system and its players on carcinogenesis and tumor growth. In ovarian carcinoma, several studies could show that different populations of intratumoral T cells positive for CD3+, CD4+ and CD8+ (Zhang et al., 2003; Sato et al., 2005), receptors on activated T cells (PD-1) and their ligands like PD-L1 (Hamanishi et al., 2007; Darb-Esfahani et al., 2015) and cytokines like IFN-γ (Marth et al., 2004) are strongly associated with

improved survival in ovarian carcinoma. Not only the adaptive but also the innate immune system seem to be involved in carcinogenesis. The apolipoprotein B mRNA-editing catalytic polypeptide (APOBEC) enzymes are part of the innate immune system. As they function as deaminases the APOBEC enzymes convert cytosine to uracil (C-to-U) in ssDNA (Vieira and Soares, 2013) and by doing so a spread of viral infections can be prevented. There are seven APOBEC3 enzymes altogether that occur in the cytoplasm or that are expressed in both the cytoplasm and nucleus (Kinomoto et al., 2007). APOBEC3B itself consists of 362 amino acids, has two catalytic domains and is located in the nucleus (Lackey et al., 2012; Vieira and Soares, 2013).

In several cancer types APOBEC3B is upregulated and thus a source of mutation such as in breast cancer (Burns et al., 2013) and in ovarian cancer (Leonard et al., 2013) with respective varying mutational signatures (Alexandrov et al., 2013). C-to-A/G transversions are obtained as the result of the APOBEC3B driven C-to-U mutation in ovarian carcinoma (Leonard et al., 2013). In breast cancer cell lines APOBEC3B mRNA was expressed most distinctively compared to other APOBEC enzymes (Taylor et al., 2013). Additionally, increased APOBEC3B mRNA was correlated with inactivated tp53 (Burns et al., 2013) in breast cancer tissue which is an often observed mutation in ovarian HGSC as well (Heim et al., 2014). In ovarian carcinoma cell lines APOBEC3B mRNA was significantly upregulated compared to normal ovarian tissue whereas all the other APOBEC enzymes showed no significant differences or even less expression (Leonard et al., 2013). Recently, it was shown that the non-canonical protein kinase C/NFϰB pathway induces APOBEC3B expression (Leonard et al., 2015). There are particular mutational signatures related to enzymes of the APOBEC family (Alexandrov et al., 2013) and especially APOBEC3B seemed to be a promising target for biomarker assessment in human cancer (Taylor et al., 2013).

Since no study has described APOBEC3B expression and its clinical relevance in the different subtypes of ovarian carcinoma before, the aim of this study is to examine APOBEC3B on protein and mRNA level in ovarian cancer. As APOBEC3B is part of the immune system it will also be analyzed as a possible correlation with prior tumor-infiltrating lymphocytes (TILs) previously assessed. We used a large and well-characterized cohort of formalin-fixed and paraffin-embedded ovarian carcinoma tumor samples to evaluate APOBEC3B expression and patient's survival.

Materials and methods

Patients and tissue samples

This study includes formalin-fixed and paraffin-embedded (FFPE) samples from altogether 482 patients with different histological subtypes of ovarian cancer collected within the Tumor Bank Ovarian Cancer Network (TOC: www.toc-network.de). According to current WHO criteria (Kurman et al., 2014) histological subtyping was performed by pathologists from the Institute of Pathology, Charité University Hospital in Berlin. APOBEC3B mRNA levels were available in 274 cases of HGSC, in 11 cases of LGSC, in 47 cases of EC and in 29 cases of CCC. Tissue microarrays (TMA) from 219 patients with HGSC, 61 patients with LGSC, 62 patients with EC and 55 patients with CCC were used for immunohistochemistry. All patients agreed to the scientific use of patient's data and tissue. Survival data on OS was available for 469 patients, data on PFS was available for 396 patients. When diagnosed patients were in mean 58.7 years old (range 21.2 till 92.6). The sectioning of the patient characteristics is summarized in Table 1.

Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

RNA was isolated from FFPE tissue samples by using the VERSANT kPCR Molecular System (Siemens Healthcare, Erlangen, Germany) and appendant VERSANT sample preparation 1.0 reagents. A liquid handling robot (Versant, Hamilton Robotics, Inc., Reno, NV, USA) extracted RNA that was subsequently stored at -80°C. TaqMan- probes were used to quantify the number of PCR-products. Sequences of primers and

Table 1. Patient characterstics.

	Epithelial ovarian carcinoma	
	no. of cases (total n=482)	%
Age, years		
≤60	249	51.7
>60	233	48.3
Histologic type (primary/relapse)		
serous high---grade	304 (303/1)	63.1
serous low---grade	61 (58/3)	12.6
endometrioid	62 (61/1)	12.9
clear cell	55 (53/2)	11.4
FIGO group		
I	92	19.2
II	28	5.9
III	294	61.5
IV	64	13.4
missing	4	–
Residual tumour		
no residual tumour	236	69.4
residual tumour	104	30.6
missing	142	–
Chemotherapy		
platinum-based	377	95.2
others	1	0.3
no chemotherapy	18	4.5
missing	86	–

probes for the reference gene RPL37A (Eurogentec, Seraing, Belgium and Microsynth, Balgach, Switzerland) and APOBEC3B (TIB Molbiol, Berlin, Germany) were as follows:
RPL37A. forward primer: 5'TGT GGT TCC TGC ATG AAG ACA3'; reverse primer: 5'GTG ACA GCG GAA GTG GTA TTG TAC3'; probe: 5'TGG CTG GCG GTG CCT GGA3' APOBEC3B; forward primer: 5'CGA GGC CAG GTG TAT TTC AAG3'; reverse primer: 5'ACA AAC CAG GTG ATC TGG AAA CA3'; probe: 5'CTC AGT ACC ACG CAG AAA TGT GCT TCC TC3'.

We tested our assay in several mammary cell lines where a clearly differential expression of APOBEC3B was detected. Different expression levels could also be quantified in ovarian cell lines (Fig. 1).

A master mix (SuperScript III Platinum One-Step Quantitative RT-PCR System with ROX, Invitrogen, Forest City, CA, USA) and a primer probe mix were pipetted in 384-well plates. RNA samples, positive (Clontech Laboratories, Saint-Germain-en-Laye, France) and negative control (aqua) were pipetted as triplicates. Quantitative RT-PCR was carried out including previous reverse transcription from mRNA to cDNA using a ViiATM 7 real-time PCR device (Life Technologies, Darmstadt, Germany) and analyzed using the ViiATM 7 software (Applied Biosystems, Waltham, MA, United States). Only plates without replication in negative controls were included in this study. Average CT- values were used for each sample in which standard deviation of triplicates had to be lower than 1 CT. The following formula was used to receive a proportional ratio of measured values and the actual RNA amount: 20 - (CT [APOBEC3B] - CT [RPL37A]).

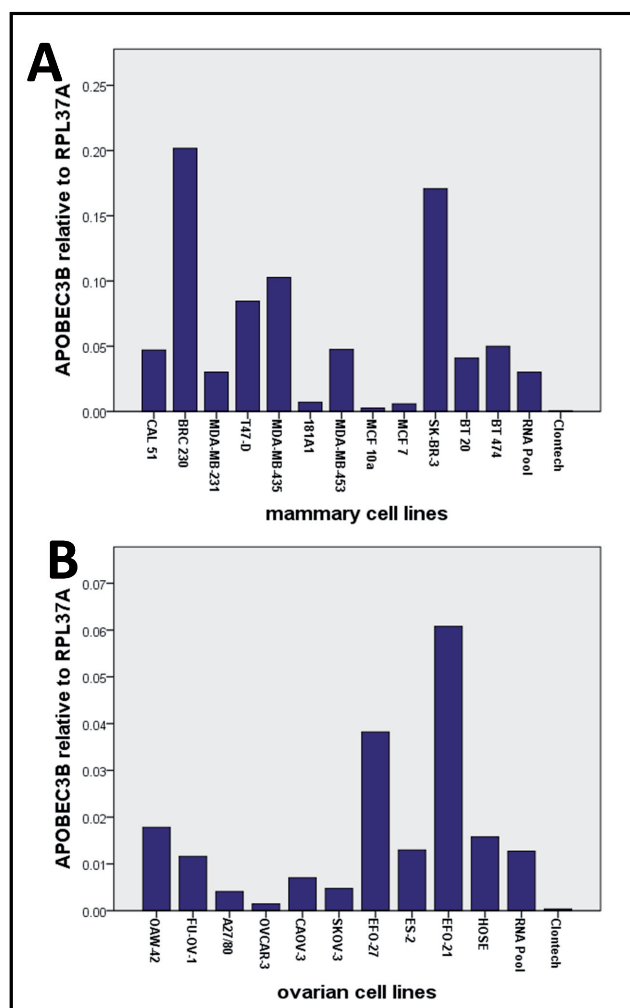

Fig. 1. Relative expression of APOBEC3B among different mammary cell lines **(A)** and ovarian cell lines **(B)**.

Immunohistochemistry (IHC)

TMA's underwent immunostaining using the BenchMark XT device (Ventana Medical Systems, Inc., Tucson, AZ, USA) and appendant reagents. APOBEC3B was stained with a rabbit polyclonal antibody (clone ab191695, Abcam, Cambridge, UK) that was pipetted manually in a dilution of 1:200 on each slide. APOBEC3B staining was visualized using 3,3'-diaminoenzidine peroxide substrate (DAB+) as chromogen. Slides were counterstained with hematoxylin and went through distilled water, ascending alcohol line and xylol before embedding. TILs were detected through the expression of CD3, CD4, CD8, PD-1 and PD-L1. Data had already been published by our group (Darb-Esfahani et al., 2015).

The dried slides were scanned within the VM Slide Explorer 2.2 and evaluated by UR and SDE using appendant software (VMscope GmbH, Berlin, Germany). In case of divergent evaluation appropriate tumor samples were analyzed together again and discussed until consensus was found. Both quantity (0=0%, 1≤10%, 2=11-50%, 3=51-80%, 4=81-100%) and intensity (0=negative, 1=weak, 2=moderate, 3=strong) of stained tumor cells were evaluated for the cytoplasmic and nuclear compartment, respectively, and the multiplication of intensity and percentage scores resulted into an immunoreactivity score (IRS) with values from 0 to 12.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 23 (IBM, Armonk, NY, USA). For correlation analyses Spearman's test, Chi-square test, Fisher's exact test and Kruskal-Wallis test were performed insofar as they were indicated. For comparison of APOBEC3B expression and clinicopathological data the Mann-Whitney-U test was applied. The cut-off finder (http://molpath.charite.de/cutoff/) was used to define the optimal cut-off for

results of qRT-PCR (Budczies et al., 2012). The Kaplan-Meier method and the Cox-regression model were used to detect a correlation of APOBEC3B expression and patient's survival. Significance was given through the results of the log-rank test according to P- values less than 0.05.

Results

APOBEC3B mRNA expression in HGSC and subtypes

APOBEC3B mRNA data ranged from 3.670 to 15.069. In histological subtypes, mRNA levels were differentially expressed (Kruskal-Wallis test, P<0.0001) with lowest expression in EC (median 6.97) and highest expression in CCC (median 9.44).

No correlations between APOBEC3B mRNA and clinicopathological data like age at diagnosis (≤/>60 years), FIGO group (I-II vs III-IV) or residual tumor after surgery could be observed in any of the histological subtypes.

APOBEC3B protein expression in HGSC and subtypes

APOBEC3B protein expression in ovarian cancer tissue was both nuclear and cytoplasmic. The distribution of IRS values ranging from 0 to 12 among all samples included in this study is shown in Fig. 2A for

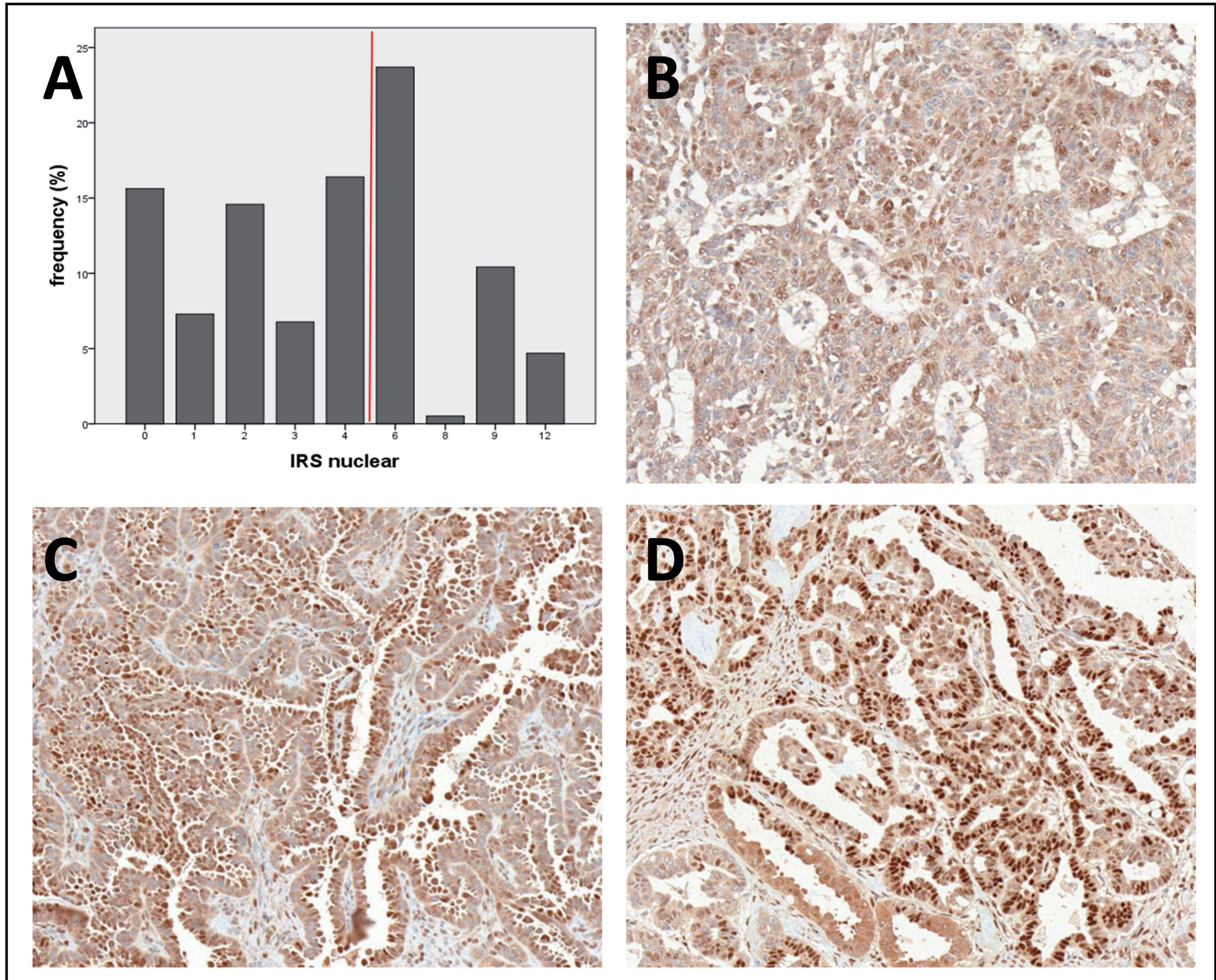

Fig. 2. A. Distribution of APOBEC3B nuclear IRS values through all ovarian cancers from this study (red line is showing the cutoff). Typical examples of APOBEC3B nuclear immunostaining in HGSC showing Weak intensity **(B)** Moderate intensity **(C)** and Strong intensity **(D)**. x 20.

the nuclear compartment and in Fig. 3A for cytoplasm. Examples for weak, moderate and strong intensity of APOBEC3B staining are shown in Fig. 2B-D for nuclear and Fig. 3B-D for cytoplasmic staining. According to results of Spearman's test nuclear and cytoplasmic staining were positively correlated (r=0.264, P<0.0001). To separate APOBEC3B positive from negative tumors, a cut-off was set at IRS>4 resulting in nuclear and cytoplasmic negative tumors corresponding to IRS values equal or lower than 4. Nuclear as well as cytoplasmic staining was different among histological subtypes (Chi^2 test, P<0.0001). In HGSC, 60 (28.6%) tumors were nuclear positive and 45 (21.4%) tumors were cytoplasmic positive. In LGSC, EC and CCC cytoplasmic positive staining was almost as frequent (55.6%, 44.3%, 54.7%, respectively) as negative staining. Nuclear staining occurred more often in LGSC and CCC (75.9%, 60.4%, respectively) and was less frequent in EC (44.3%).

Regarding the nuclear APOBEC3B expression no association of clinicopathological data such as age at diagnosis (≤/>60 years), FIGO group (I-II vs III-IV) and residual tumor after surgery was seen in any type of ovarian cancer. Regarding APOBEC3B expression in cytoplasm, higher IRS values were associated with age equal to or lower than 60 years in HGSC (Mann-Whitney-U test, P=0.001) and with age older than 60 years in EC (Mann-Whitney-U test, P=0.032).

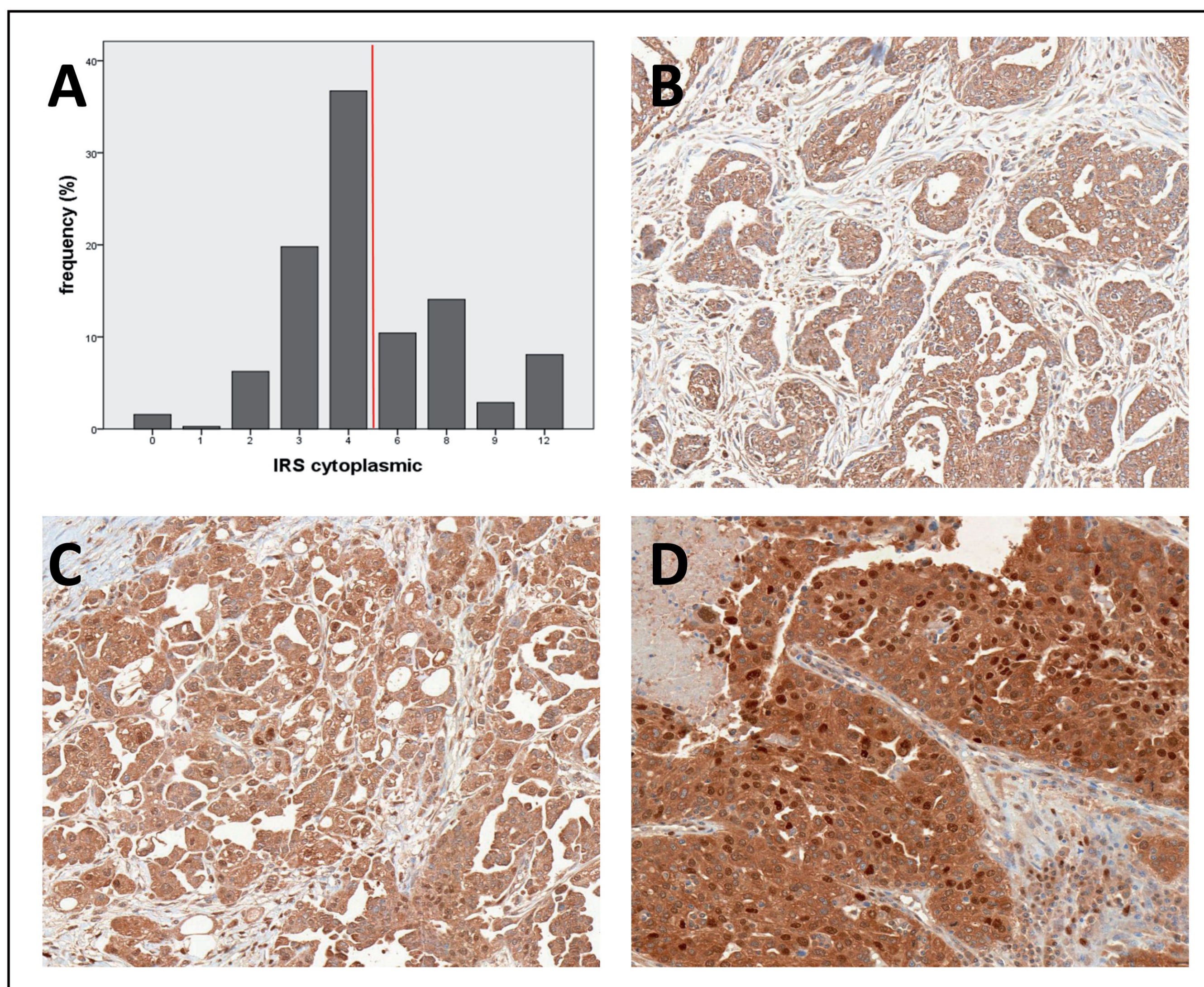

Fig. 3. A. Distribution of APOBEC3B cytoplasmic IRS values through all ovarian cancers from this study (red line is showing the cutoff). Typical examples of APOBEC3B cytoplasmic immunostaining in HGSC showing Weak intensity **(B)** Moderate intensity **(C)** and Strong intensity **(D)**. x 20.

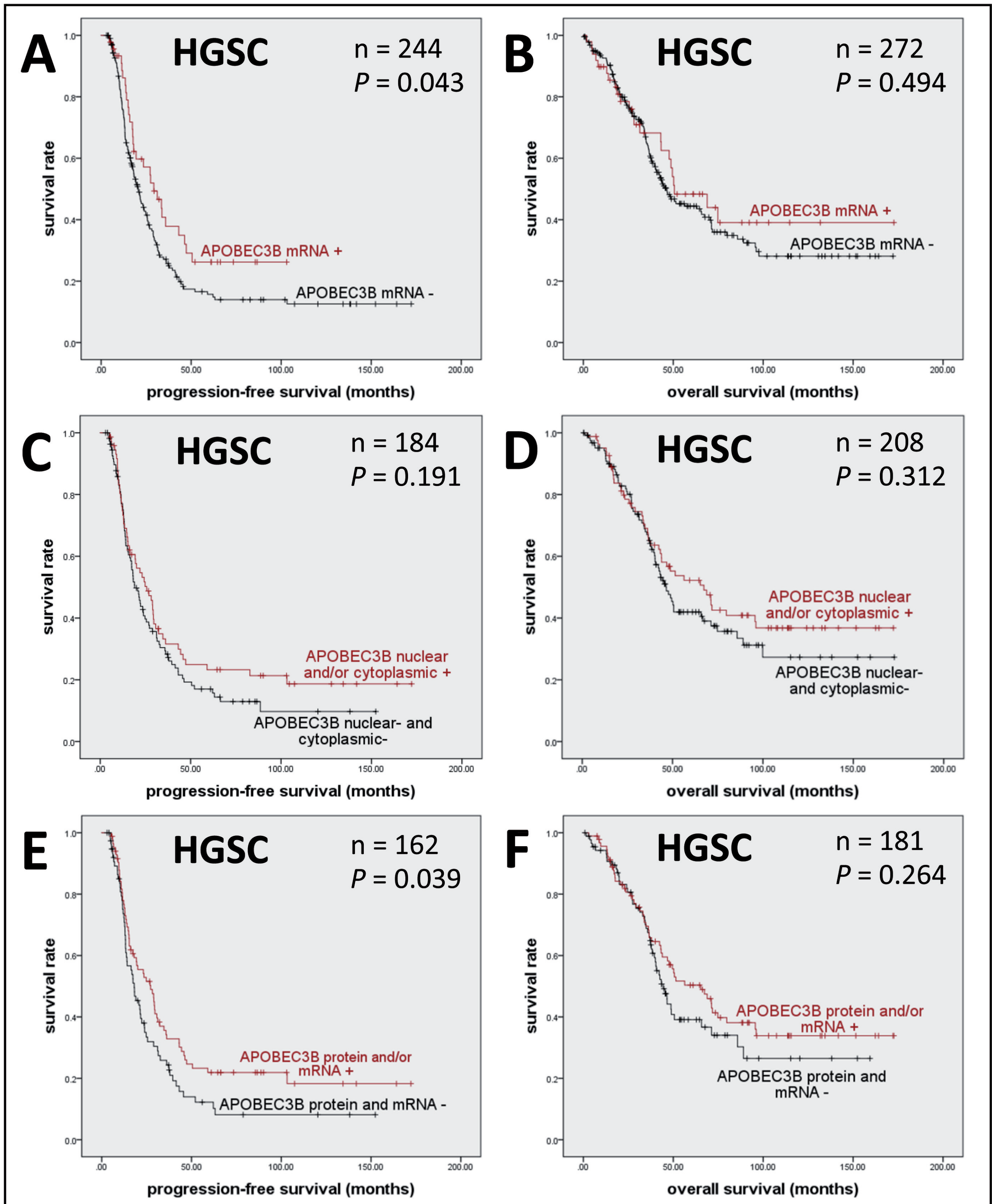

Fig. 4. Kaplan-Meier survival curves in HGSC for progression-free survival and overall survival, respectively, for APOBEC3B mRNA analyses **(A, B)**, APOBEC3B combined protein expression **(C, D)** and APOBEC3B combined mRNA and protein expression **(E, F)**.

Impact of APOBEC3B mRNA expression on patient's survival in HGSC

We performed survival analyses only in HGSC, due to the small sample sizes in LGSC, EC and CCC. Continuous Cox regression showed no significant impact of APOBEC3B mRNA expression on OS or PFS. Similarly, dichotomization of the study cohort at median, the 25th and 75th percentile of ΔΔCT values yielded no significant results in survival analysis. Using the cut-off finder software (http://molpath.charite.de/cutoff/, Budczies et al., 2012) we determined 10.12 as the cut-off with prognostic relevance in HGSC. Using this cut-off 224 tumors (82.1%) were classified as APOBEC3B negative. Patients with negative APOBEC3B mRNA relapsed significantly earlier within 41.36 months compared to 44.61 months in patients with positive APOBEC3B mRNA status (P=0.043, Fig. 4A, Table 2A). Regarding OS, this difference was not significant (P=0.494, Fig. 4B, Table 2B).

Impact of APOBEC3B protein expression on patient's survival in HGSC

While for cytoplasmic staining a trend towards better PFS (P=0.154, Fig. 5A) and OS (P=0.206, Fig. 5B) was seen in patients with APOBEC3B positive HGSC, no significant difference was observed for PFS

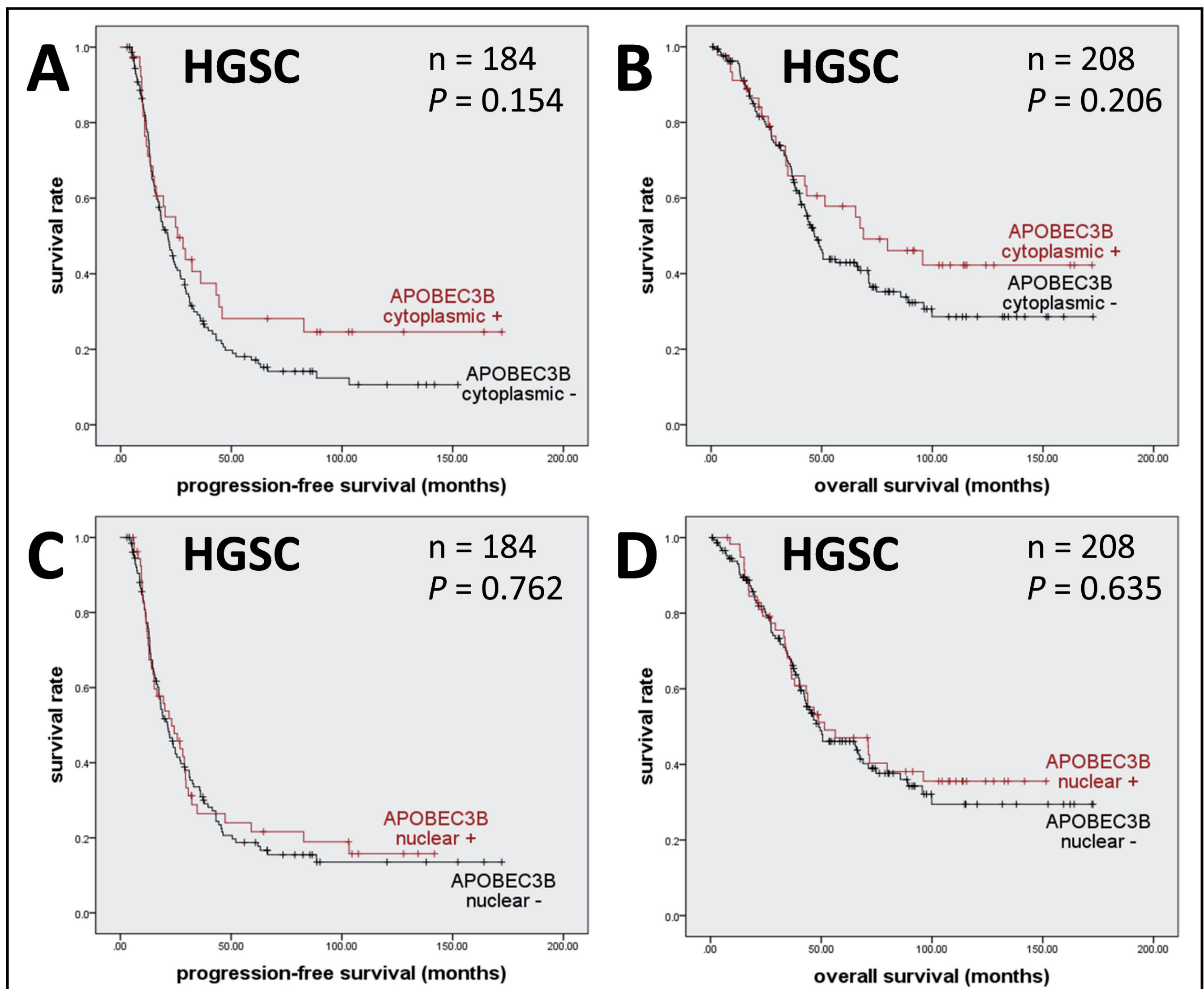

Fig. 5. Kaplan-Meier survival curves in HGSC for of cytoplasmic and nuclear immunostaining, respectively, for progression-free survival **(A, C)** and Overall survival **(B, D)**.

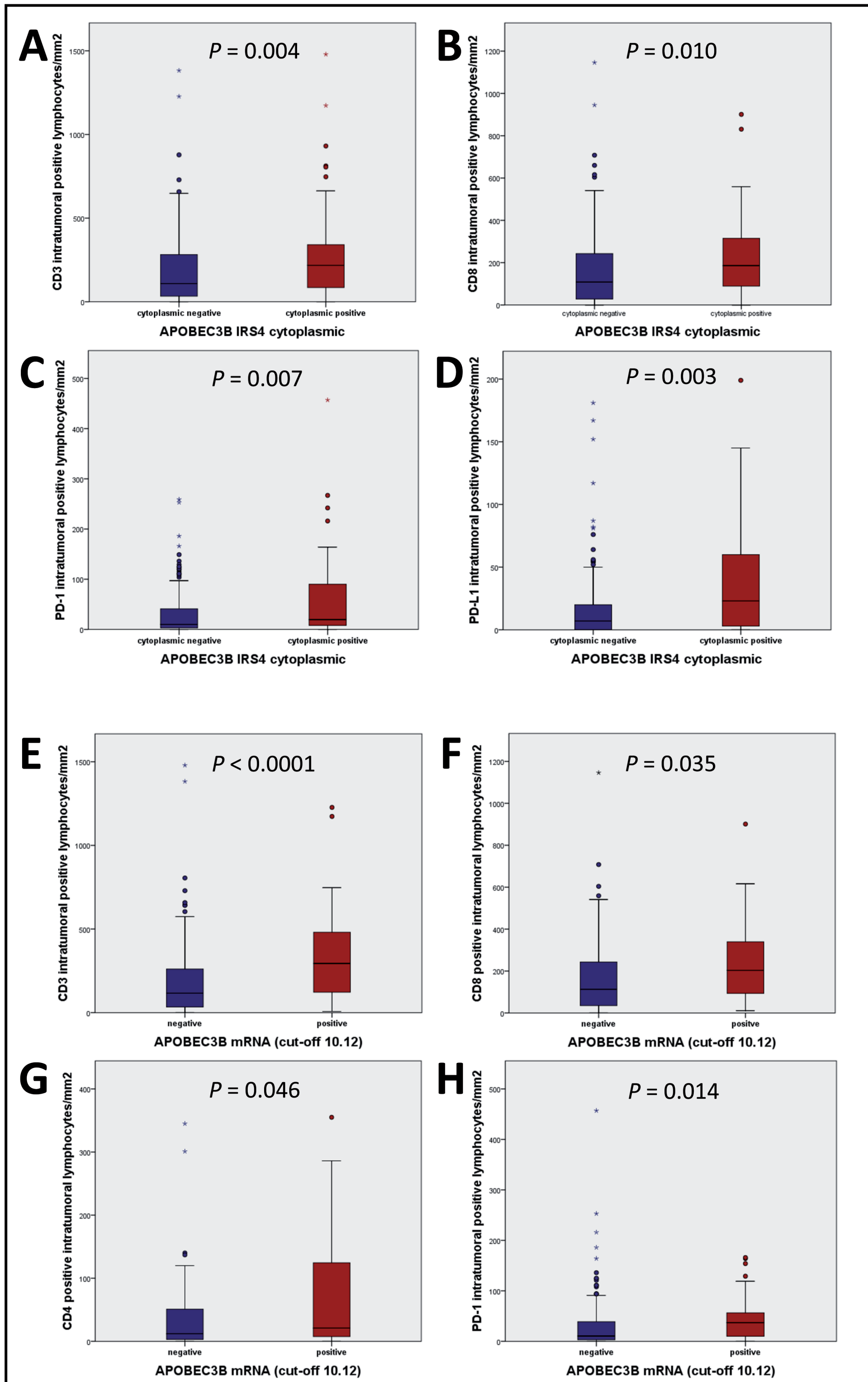

Fig. 6. Correlation of cytoplasmic APOBEC3B expression with CD3 **(A)**, CD8 **(B)**, PD-1 **(C)** and PD-L1 **(D)** and for APOBEC3B mRNA with CD3 **(E)**, CD8 **(F)**, CD4 **(G)** and PD-1 **(H)**.

(P=0.762, Fig. 5C) and OS (P=0.635, Fig. 5D) for nuclear staining in this histological type. When comparing tumors with both nuclear and cytoplasmic negative staining against all others, differences in survival were not significant for PFS (P=0.191; Fig. 4C, Table 2A) or OS (P=0.312, Fig. 4D, Table 2B).

Impact of combined APOBEC3B mRNA and protein expression on patient's survival

In HGSC, a trend towards APOBEC3B as a favorable prognostic factor could be seen for mRNA and protein expression. Therefore, we decided to analyze combined mRNA and protein expression. Patients with tumors negative for both APOBEC3B mRNA and protein expression relapsed significantly earlier (within 32.10 months) than patients with APOBEC3B positive tumors (within 52.56 months; P=0.039, Fig. 4E). According to OS combined APOBEC3B expression was not a significant prognostic factor (P=0.264, Fig. 4F).

Using a multivariate cox-regression model including APOBEC3B expression, age (continuous data), FIGO group (I-II versus III-IV) and residual tumor (none versus present) APOBEC3B mRNA levels were an independent prognostic marker for PFS (HR, 0.55; 95% CI, 0.35-0.88; P=0.012, Table 3A) together with FIGO group and residual tumor, but not for OS (HR, 0.83; 95% CI, 0.51-1.34; P=0.452).

Correlation of APOBEC3B with TILs in HGSC

APOBEC3B cytoplasmic protein expression (IRS) was significantly associated with tumor-infiltrating lymphocytes (TILs) such as CD3 (r=0.167, P=0.017), CD8 (r=0.176, P=0.020) and PD-1 (r=0.172, P=0.014). Using the Mann-Whitney-U test with categorized expression data, we could show that in case of positive cytoplasmic APOBEC3B staining the TILs numbers (CD3, CD8, PD-1, PD-L1) were significantly higher (P=0.004, P=0.010, P=0.007, P=0.003, respectively, Fig.

Table 2. Univariate Kaplan-Meier analyses for survival in HGSC.

	Events	n	mean survival (months)	SE	P (log rank)
A, progression-free survival					
APOBEC3B mRNA expression					
Positive	29	46	44.61	5.89	
Negative	145	198	41.36	4.30	0.043
APOBEC3B protein expression					
Nuclear					
Positive	41	54	43.73	6.85	
Negative	100	130	44.02	5.16	0.762
Cytoplasmic					
Positive	27	39	60.11	11.20	
Negative	114	145	38.63	4.01	0.154
APOBEC3B combined protein expression					
nuclear-/cytoplasmic-	87	111	37.10	4.57	
nuclear and/or cytoplasmic+	54	73	52.81	7.56	0.191
APOBEC3B protein/mRNA expression					
protein and/or mRNA+	62	84	52.56	7.07	
protein-/mRNA-	62	78	32.10	4.88	0.039
B, overall survival					
APOBEC3B mRNA expression					
Positive	23	49	88.91	11.68	
Negative	117	223	77.13	5.25	0.494
APOBEC3B protein expression					
Nuclear					
Positive	34	59	79.17	7.81	
Negative	79	149	80.24	6.32	0.635
Cytoplasmic					
Positive	22	45	95.69	11.10	
Negative	91	163	78.51	5.82	0.206
APOBEC3B combined protein expression					
nuclear-/cytoplasmic-	68	126	77.19	6.94	
nuclear and/or cytoplasmic+	45	82	88.93	7.93	0.312
APOBEC3B protein/mRNA expression					
protein and/or mRNA+	51	92	86.22	7.48	
protein-/mRNA-	49	89	71.30	7.36	0.264

6A-D). No association between nuclear APOBEC3B expression and TILs was found (neither for continuous nor for categorized data). APOBEC3B mRNA was positively correlated with following TILs subpopulations: CD3 (r=0.280, P<0.0001), CD4 (r=0.186, P=0.023), CD8 (r=0.172, P=0.033) and PD-1 (r=0.244, P=0.001). Having used the Mann-Whitney-U test with categorized data we could show that in case of positive mRNA APOBEC3B status (cut-off 10.12) the TILs numbers (CD3, CD4, CD8, PD-1) were significantly higher (P<0.0001, P=0.046, P=0.035, P=0.014, respectively, Fig. 6E-H). We further performed a multivariate cox-regression model with APOBEC3BmRNA, age (continuous data), FIGO group (I-II versus III-IV), residual tumor (none versus present) and TILs subpopulations (CD3, CD4, CD8, PD-1) that were positively correlated with APOBEC3B mRNA. APOBEC3B mRNA was still an independent prognostic factor (HR, 0.45; 95% CI, 0.20-0.97, P=0.042) taken together with FIGO group and CD4 expression.

Discussion

To our knowledge this is the first study on APOBEC3B expression in different subtypes of ovarian carcinoma at both protein and mRNA levels. Since ovarian HGSC is the most common subtype among ovarian carcinoma (Gilks, 2004) and the one with the worst prognosis, the impact of APOBEC3B on patient's survival was of high interest in this group. In HGSC, high APOBEC3B mRNA expression was significantly prognostic for prolonged PFS in univariate and multivariate analyses. This is in line with a study by Leonard et al. which obtained a trend towards APOBEC3B mRNA as favorable prognostic factor but without significance in ovarian HGSC (Leonard et al., 2016).

In contrast to that, in estrogen-receptor positive (ER+) breast cancer high APOBEC3B mRNA levels were significantly correlated to worse survival, whereas in ER- breast cancer APOBEC3B expression had no

Table 3. Multivariate Cox regression analyses for PFS in HGSC.

	HR	95% CI	P
APOBEC3B mRNA expression			
APOBEC3B status			
APOBEC3B mRNA-	1		
APOBEC3B mRNA+	0.55	0.35-0.88	0.012
Age (per year)	0.99	0.98-1.01	0.434
FIGO			
I-II	1		
III-IV	7.29	2.67-19.86	<0.0001
Residual tumor			
No residual tumor	1		
Residual tumor	1.79	1.27-2.53	0.001
APOBEC3B protein expression			
APOBEC3B status			
APOBEC3B nuclear and cytoplasmic negative	1		
APOBEC3B nuclear and/or cytoplasmic positive	0.86	0.59-1.26	0.440
Age (per year)	1.00	0.98-1.02	0.811
FIGO			
I-II	1		
III-IV	2.42	0.88-6.62	0.086
Residual tumor			
No residual tumor	1		
Residual tumor	1.41	0.94-2.12	0.098
APOBEC3B combined mRNA/protein expression			
APOBEC3B status			
protein-/mRNA-	1		
protein and/or mRNA+	0.81	0.55-1.21	0.301
Age (per year)	1.00	0.98-1.03	0.726
FIGO			
I-II	1		
III-IV	2.09	0.75-5.82	0.157
Residual tumor			
No residual tumor	1		
Residual tumor	1.34	0.87-2.08	0.189

impact on survival (Sieuwerts et al., 2014). Also, Cescon et al. could show that the prognostic impact of APOBEC3B on survival is limited to the luminal breast tumor type (Cescon et al., 2015). Since ER- breast tumors are molecularly similar to ovarian HGSC showing both frequent tp53 and BRCA mutations, a high level of copy number alterations and aberrations in AKT3 and c-myc pathways (Alexandrov et al., 2013), these results correspond with our data.

APOBEC3B protein expression showed a distinctive, both nuclear and cytoplasmic staining, in our study population. Cytoplasmic expression was rather unexpected given the described function of APOBEC3B as a nuclear enzyme. However, there are reports revealing that only the N-terminal domain of APOBEC3B is located to the nucleus whereas the C-terminal half of APOBEC3B show cytoplasmic localization (Bogerd et al., 2006; Lackey et al., 2013). This might explain the cytoplasmic staining in our study population. Interestingly, only cytoplasmic staining showed associations with survival, clinical parameters and TILs.

Cytoplasmic APOBEC3B expression has already been described in other entities. In renal cell carcinoma (RCC) APOBEC3B staining was only obtained in the cytoplasm. Nearly a quarter of tumors were positive in this study and at least in clear-cell RCC high APOBEC3B expression was significantly related to worse survival (Xu et al., 2015). In non-small-cell-lung-cancer (NSCLC) APOBEC3B expression was observed in 50.7% of tumors and again exclusively stained in cytoplasm (Yan et al., 2016). This study could also show, that high levels of APOBEC3B are associated with worse survival. Strikingly, in both studies no APOBEC3B was described in the nuclei. This, together with the results of our project point to a biological role of cytoplasmic localization of APOBEC3B. Other groups found APOBEC3B expression to be significantly linked to a worse prognosis in chondrosarcoma (Jin et al., 2014) and in gastric cancer (Zhang et al., 2015), yet details on distribution of APOBEC3B immunostaining were missing in these studies.

The correlation of APOBEC3B with markers for an active immune infiltrate is in line with results of Leonard et al. (2016) who, apart from the correlation of immune parameters with APOBEC3B, detected a stronger and highly significant correlation with TILs (CD3D, CD4, CD8A, GZMB, PRF1) and APOBEC3G based on mRNA levels. To distinguish negative from positive expression, APOBEC3B mRNA levels were split at the median in this study. In 354 cases of primary serous HGSC, only a trend for APOBEC3B mRNA as a favorable prognostic factor for prolonged PFS ($P=0.034$) rather than for OS ($P=0.06$) was detectable. However, APOBEC3G was a favorable prognostic factor and in addition to estimated TILs the most indicative marker for improved survival for both PFS ($P<0.0001$) and OS ($P=0.0003$) in this study. Thus, the impact of APOBEC3 enzymes on survival in epithelial ovarian carcinoma is probably based on the expression of APOBEC3G rather than APOBEC3B.

Both APOBEC enzymes and TILs are part of the immune system and thus their similar influence on survival in HGSC could be explained by a cancer-driven immune stimulation in general. On the other hand, one could speculate about a causal relationship between APOBEC3B and TILs which are both positively correlated with each other. APOBEC3B is also shown to be involved in mechanisms of antiviral defense such as in reducing HIV infectivity (Doehle et al., 2005) and HBV replication (Zhang et al., 2008). There are conflicting reports about the role of human papilloma viruses (HPV) in ovarian carcinoma (Ingerslev et al., 2016; Zhang et al., 2016) but altogether a migration of dysplastic cells from the fallopian tube to the ovary seems to be more likely responsible for ovarian carcinogenesis than viral infections (Piek et al., 2001). Nevertheless, it is interesting to know that high-risk HPV induces upregulation of APOBEC3B in vitro and in vivo (Vieira et al., 2014).

The strengths of our study are that we detected APOBEC3B at both protein and mRNA levels using IHC and qRT-PCR, as well as the rather large sample size for the HGSC subtype. The small samples sizes of the special histological subtypes are limitations of our study. We must state that APOBEC3B mRNA seems to be of a rather minor relevance as a prognostic marker in epithelial ovarian cancer. A robust biomarker should have shown several significant cut-offs and more distinctive results in survival analyses. Furthermore, we had no data on mutational signatures in our cohort. The investigation of a potential relationship between protein expression of APOBEC3B (and potentially other APOBEC isoforms) with special emphasis on cytoplasmic expression and APOBEC signatures in ovarian carcinoma would be an attractive topic for future studies.

All in all, we can conclude that APOBEC3B is differentially expressed in epithelial ovarian cancer tissue. Due to correlations with markers for an active immune infiltrate, a link to immune activation seems to be plausible. However, we must consider that in ovarian HGSC there is no evidence for APOBEC3B as a suitable biomarker for patient's survival or patient's response to immunotherapies given through the findings of our study.

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Compliance with ethical standards. All patients included in TOC gave informed consent for scientific use of their samples and data. The current study has been confirmed in the current amendment (AVD-no 2004-000034) of the TOC ethical vote 207/2003 by the ethics committee of the Charité. Approval for patients not included into TOC was given by the Charité ethics committee by the 2013 amendment to ethical vote EA1/139/05. According to this vote, informed consent was not necessary for older samples (<2010) but necessary and available for younger samples.

Conflict of interest. The authors declare that they have no conflicts of interest.

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