Reviewer’s report

Title: Cancer-testis antigen Cyclin A1 is broadly expressed in ovarian cancer and is associated with prolonged time to tumor progression after platinum-based therapy

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Reviewer: Marek Cybulski

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Cancer-testis antigen Cyclin A1 is broadly expressed in ovarian cancer and is associated with prolonged time to tumor progression after platinum-based therapy.

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The authors compared Cyclin A1 mRNA expression in 20 epithelial ovarian cancer (EOC) and different healthy tissues from microarray data sets (NCBI GEO database). Additionally, authors evaluated Cyclin A1 mRNA and protein expression, in serous EOCs from patients who received cytoreductive surgery followed by platinum-based chemotherapy, by quantitative Real-Time PCR (qRT-PCR; N=17) and immunohistochemistry (IHC; N=72), respectively. Cyclin A1 expression was correlated with clinical features of EOC.

I. Major Compulsory Revisions

Results

1) Lines 229-231: "... at least moderate Cyclin A1 expression (Cyclin A1 high) was associated with prolonged TTP in an univariate survival analysis (p=0.018, 27.5 vs 14.6 months) (Figure 6)."

a) Check figure 6 legend and Kaplan-Meier (KM) plots. Legends for panels A/B, C/D or KM plots are switched. Check labels on Y axes of KM plots.

b) Indicate in Fig. 6 and 7 legends the meaning of "+" signs. Are they censored cases? If yes why there are no censored cases on Kaplan-Meier plot B (Fig. 6)?

c) Lines 245-246: "Homogeneous positivity for Cyclin A1 was associated with longer OS in the univariate analysis (p=0.044, 65.3 vs 42.2 months)"

Is it presented on Fig. 6, panel D?

2) Lines 234-237: "In that population, the difference in TTP between Cyclin A1 high and Cyclin A1 low patients was even greater (median TTP 26.1 vs 13.0 months) suggesting that Cyclin A1 expression is predictive of patient responsiveness to the standard first-line chemotherapy regimen (Figure 7F)."

To draw such conclusion authors should additionally compare cyclin A1 expression in EOCs in relation to tumor platinum-sensitivity and patients response to chemotherapy (RECIST criteria).
3) Lines 241-244: "In a multivariate analysis of Cyclin A1 staining intensity, the percentage of Cyclin A1-positive cells, tumor grade, macroscopic residual tumor after debulking, FIGO stage, and age at first diagnosis, only Cyclin A1 high staining intensity was an independent indicator for prolonged TTP (p=0.012). (Supplementary Figure 2)."

Additionally, EOC platinum-sensitivity and peritoneal carcinomatosis should be included in Cox regression analysis. The authors should describe criteria used for inclusion or exclusion of prognostic factors in Cox model. Moreover, the authors should add HR, and lower and higher 95% confidence interval values for all factors included in the final Cox model to Supplementary Table 2.

4) Lines 263-264: "Consequently, the data imply that the impact of Cyclin A1 expression on TTP is not dependent of the molecular subtype."

This conclusion should be supported by the results of the multivariate survival analysis including "C" molecular subtypes of EOC. As there are several different C subtypes, they can be entered into Cox model as dichotomous variable (C1 (most common subtype) vs combined other high-grade subtypes).

Discussion

1) Lines 325-328: "The longer TTP in patients with higher Cyclin A1 levels might reflect responsiveness to cytostatic treatment rather than an association between more aggressive tumor biology and later-stage and/or metastatic disease and peritoneal carcinomatosis at first diagnosis."

Update discussion according to the comments to Results.

2) Lines 347-349: "Given that C1 is characterized by short TTP, its association with high Cyclin A1 expression implies that the impact of Cyclin A1 expression on TTP is independent of the molecular subtype (Supplementary Figure 3)."

Update discussion according to the comments to Results.

3) Lines 354-355: "Cyclin A1 acts as predictive marker for response to standard platinum based cytostatic therapy translating into prolonged TTP."

Update discussion according to the comments to Results.

II. Minor Essential Revisions

Introduction

Lines 115-116: "Currently, we have only sparse data on the impact of Cyclin A1 on proliferation, invasiveness, and resistance to apoptosis in EOC[21]."


Material and Methods

1) Paragraph "Patients and specimens"

The authors should indicate if EOC samples (frozen and paraffin-embedded) used for qRT-PCR and IHC were collected from patients before or after
chemotherapy.

2) Lines 142-144: "Samples exceeding the mean expression level plus three standard deviations of the healthy, non-testicular tissue samples were considered positive."

According to figure legends (lines: 388 and 392-393) median+3SD values are marked by horizontal bar in Figure 1 and 2.

3) Lines 172-173: "The staining intensities were expressed as weak (1), weak to moderate (1.5), moderate (2), moderate to strong (2.5), or strong (3)."

This sentence belongs to the "Immunohistochemistry staining" paragraph.

4) Paragraph “Immunohistochemistry staining”
The authors should describe who evaluated IHC staining and if there were two or more observers how discrepancies were handled.

5) Paragraph "Statistics"
Provide the name of statistical software used for statistical analysis.

Results

1) Lines 213-214: "Homogenous Cyclin A1 positivity observed in 43 of 61 grade 3 specimens but in only one of 11 grade 2 specimens (p=0.005, Figure 4)."

According to Table 1 and Figure 4 there were 10 grade 2 and 62 grade 3 EOCs. If "Homogenous Cyclin A1 positivity" is shown as red bars there are 42 grade 3 EOCs (Fig. 4).

2) Lines 215-216: "The percentage of positive cells but not staining intensity was significantly higher in the grade 3 specimens (Figure 5B,D)."

Add p value for this comparison in text and add p values for all 4 panels (A-D) in Fig. 5.

3) Paragraph "Cyclin A1 expression is associated with prolonged time to progression"

The authors should calculate and give the mean or median and min-max time of TTP and OS for all patients.

4) Lines 228-229: "While grading, age, macroscopic residual tumor after debulking, and peritoneal carcinomatosis / distant metastasis at first diagnosis had no impact on TTP or OS, ..."

The authors should calculate and indicate if FIGO stage and tumor platinum-sensitivity had significant impact on TTP and OS in univariate analysis.

5) Lines 238-241: "... online-accessable tool ‘Kaplan-Meier-Plotter’ (www.kmplot.com/ovar[23] to evaluate the impact of Cyclin A1 expression on TTP in an independent data set of 264 patients with serous ovarian cancer after suboptimal debulking and platinum-based chemotherapy. Again, higher Cyclin A1 expression levels were associated with longer TTP (p=0.0088, Supplementary Figure 1)."

The authors should indicate the method for cut-off selection and database
version used for analysis with KM Plotter as the analysis with following settings (Affy id: 205899_at, survival: PFS, split patients by: Auto (best cutoff), restrictions: histology (serous), debulk (suboptimal), and chemotherapy (contains platin), database: 2015 version n=1648) can be performed on 266 patients and yields HR=0.64 (lower risk), p=0.0014 (603 cut-off value). Interestingly, the analysis made on the group of patients after optimal debulking (n=496) gives HR=1.27 (higher risk), p=0.0349 (cut-off value 483), which should be discussed by authors. 

6) Lines 246-247: “However, in the multivariate analysis, none of the parameters mentioned above was an independent prognostic marker for OS (data not shown”).

There are shown in supplementary Table 2 "(Supplementary Figure 2)".

Discussion

1) Line 307: "(Heiko Schuster, Tübingen, personal communication) [20]"
Wrong citation "[20]" and the missing reference "Heiko Schuster, Tübingen, personal communication".

2) Line 315: "... Cyclin A1/CDC2 complex mediates ...."
Restle et al. [36] proposed that cyclin A1/cdk2-mediated phosphorylation of p53 enables stable complex formation with topo I, thereby causing hyper-recombination in p53 mutant cells (Fig. 9).

3) Lines 328-330: "Cyclin A1 directly interacts not only with p53 but also with at least two members of the Retinoblastoma gene product (pRB) pathway, pRB and E2F-1, which regulates proliferation and is itself modulated by p53 [39-41]."

4) Line 332: "rRB"
Correct the name.

References


4) Some references need reformatting according to the journal reference style.

III. Discretionary Revisions

Abstract

Results: "Cyclin A1 was homogeneously expressed in 43 of 61 grade 3 tumor samples and in 1 of 11 grade 2 specimens (p<0.001). Survival analysis showed longer time to progression (TTP) among 49 patients with at least moderate Cyclin A1 expression (univariate: p=0.018, multivariate: p=0.012)."

Was it cyclin A1 protein expression?

Introduction

1) Lines 71-72: "Epithelial ovarian cancer (EOC) is the sixth most common cancer and the seventh most common cause of cancer-related death among women worldwide[1], ...

Ovarian cancer is the seventh most common cancer and the eighth most common cause of cancer-related death among women worldwide [http://globocan.iarc.fr/Pages/fact_sheets_population.aspx]; data in Table "Estimated incidence, mortality and 5-year prevalence: women".

2) Lines 73-74: “About two thirds of patients with EOC are diagnosed in an advanced stage with peritoneal or visceral spread[3].”


3) Lines 75-77: "Despite high response rates to first-line systemic treatment, all patients with initially advanced or secondary metastatic disease relapse, develop platinum resistance, and eventually die from the disease[4]."

Approximately 80% of women with advanced ovarian cancer will have tumour progression, or more commonly a recurrence, which is usually eventually fatal due to the emergence of drug resistance [Luvero D, Milani A, Ledermann JA. Treatment options in recurrent ovarian cancer: latest evidence and clinical potential. Ther Adv Med Oncol. 2014 Sep;6(5):229-39. doi: 10.1177/1758834014544121].

Results


Table 1
Last row "Primary platinum sensibility"
Change "sensibility" to "sensitivity"

Legend for Figure 1
Add the median+3SD value in the legend to this Fig.

Figure 2
1) Several names of healthy tissues on X axis are cut.
2) Add label to Y axis e.g. CCNA1 expression (%)
3) Add the median+3SD value in the legend to this Fig.

Supplementary Figure 3
1) Figure Legend
Describe the meaning of red horizontal lines on dot plots. Are they median or mean values?
2) Supplementary Figure 3
Add Label to Y axis e.g. CCNA1 expression

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.