Cells 2025 Conference
The 3rd International Online Conference on Cells
25–27 March 2025 | Online
MDPI

Clinicopathological significance of DNA damage signalling and repair (DDR) regulating E3 ubiquitin ligases and de-ubiquitinases in ovarian cancer

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INTRODUCTION & AIM

- Ovarian cancer is the second leading cause of gynaecologic cancer related mortality. Late diagnosis and therapy related resistance contribute to poor clinical outcomes for patients.
- DNA damage signalling and response (DDR) is critical for maintaining genomic stability.
- In platinum sensitive or homologous recombination deficient (HRD) or BRCA1/2 germ-line mutated ovarian cancers, PARP inhibitor maintenance therapy (olaparib, niraparib, rucaparib) is approved for clinical use in high grade serous ovarian cancer. However, PARP inhibitor resistance is a growing challenge in clinical settings. Therefore, development of novel predictive biomarkers and therapeutic targets is a high priority.
- Protein regulation by ubiquitination (by ubiquitin ligases) and de-ubiquitination (by de-ubiquitinases) is critical for cellular homeostasis.
- E3 ubiquitin ligases (E3 UBL) and de-ubiquitinases (DUBs) regulate DDR and are potential therapeutic targets for cancer treatment.
- In this study, we evaluated the clinicopathological impact of DDR specific E3 UBLs and DUB in ovarian cancer.

METHOD

- Five E3 UBLs [DDB2, CUL4A, RAD18, HLTF, and HUWE1] and 3 DUBs [USP5, USP7, PSMD14] were investigated in ovarian cancer.
- Western blot: A panel of platinum sensitive and resistant ovarian cancer cell lines were used to validate the specificity of the used antibodies and to study their protein expression level.
- The immunohistochemical study was conducted on 331 consecutive cases of ovarian cancer patients who were diagnosed and treated at Nottingham university hospitals (NUH) between 1997 and 2010.
- Transcriptomic study: RNA-seq data of 424 *TP53* mutant, platinum treated with advanced stage (III & IV) ovarian cancer patients was explored using KM plotter online tool.

RESULTS & DISCUSSION

- A single specific band was detected in ovarian cancer cell lines of all studies targets as shown in figure (1).

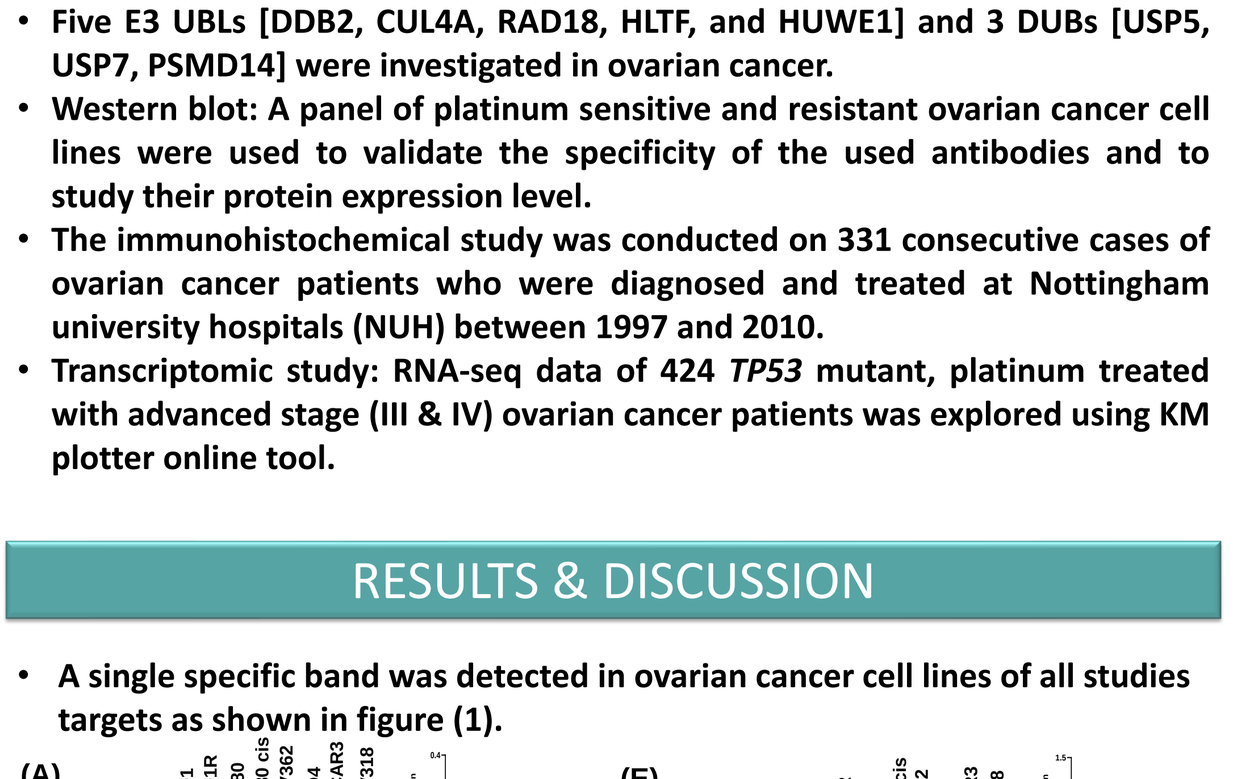

Figure (1): Expression of different E3 UBLs and DUBs in ovarian cancer cell lines. (A) DDB2, (B) CUL4A, (C) HLTF, (D) RAD18, (E) HUWE1, (F) USP5, (G) USP7, (H) PSMD14.

- All used antibodies showed nuclear and cytoplasmic expression except DDB2 and USP7 which were exclusively expressed in the nucleus as shown in figure (2).
- Patients with high grade serous carcinoma (HGSOC) showed significantly low DDB2 (*p* value <0.001), high cytoplasmic CUL4A (p value = 0.02) and high USP5 expression either cytoplasmic (*p* value = 0.003) or nuclear (*p* value = 0.02). High nuclear and cytoplasmic co-expression (*p* value = 0.004) was also associated with HGSCOC.
- Advanced stage tumors (stage III) was significantly linked with low DDB2 (*p* value = 0.01), high USP5 cytoplasmic (*p* value = 0.05) and high PSMD14 cytoplasmic expression (*p* = 0.02).

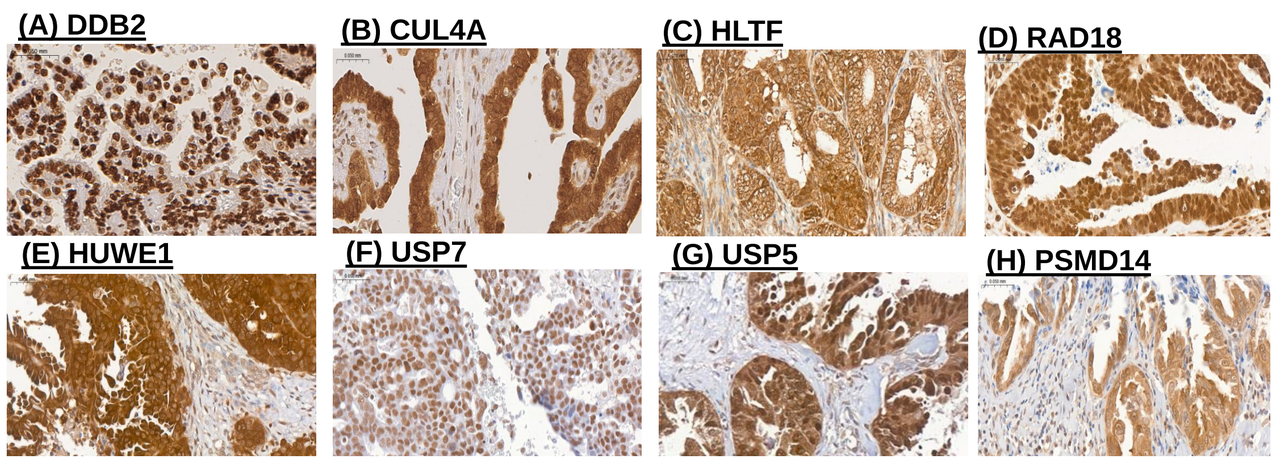

Figure (2): photomicrographs of UBLs and DUBs immunohistochemically stained in ovarian TMA. (A)DDB2, (B) CUL4A, (C) HLTF, (D) RAD18, (E) HUWE1, (F) USP7, (G) USP5 and (H) PSMD14. All photos are taken at 40x magnification.

- Outcome analysis revealed that among studied E3 UBLs, DDB2 deficiency, CUL4A and HLTF overexpression predicted poor outcome of OC patients. Additionally, all studied DUBs were poor predictors of patient survival as shown in figure (3).

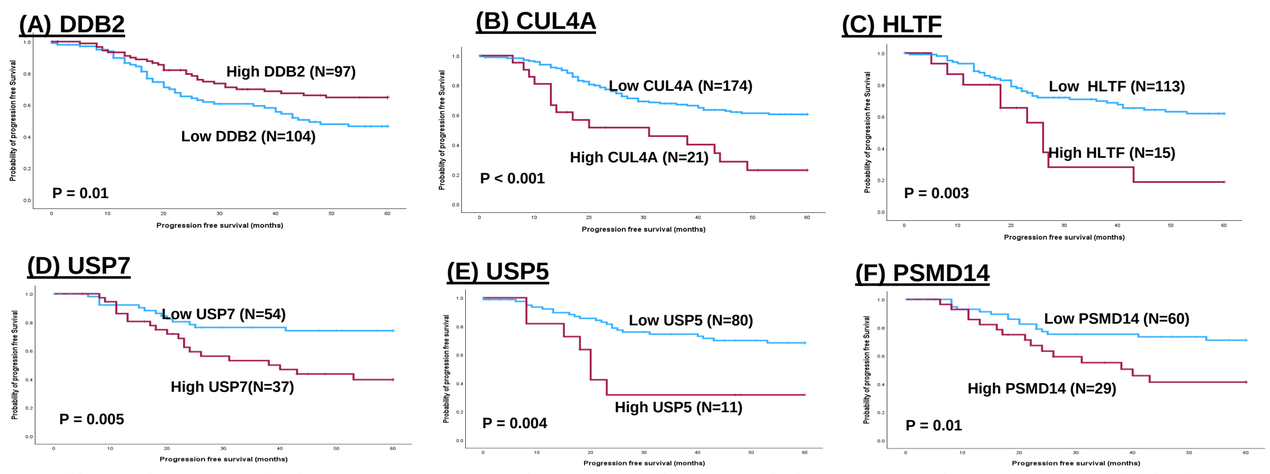

Figure (3) Kaplan Meier curves of progression free survival analysis in whole ovarian cohort. (A) DDB2, (B) CUL4A, (C) HLTF, (D) USP7, (E) USP5, (F) PSMD14.

- Outcome analysis of patients with *TP53* mutation revealed that USP5, PSMD14 and HLTF overexpression was poor predictors of patients' outcome at proteomic and transcriptomic levels as shown in figure (4)

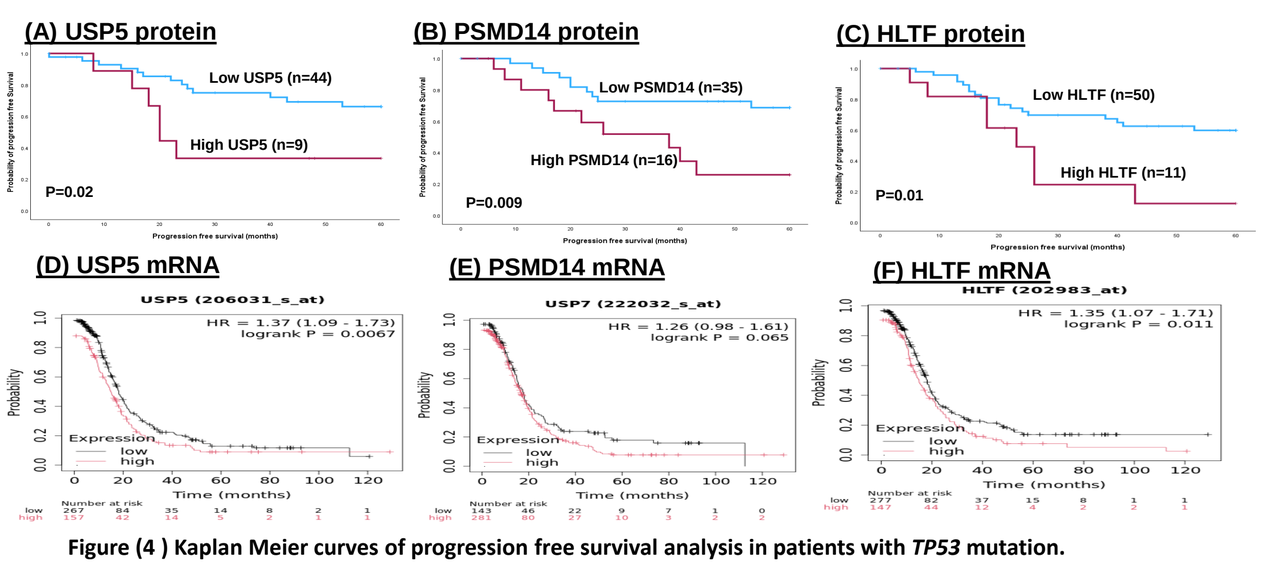

Figure (4) Kaplan Meier curves of progression free survival analysis in patients with *TP53* mutation.

- Multivariate survival analysis of all studied genes revealed that only USP5 was independent predictor of poor progression free survival (p = 0.03) when all of them were tested against pathological stage (p = 0.001).

Conclusion

- Our data suggest a complex role for E3 UBLs and DUBs in ovarian cancers. USP5 may be an attractive target for patient stratification and therapeutics in ovarian cancer.