Editorial Commentary

Proteomic analysis of PAX8 alterations provides new insights into its role as a master regulator of migration in high-grade serous ovarian cancer

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High-grade serous ovarian cancer (HGSOC) is the most common subtype of ovarian cancer (1). This cancer is a heterogeneous disease associated with few shared genetic mutations (BRCA1, BRCA2, p53) (2) and with widely accepted origination from the fallopian tube epithelium (FTE). Eighty percent to 90% of HGSOCs, after malignant transformation, express the paired box transcription factor 8 (PAX8) (3). This transcriptional factor is normally expressed in the FTE but not in the healthy ovarian surface epithelium (OSE) (4). Several studies have recently reported PAX8 pivotal function in the migration, invasion and tumorigenic ability of ovarian cancer cells. Loss PAX8 in ovarian cancer cell lines decreases *in vitro* and *in vivo* tumor phenotype and promotes apoptosis (4-6). Hence, there is a growing interest to evaluate PAX8 not only as a diagnostic biomarker but also as a potential therapeutic target for HGSOC. However, the key pathways that allow PAX8 to regulate cellular processes in FTE and in HGSOC is still poorly understood.

In this scenario, Hardy *at al*. recently reported the first proteomic study of PAX8 regulated pathways in human ovarian cancer cell models and in normal OSE cell models after PAX8 alterations (7). They used as model cells the OVCAR8^{RFP} human ovarian cancer cell line and the normal murine ovarian surface epithelium (MOSE) cells. This choice was based on the findings of their previous work that evaluated the role of PAX8 in cells derived from the two progenitor sites of HGSOC: OSE and FTE (5). The work demonstrated that PAX8 deletion in the adult FTE, where it is normally expressed, did not have evident phenotypic effects and gene expression alterations. On the contrary, in OSE cells the forced PAX8 expression led to a malignant phenotype (5). Hence, the proteomic analysis was performed on OVCAR8^{RFP} versus OVCAR8^{RFP} cells after PAX8 deletion (OVCAR8^{RFP}PAX8$^{-/-}$) and on MOSE versus MOSE cells stably expressing PAX8 (MOSE-PAX8) by using isobaric labelling strategy (SILAC and iTRAQ) (7). The Gene Ontology (GO) analysis of the differentially expressed proteins in OVCAR8^{RFP} and MOSE cells pointed out the enrichment of pathways involved in cytoskeletal structure, cadherin binding and ubiquitin protein ligase binding, but only 28 proteins were regulated by PAX8 in both cell lines. In line with their previous study, N-Cadherin (CDH2) a key mediator of cell adhesion, was upregulated in both MOSE and OVCAR8 cells. Conversely, Protein kinase Cα (PKCα) was increased only in OVCAR8 cells while the actin filaments ACTN1 and ACTN4 were upregulated only in MOSE cells. The authors explained that these differences could be due to the marked reprogramming of the PAX8 cistrome across FTE and HGSOC cell lines as demonstrated by Elias *et al.* (8). The proteomic data obtained in MOSE-PAX8 cells were confirmed by RNA-sequencing analysis. Among the 4,257 transcripts modified by PAX8, 187 were found to be also affected in the proteomic analysis. Gene set enrichment analysis (GSEA) of these datasets pointed out the KEGG adherens junction pathways as differentially regulated by PAX8. GSEA analysis identified ACTN1 and ACTN4 as drivers

of adherens junction in MOSE-PAX8 cells. These findings were supported by functional assays. The proteomic data were also validated in HGSOC cells by *in vitro* migration assay. The results confirmed that PAX8 increased migration and metastasis in HGSOC cells. Based on these findings, the authors decided to validate the influence of PAX8 on cytoskeleton analysing the migratory and invasive ability of serous tumor cells derived from the OSE, FTE and human HGSOC. They knocked down PAX8 in spontaneously transformed murine OSE (STOSE) tumor cells and in murine oviductal epithelium (MOE $PTEN^{shRNA}$/$KRAS^{G12V}$) cells. They demonstrated that PAX8 increased migration and metastasis regardless of cell of origin. Despite this, the PAX8 downstream transcriptional targets are dependent on tumor's cell of origin. The authors proved that PAX8 increased migration in HGSOC cell lines by upregulation of PCK_{α} but not in MOSE cells. Conversely, in MOSE cells PAX8 regulated ACTN1 and ACTN4 to promote adherens junctions but not in OVCAR8. The authors stated that these unique downstream targets of PAX8 in OVCAR8 and MOSE cell lines could be due to lineage and tumor specific DNA binding sites. This hypothesis is supported by previous work of Elias *et al.* (8). Through whole transcriptome shotgun sequencing (RNA-Seq) after PAX8 knockdown and ChIP-Seq in fallopian tube secretory epithelial cells (FTSECs) and HGSOCs, they pointed out that ovarian cancer cells displayed marked reprogramming of the PAX8 cistrome (8). Based on these data, Hardy *et al.* hypothesized the acquisition of a DNA binding near PCK_{α} in OVCAR8 cells during the malignant transformation.

Despite the lineage and tumor specificity of PAX8 binding sites, the ability of PAX8 to regulate the migration is conserved across the cell types examined. For this reason, Hardy *et al.* suggested PAX8 as valuable drug target to reduce the metastasis and increase survival of HGSOC patients regardless of cell of origins. The authors proposed the use of drug thiostrepton, a thiazole antibiotic to target PAX8. This natural product inhibits prokaryotic translational through the binding of 70S ribosome and it is known to suppress breast cancer cell migration, metastasis, and transformation, through downregulation of the forkhead transcription factor FOXM1 expression (9,10). Moreover, FOXM1 is an oncogene activated in over 80% of HGSOC (11). Hardy *et al.* demonstrated that thiostrepton did not affect the PAX8 transcription but destabilised the PAX8 protein level both in OVCAR8 and in MOSE-PAX8 cells. This PAX8 protein reduction was not affected by FOXM1 expression or proteasome activity. The authors attempted to confirm *in vivo* the ability of thiostrepton to target PAX8 and reduce tumor progression and aggressiveness. They used six-week-old female athymic nude mice inoculated i.p. with OVCAR8 cells and after a week treated with thiostrepton. The administration of un-encapsulated drug to reduce the tumor was unsuccessful due to toxic effects and solubility issues. Conversely, the treatment with thiostrepton encapsulated in sterically stabilised micelles (SSM) composed of PEGylated phospholipid, DSPE-PEG_{2000} led to a significantly reduction of tumor burden. Moreover, immunoblotting analysis on tumor tissue pointed out the ability of thiostrepton-SSM to reduce both FOXM1 and PAX8 levels. The decreased PCK_{α} levels associated with PAX8 reduction by thiostrepton-SSM treatment *in vivo* was confirmed through immunohistochemistry assays.

The innovative feature of the work, presented by Hardy *et al.*, is the use of "omic" approach (proteomics and transcriptomics) to examine the role of PAX8 in peritoneal colonization of HGSOC.

Previously published papers dealt with transcriptomic analysis of ovarian cancer cells with PAX8 alterations, an approach that provides information only about the effects on gene expression. The "omic" data have allowed pointing out the unifying role of PAX8 as a master regulator of migration regardless of cell of origin but with unique transcriptional targets depending on the cell's site of origin. They proposed PCK_{α} as one of the unique differentially regulated proteins in HGSOC cells while ACTN1 and ACTN4 as distinctive of MOSE cells. The strength of the work depends on being able to validate the results through both *in vitro* and *in vivo* functional assays. The results led the authors to propose PAX8 as an effective drug target to treat HGSOC derived from both the OSE and FTE. Promising results were obtained on murine models treated with the micelle encapsulated drug thiostrepton.

Some critical technical aspects of the study are still lacking. The proteomic analysis revealed 28 proteins differentially expressed in both HGSOC and MOSE cells. However, the authors decided not to include all these proteins in the subsequent validation analysis. Indeed, they confirmed only CDH5, a protein found to be affected by PAX8 in their previous work. The same choice was applied on the proteins exclusively differentially expressed in HGSOC and MOSE cells. They investigated the effects of PAX8 on PCK_{α} in HGSOC cells or ACTN1 and ACTN4 in MOSE cells. The analysis of the entire "omic" dataset is needed to completely elucidate the regulated pathways by PAX8 in ovarian cancer.

Another critical aspect of the work is the use of a single human ovarian cancer cell line (OVCAR8). Genomic differences between ovarian cancer cell lines and tissue samples have been pointed out in several studies (12). Hence, further studies on other HGSOC cell line models as well as on cells derived from patients are needed to confirm the proposed role of PAX8 as a master regulator of migration in ovarian cancer. New findings will allow undoubtedly proposing PAX8 as drug target for HGSOC therapy.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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