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Transcriptional pattern enriched for synaptic signaling is associated with shorter survival of patients with high-grade serous ovarian cancer

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Arkajyoti Bhattacharya, Thijs S Stutvoet, Mirela Perla, Stefan Loipfinger, Mathilde Jalving, Anna KL Reyners, Paola D Vermeer, Ronny Drapkin, Marco de Bruyn, Elisabeth GE de Vries, Steven de Jong, Rudolf SN Fehrmann

Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands • Cancer Biology and Immunotherapies Group, Sanford Research, Sioux Falls, United States • Penn Ovarian Cancer Research Center and Basser Center for BRCA, University of Pennsylvania, Perelman School of Medicine, Philadelphia, United States • Department of Obstetrics and Gynecology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

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eLife Assessment

This **valuable** study uses consensus-independent component analysis to highlight transcriptional components (TC) in high-grade serous ovarian cancers (HGSOC). The study presents a **convincing** preliminary finding by identifying a TC linked to synaptic signaling that is associated with shorter overall survival in HGSOC patients, highlighting the potential role of neuronal interactions in the tumour microenvironment. This finding is corroborated by comparing spatially resolved transcriptomics in a small-scale study; a weakness is it being descriptive, non-mechanistic, and requires experimental validation.

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Abstract

Background

Bulk transcriptomic analyses of high-grade serous ovarian cancer (HGSOC) so far have not uncovered potential drug targets, possibly because subtle, disease-relevant transcriptional patterns are overshadowed by dominant, non-relevant ones. Our aim was to uncover disease-outcome-related patterns in HGSOC transcriptomes that may reveal novel drug targets.

Method

Using consensus-independent component analysis, we dissected 678 HGSOC transcriptomes of systemic therapy naïve patients—sourced from public repositories—into statistically independent transcriptional components (TCs). To enhance c-ICA's robustness, we added 447 transcriptomes from non-serous histotypes, low-grade serous, and non- cancerous ovarian tissues. Cox regression and survival tree analysis were performed to determine the association between TC activity and overall survival (OS). Finally, we determined the activity of the OS-associated TCs in 11 publicly available spatially resolved ovarian cancer transcriptomes.

Results

We identified 374 TCs, capturing prominent and subtle transcriptional patterns linked to specific biological processes. Six TCs, age, and tumor stage stratified patients with HGSOC receiving platinum-based chemotherapy into ten distinct OS groups. Three TCs were linked to copy-number alterations affecting expression levels of genes involved in replication, apoptosis, proliferation, immune activity, and replication stress. Notably, the TC identifying patients with the shortest OS captured a novel transcriptional pattern linked to synaptic signaling, which was active in tumor regions within all spatially resolved transcriptomes.

Conclusion

The association between a synaptic signaling-related TC and OS supports the emerging role of neurons and their axons as cancer hallmark-inducing constituents of the tumor microenvironment. These constituents might offer a novel drug target for patients with HGSOC.

Background

Epithelial ovarian cancer encompasses five primary histological subtypes, with high-grade serous ovarian carcinoma (HGSOC) constituting about 75% of all cases (1). The standard treatment for HGSOC diagnosed at stage IIB and beyond involves a combination of surgery and chemotherapy, primarily using platinum-based compounds and taxanes (2,3). While initial chemotherapy results in tumor response in most patients with HGSOC, there is a very high recurrence rate (4). The addition of poly-ADP ribose polymerase and vascular endothelial growth factor A inhibitors to chemotherapy for subsets of patients currently results in a 5-year disease-specific overall survival (OS) rate of approximately 45% for patients with HGSOC. This rate has hardly improved in the last three decades (5–8). Therefore, new insights into the complex biology underlying HGSOC are urgently needed to develop more effective treatment strategies.

Previous studies using bulk transcriptomes of patients with HGSOC have identified expression-based molecular subtypes. However, these subtypes did not provide insights that have translated into novel drug targets (9–11). A common limitation of such studies is their reliance on bulk transcriptomes, containing both tumor cells and tumor microenvironment (TME) components, thus reflecting the average transcriptional patterns of the combination of all biological processes present in the tumors. This averaging often masks subtle transcriptional patterns pivotal to

understanding HGSOC biology, especially when these are overshadowed by dominant patterns from other less relevant (non-)biological processes (12). Consensus-independent component analysis (c-ICA) offers an alternative by decomposing such bulk transcriptomes into statistically independent transcriptional patterns (i.e., transcriptional components; TCs)(13,14). This approach reveals both dominant and subtle patterns and provides a measure of TC activity for each sample (15).

In the present study, our aim was to utilize c-ICA to dissect HGSOC transcriptomes to identify as many TCs associated with patient OS as possible, which could reveal potential novel drug targets.

Methods

See Supplementary methods for the extended methods.

Data acquisition

Raw microarray bulk transcriptomes and clinicopathological details for patients with HGSOC, low-grade serous ovarian cancer (LGSOC), non-serous ovarian cancer, and benign ovarian tissues were sourced from the Gene Expression Omnibus (GEO)(16). We exclusively utilized transcriptomes generated from primary tumor samples. Our analysis was confined to samples on the Affymetrix HG-U133 Plus 2.0 platform (GEO accession identifier: GPL570) and excluded cell line samples. The datasets were pre-processed, and quality controlled as previously described (17). Furthermore, for comprehensive analyses, we incorporated transcriptomes from five distinct resources: the Cancer Cell Line Encyclopedia (CCLE, n = 969), Genomics of Drug Sensitivity in Cancer (GDSC, n = 959), Gene Expression Omnibus (GEO, n = 13,810), and The Cancer Genome Atlas (TCGA, n = 8,150), and spatially resolved transcriptomes from 10xGenomics (16,18–20).

Consensus-independent component analysis (c-ICA)

To preprocess the bulk transcriptome data, we applied a whitening transformation to prepare it for subsequent analysis. Consensus-ICA was conducted as described previously (Knapen et al., 2024). The output of an c-ICA includes two matrices: (i) transcriptional components (TCs) with gene weights, where each weight within the TC represents both the direction and magnitude of its effect on the expression levels of each gene, and (ii) a consensus mixing matrix (MM) with its coefficients representing the activity scores of TCs across samples.

Survival analysis

To discern the relationship between TC activity and patient OS, a univariate Cox proportional hazards analysis was conducted on a select group of patients with available follow-up data (n = 541, Supplementary Table S1). In addition, a multivariate Cox proportional hazards analysis was carried out, including covariates such as age, stage, debulking status, and tumor grade. This latter analysis was based on a subset of patients with comprehensive clinicopathological data available (n = 373, Supplementary Table S1). We implemented a multivariate permutation framework encompassing 10,000 permutations to mitigate the risk of false discoveries. We established the acceptable false discovery rate (FDR) at 1%, maintaining an 80% confidence level, applicable for both the univariate and multivariate analyses.

Survival tree analysis

We performed a survival tree analysis to delineate groups of patients with HGSOC treated with platinum-based chemotherapy based on distinct transcriptional and clinicopathological attributes. The analysis utilized activities of TCs associated with OS (either from univariate or multivariate survival analysis as mentioned in supplementary methods) in conjunction with relevant

clinicopathological factors, such as age, tumor stage, debulking status, and grade, as potential classifiers. We divided patients into two subsets using every plausible cut-off point for each classifier and compared the resulting survival curves employing the log-rank statistic. Consequently, the division was based on the most significant classifier at its optimal cut-off based on the smallest p-value of the log-rank test mentioned above. This divisional process was successfully reiterated on the derived subsets until any of the following stipulated conditions was satisfied: *i*) the total patient count across both subsets fell below 50, *ii*) the collective number of uncensored events in both subsets was < 25, or *iii*) one of the subsets contained < 17 patients. To gauge the stability of our classifiers, we performed 20,000 iterations, randomly selecting 80% of the patient group in each iteration. The significance-based ranks of classifiers in these iterations were correlated with those from the primary survival tree.

Associating the identified transcriptional components with biological processes

To discern the biological processes associated with the TCs, we adopted a multifaceted approach encompassing *i*) Transcriptional Adaptation to Copy Number Alterations (TACNA) profiling, targeting the identification of TCs that reflect the downstream implications of copy number alterations (CNAs) on gene expression levels(21); *ii*) Execution of gene set enrichment analysis (GSEA) for each TC, utilizing gene set collections (n = 16) from The Human Phenotype Ontology (The Monarch Initiative), the Mammalian Phenotypes (Mouse Genome Database), and the Molecular Signatures Database (MsigDB)(22,23); *iii*) The formation of co-functionality networks on the top and bottom genes of each TC, achieved using the GenetICA methodology, accessible via *https://www.genetica-network.com*(24). For clusters comprising ≥ 5 genes, the enrichment of the predicted functionality was quantified. This served as the foundation for determining the biological process associated with the TC being examined.

Cross-study transcriptional component projection

To determine whether a biological process captured by an identified TC is also active in other cancer types and to investigate if it is more active in tumor cells or in the tumour microenvironment (TME), we collected raw expression profiles from multiple sources: the Cancer Cell Line Encyclopedia (CCLE, n = 969), Genomics of Drug Sensitivity in Cancer (GDSC, n = 959), Gene Expression Omnibus (GEO, n = 13,810), and The Cancer Genome Atlas (TCGA, n = 8,150) (16,18–20). While the CCLE and GDSC datasets comprise cell line profiles across many solid and hematologic malignancies, the GEO and TCGA datasets offer an extensive set of bulk transcriptomes derived from patient samples spanning 27 tumor types. We pre- processed the raw data as previously described (21). Next, we projected the TCs identified via c-ICA onto the cell line expression profiles from CCLE and GDSC and the patient-derived expression profiles from GEO and TCGA. This projection methodology has been described in more detail previously (21). To identify potential variations in the activity scores of the TCs, we compared the activity scores among cell lines and samples derived from patients within all four repositories. We used an absolute activity score threshold of 0.05 for each TC to pinpoint outlier cell lines and patient tumors with heightened activity.

Determination of spatial transcriptomic profiles' significant activity locations for individual transcriptional components

To further assess whether a biological process captured by an identified TC is more active in tumor cells or in the TME, we collected publicly available spatial resolved transcriptomic profiles of ovarian cancer samples. Eight were sourced from GEO (study ID GSE211956), and three were sourced from the public dataset repository of 10xGenomics (see supplementary methods for details) generated using the 10xGenomics Visium platform. The samples were from patients with HGSOC, serous papillary, and endometrioid ovarian cancer. Activity for each TC across every location within the spatial samples was ascertained through the cross- study projection

methodology referred to in the previous method section (21). We incorporated a permutation-driven approach to discern the markedly active areas within the spatial samples for each TC. We derived a null distribution of activities for each TC-location pairing by performing 3,000 permutations and subsequent projections. The p-value of each observed TC activity quantifies the significance of the deviation of the TC activity at a given location from its baseline null distribution. After this, we visualized the z-transformed p- values using a heatmap, followed by obtaining colocalization scores for each combination of TCs in the spatial transcriptomic profiles for each ovarian cancer sample (25). This visualization aided in highlighting the areas with notable activity aligned against the stained representation of the tissue sample.

Results

An integrated data set containing 1,125 bulk transcriptomes from ovarian tissues

We curated 1,193 bulk transcriptomes from the GEO, including patients with HGSOC, low- grade serous ovarian cancer (LGSOC), non-serous ovarian cancer, and benign ovarian tissues (16). These were extracted from 32 distinct studies and represented the entire spectrum of ovarian cancer types, stages, and grades, and included 43 samples from non-malignant ovarian tissue. Pre-processing, which included removing duplicates and quality checks, culminated in a refined dataset of 1,125 samples (17). Supplementary Tables S1 and S2 provide detailed breakdowns of these samples, showcasing the comprehensive coverage of ovarian cancer types, stages, and grades within this dataset. The ovarian cancer dataset comprised bulk transcriptomes of patients with HGSOC (n = 678), other serous (n = 110), endometrioid (n = 110), and clear-cell ovarian cancer samples (n = 96). Additionally, for 541 patients, comprehensive survival data was available, as well as additional clinicopathologic information, including age, grade, stage, subtype, treatment schedule, and debulking status for 373 patients (**Fig. 1**).

Consensus-independent component analysis identifies 374 transcriptional components (TCs)

c-ICA on the 1,125 bulk transcriptomes revealed 374 independent TCs. Notably, 135 TCs captured the impact of copy number alterations on gene expression levels. Each TC displayed enrichment for at least one gene set from the 16 gene set collections, with an absolute Z-score of more than two. For example, the number of enriched gene sets from the Hallmark gene set collection in an individual TC ranged from zero to 28 enriched gene sets (interquartile range 3 – 7). The median top Z score for Hallmark gene sets was 3.21 (range 1.55 – 37.54, interquartile range 2.6 – 4.25). A comprehensive database, including all TCs and GSEA outcomes, has been made accessible at *http://transcriptional-landscape-ovarian.opendatainscience.net*.

The activities of 13 TCs were associated with patient overall survival (OS) in a univariate analysis, with an additional TC (TC166) identified in a multivariate analysis accounting for age, stage, debulking, and tumor grade. Combined, these 14 OS-associated TCs were enriched for gene sets associated with diverse biological processes and clinicopathological characteristics, with four TCs capturing the effects of copy number alterations on gene expression levels.

The activities of six transcriptional components are associated with patient overall survival

For a selected subset of 541 patients—including HGSOC, LGSOC, and non-serous ovarian cancer—with available OS information (Supplementary Table S1), 13 TC activities displayed an association with OS univariately (false discovery rate 5%, confidence level 80% in permutation-based multiple

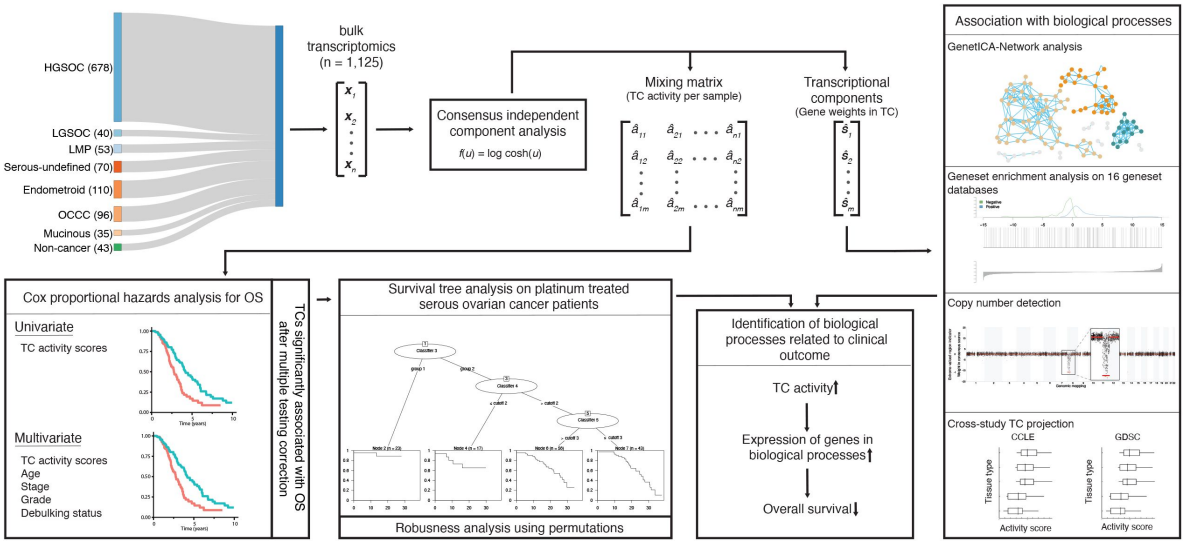

Figure 1.

Workflow indicates the data acquisition and relations between the methods.

testing framework Supplementary Table S3; **Fig. 2**). For patients with serous ovarian cancer, treated with platinum-based therapy (n = 301, Supplementary Table S1), lower activity of one additional TC (TC166) was associated with worse OS independent of age, stage, debulking, and tumor grade (Supplementary Table S4). Combined, these 14 OS-associated TCs were enriched for gene sets associated with diverse biological processes and clinicopathological characteristics. Four of these TCs captured the downstream effects of CNAs on gene expression levels (**Fig. 2**, Supplementary Fig. S1-S3). Survival tree analysis identified ten groups of patients with platinum-treated HGSOC based on the activity of six OS-associated TCs and the presence of two clinicopathological characteristics, namely age and stage (**Fig. 3**, Supplementary Table S5, median robustness statistic of survival tree = 0.52, interquartile range = 0.36 - 0.69). The survival tree demonstrated good classification power (concordance statistic = 0.72, standard error = 0.021). As expected, patients were divided into separate survival groups based on stage (1/2 vs. 3/4) and age (<53.7 vs. ≥53.7 years). The most significant difference in OS was observed between the cohorts with low and high TC121 activity (Supplementary Table S5). Patients with high TC121 tumor activity exhibited the shortest OS, also observed for the subset of patients with advanced-stage HGSOC (Supplementary Fig. S4, Supplementary Table S6). Supplementary Fig. S5 indicates that TC121 activity is highest in patients with HGSOC compared to other ovarian cancer subtypes. Notably, all subtypes contain subsets of samples with elevated TC121 activity. These robust associations with OS for TC121 in these two subsets of patients indicate the relevance of TC121, irrespective of stage.

Distinct biological processes show enrichment in the transcriptional components associated with overall survival

Three of the six TCs associated with OS—TC166, TC247, and TC76—captured the effects of CNAs on the expression levels of genes mapping to chromosome regions 13q12-q14, 11q13-q14, and 9p13-p21, respectively (Supplementary Fig. S6, Supplementary Table S7) (6). The higher activity of TC166 was associated with better OS, whereas the higher activities of TC121, TC247, TC250, TC76, and TC146, were associated with worse OS. Among the 14 OS- associated TCs, only TC166 showed a significant association with OS in an independent cohort of patients with ovarian clear cell carcinoma (Bonferroni corrected p-value < 0.05; see supplementary methods and Supplementary Table S8) (26). The top genes from TC166 were enriched for genes involved in replication and apoptosis. The chromosomal region 13q12- q14 linked to the TC166, contains the tumor suppressor genes retinoblastoma 1 (*RB1*) and Breast Cancer Type 2 Susceptibility Protein (*BRCA2*). Loss of heterozygosity of this chromosomal region is frequently observed in both sporadic and hereditary serous ovarian cancers (27,28). The top genes from TC247 were enriched for genes involved in proliferation and immune cell activation, TC76 in replication stress, TC250 in extracellular matrix (ECM) interactions, and TC146 in neurotransmitter signaling.

Intriguingly, the top 100 genes in TC121 revealed a co-functional cluster enriched for genes involved in synaptic signaling, with the corresponding proteins reported to localize to the synaptic membrane of neurons (**Fig. 4**). Among these were pre-synaptic protein neurexin-1 (*NRXN1*) and its post-synaptic ligand leucine-rich repeat transmembrane protein 2 (*LRRTM2*), which regulates excitatory synapse formation (top 20 genes are described in Supplementary Table S9, for more details: *http://transcriptional-landscape-ovarian.opendatainscience.net*) (29,30). Furthermore, this co-functional cluster included neuron-specific synaptic structure proteins, neurofilament light, and medium chain. Moreover, genes encoding for potassium ion channel proteins integral to membrane repolarization during synapse signal transduction carried high weights in TC121. These genes included *KCNC1, KCNN2*, and *KCNIP1*(31–33). Several genes in TC121 encoded proteins related to glutamate receptor signaling, including *GRIN2C* and *SLC7A10* (34). In line with this proposed function, high activity of TC121 was observed in neuroblastoma cell lines but not in ovarian or central nervous system cancer cell lines in the GDSC and CCLE datasets (**Fig. 5A**,

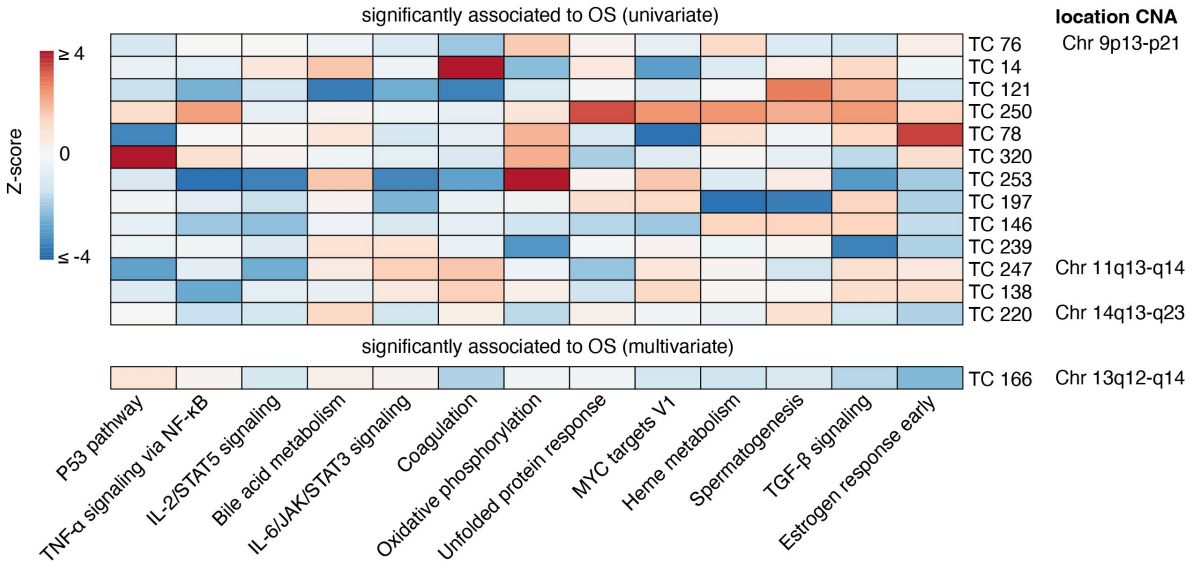

Figure 2.

Enrichment heatmap of hallmark gene sets in transcriptional components associated with patient overall survival.

Gene Set Enrichment Analysis for 14 TCs associated with OS identified through univariate or multivariate survival analyses are presented. Only Hallmark gene sets with significant enrichment (Bonferroni-corrected p-value) for at least one TC are shown. The heatmap displays Z-scores, which indicate the relative enrichment strength, with values truncated at a maximum of 4 for visualization purposes. The gene sets were clustered based on Pearson correlation using the Ward D2 method, providing insights into related biological processes captured by different TCs. In the right column, chromosomal locations of copy number alterations (CNAs) are shown, reflecting the downstream effects on gene expression that each TC captures. This integration of CNA information highlights the biological relevance of each TC and its contribution to gene expression variability and patient outcomes.

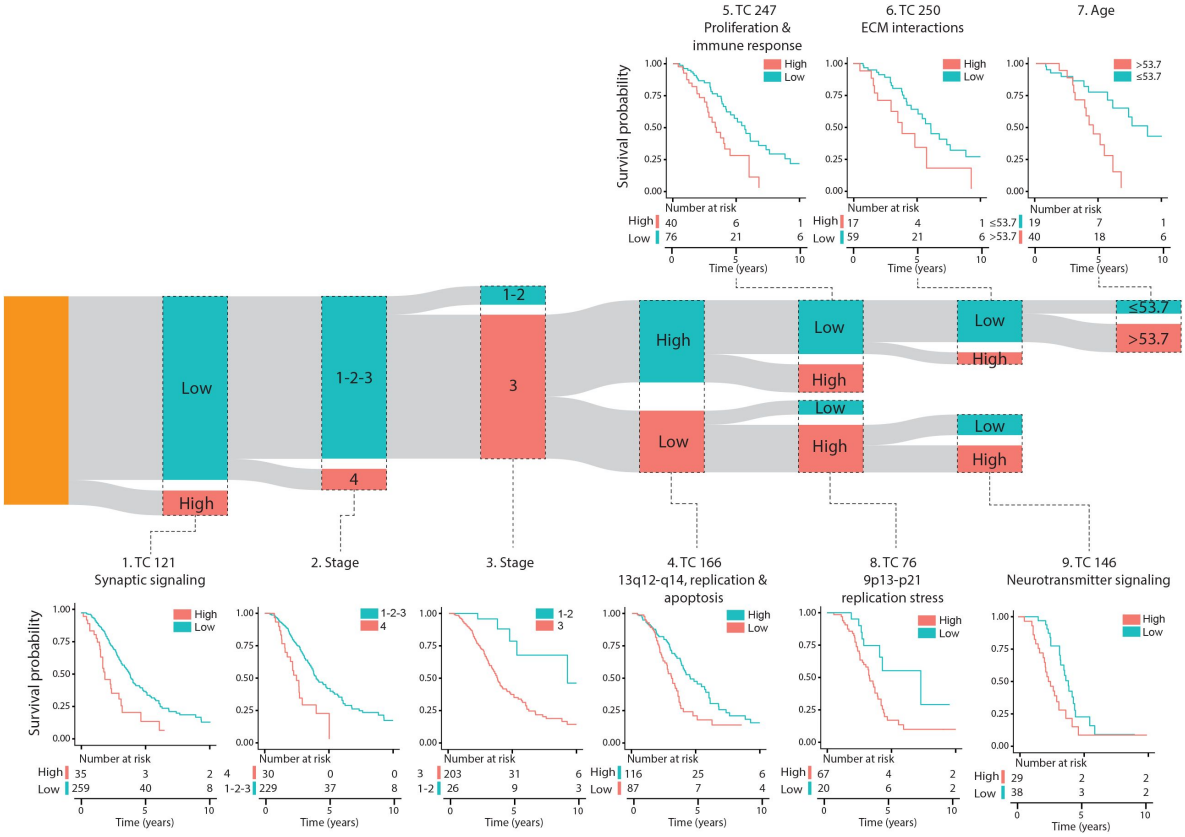

Figure 3.

Survival tree analysis of patients with platinum-treated HGSOC defines survival cohorts with distinct clinicopathologic and biological characteristics.

The results of survival tree analysis of 294 patients with high-grade serous ovarian cancer (HGSOC) treated with platinum-based chemotherapy are presented. The analysis utilized 14 transcriptional components (TCs) associated with overall survival (OS), along with other clinicopathologic factors, including age, tumor stage, grade, and debulking status. The resulting tree identified nine distinct survival cohorts, each represented as a bar in the Sankey diagram, where the bar height corresponds to the number of patients in each cohort. Kaplan-Meier survival curves with accompanying number-at-risk tables are shown for each cohort, with survival data censored at 10 years. The names of the survival cohorts were based on enriched biological processes in the TCs, as determined by the chromosomal location of genes captured by a TC, GSEA, and co-functionality analysis of the top genes. The p-values in the Kaplan-Meier plots were derived from log-rank tests comparing survival distributions between groups. Abbreviations: TC = transcriptional component, ECM = extracellular matrix.

Supplementary Fig. S7, S8). In the GEO and TCGA datasets, high activity of TC121 was observed in glioblastoma multiforme and lower-grade glioma but not in ovarian cancer patient samples (**Fig. 5B**).

Distinct cluster of patients from TCGA overlap with elevated activity of TC121

To explore if pre-existing classifications of patients with ovarian cancer correspond to the contrasting activities of the TCs, we investigated the classification provided by TCGA. TCGA identified four optimal clusters describing the patients with ovarian cancer using transcriptional profiles.(9) To explore associations between these clusters and TC activity, we performed a Kruskal-Wallis test using TCGA sample data. Supplementary Fig. S9 highlights the associations between each cluster set and the TCs, represented by log-transformed p-values. A significant association between a TC and a cluster set indicates that at least one cluster within the cluster set exhibited significantly different activity scores for the corresponding TC compared to the other clusters. Notably, samples with high TC121 activity were not captured by any of the clusters of the four-cluster set. Interestingly, the eight-cluster set predefined by TCGA was able to identify a cluster that corresponded to samples with elevated TC121 and TC146 activity. This finding suggests that while TCGA's analysis identified this patient group based on transcriptional profiles, it didn't characterize them further.

Distinct spatial and single cell transcriptional profiles with high activity of OS-associated TCs

Cross-study TC projection onto spatial transcriptomic profiles from 11 ovarian cancer samples revealed that TC121 was highly active in profiles from the tumor region of the 11 ovarian cancer samples (**Fig. 6A**; Supplementary Fig. S10). Additionally, TC121 showed markedly higher activity in the transcriptional profiles of a subset of the unannotated single cells from HGSOC patients (study ID GSE158722; see supplementary methods and Supplementary Fig. S11).(35) This finding suggests that some of these unannotated cells could be neurons. Furthermore, the unannotated single cell transcriptional profiles showed contrasting activity scores of different OS-associated TCs (supplementary Fig. S11). These contrasting activities indicate that these TCs could provide insights into the biology of previously uncharacterized cell types. Distinct regions with high activity of the copy number TCs (TC166, TC247, and TC76) in the HGSOC sample overlapped with the region containing cancer cells, as expected. TC250, enriched for extracellular matrix interactions, was also active in the stromal region. The strongest inverse colocalization (colocalization score -2.43) was observed between the activity scores of TC146, enriched for neurotransmitter signaling, and TC76, captured the effect of copy number alterations at chromosome 9p13-p21, at the serous ovarian cancer sample (**Fig. 6B**, Supplementary Table S10).

Discussion

In this study, we identified 374 TCs, each enriched for gene sets representing various biological processes in HGSOC samples. Six could stratify patients with HGSOC who had received platinum-based treatment into ten distinct OS groups.

The most significant TC in the survival tree analysis, TC121, captured a clinically relevant subtle transcriptional pattern linked to synaptic signaling not previously recognized in HGSOC. In the survival tree, TC121 identified 12% of the HGSOC patients with the shortest OS and, based on spatially resolved transcriptomic analyzed samples, is active in tumor regions. This observation supports the emerging role of neurons and neuronal projections as cancer hallmark-inducing constituents of the TME (36–38).

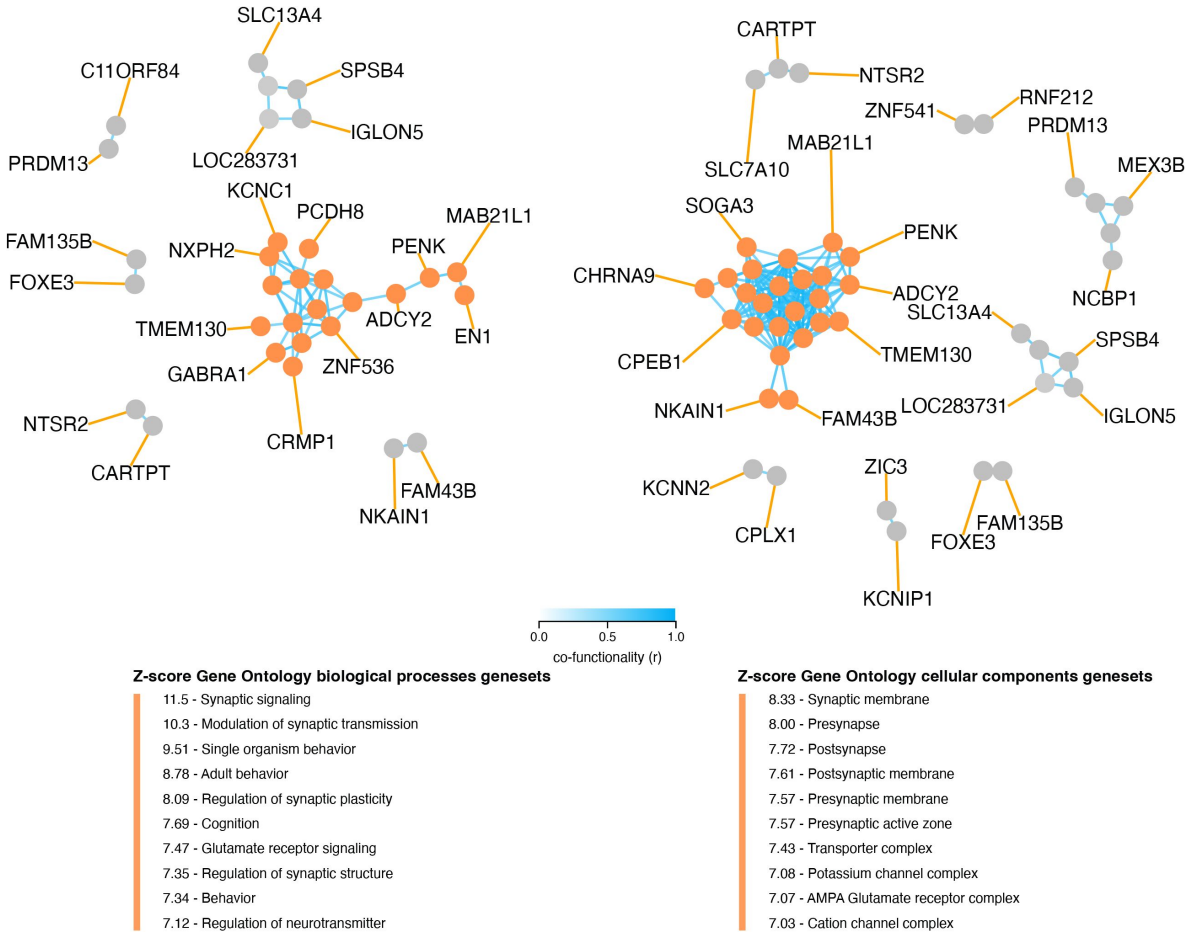

Figure 4.

Co-functionality network of top 100 absolute weighted genes in TC121.

Co-functionality network for the top 100 genes with the highest absolute weights in TC121 is presented. Genes were clustered based on predicted co-functionality ($r > 0.7$) across datasets, with clusters identified using both Gene Ontology (GO) Biological Processes and Cellular Components databases. One primary cluster, containing more than five genes, exhibited strong enrichment for synaptic signaling in the GO Biological Processes database and for synaptic membranes in the GO Cellular Components database. This highlights the biological specificity of TC121 in regulating gene expression linked to synaptic functions.

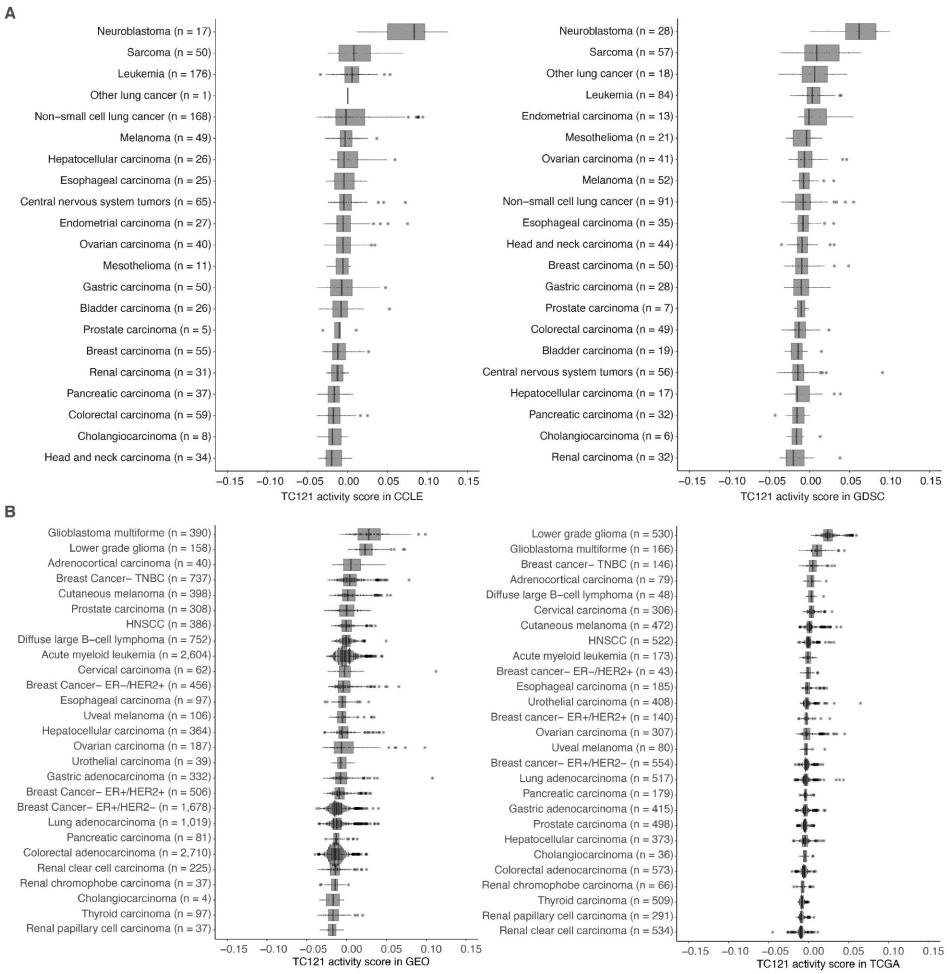

Figure 5.

The activity of TC121 in bulk transcriptomes of CCLE, GDSC cell lines, and GEO and TCGA patient-derived samples.

A. Cross-study TC projection of TC121 on CCLE and GDSC cell lines. The boxplots display the activity scores of TC121 in different tissue types, which are ordered based on their corresponding median activity scores. **B**. Cross-study TC projection of TC121 on GEO and TCGA bulk transcriptomes resulted in the activity scores presented in the boxplots. Cancer types were ordered based on corresponding medians of TC121 activity scores. Abbreviations: TC = transcriptional component.

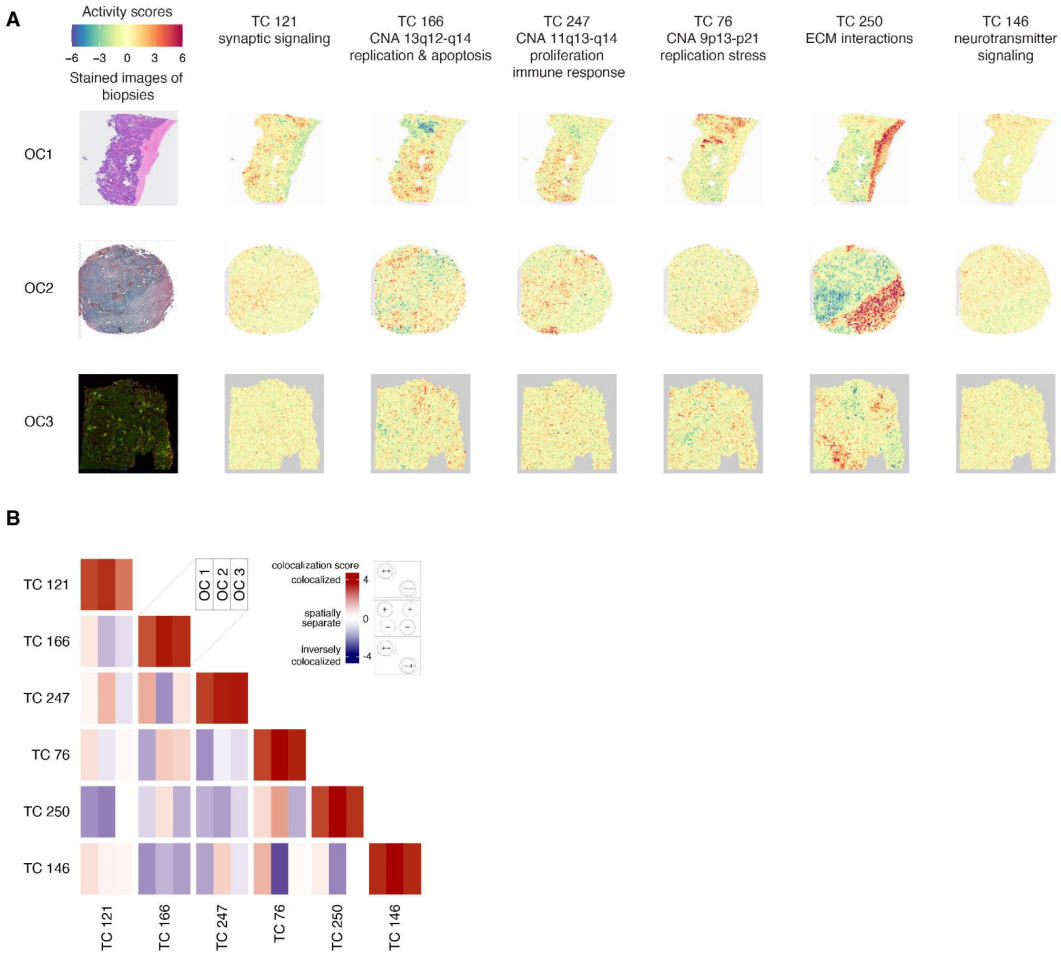

Figure 6.

Spatial transcriptomic profiles in ovarian cancer samples.

A. We employed a permutation-based approach to pinpoint the areas of significant TC activity in spatial transcriptomic profiles. We ran 5,000 permutations for each TC-profile combination, yielding a p-value that indicates the extent to which the TC activity in the corresponding profile differs from what would be expected by chance (the null distribution). We then transformed these p-values into logarithmic values and represented them using a heatmap. Heatmaps of activity scores of the TCs are presented in individual rows for the HGSOC, serous papillary, and endometrioid adenocarcinoma of ovary samples. The first column represents the stained images of the samples. The second to seventh columns show heatmaps corresponding to the mentioned TCs. **B**. The heatmap illustrates the colocalization between two TC activities on spatial transcriptomic profiles from ovarian cancer samples. For each cell, the colocalization scores of the TCs at each of the three spatial transcriptomics samples OC 1, OC 2, and OC 3 are arranged in columns. A colocalization score of 4 between two TCs (red) indicates that the positively (+) and negatively (−) active regions of both TCs are perfectly colocalized. Conversely, a colocalization score of -4 between two TCs (blue) also indicates colocalization. Still, with inverse activity, i.e., the positively active regions of one TC are colocalized with the negatively active regions of the other TC or vice versa. A colocalization score close to 0 between two TCs (white) indicates that the activities of two TCs are spatially separated. The dashed and solid circle in the panel on the right side of the color bar represents two different TCs. Abbreviations: TC = transcriptional component.

Further investigation on whether the activity of TC121 originated from tumor cells or in the TME revealed that the TC121 signal is coming from cells within the TME. The high activity of TC121 in low-grade glioma and glioblastoma multiforme patient samples (**Fig. 5B**) is in agreement with the presence of neurons in large numbers within the TME of gliomas, where they form functional synapses with tumor cells(39,40). Moreover, TC121 activity was lower in non-brain cancers, such as ovarian cancers, which contain fewer neurons and synapses in the TME compared to brain cancers. We expected TC121 activity to be low in the bulk transcriptomes of all cell lines, since they lack TME. TC121 activity in most cell lines, which includes glioblastoma and ovarian cancer cell lines, was indeed low. Neuroblastoma cell lines, however, exhibited high TC121 activity, which is likely due to retained synaptic formation capacity originating from neuroblast cells (41,42). Lastly, TC121's high activity observed in small, scattered regions within the tumor of spatially resolved transcriptomic ovarian cancer samples also supports TC121's role in the TME.

TC121's significant association with OS underscores the potential significance of synaptic signaling in HGSOC biology. Yet, the neuronal subtype and the molecular mechanisms associated with TC121 remain to be elucidated. A study in human ovarian cancer-bearing mice demonstrated that sympathetic innervation in HGSOC involves adrenergic signaling: norepinephrine released by sympathetic neurons binds to beta- adrenergic receptors on the cancer cells (43–45). This binding triggers the tumor cells to release brain-derived neurotrophic factor (BDNF), which enhances cancer innervation via activation of host neurotrophic receptor tyrosine kinase B receptors (NTRK2), thereby establishing a feed-forward loop of sustained signaling. BDNF and the nerve marker neurofilament protein expression were examined in 108 human ovarian tumors (41). This study revealed that increased intratumoral nerve presence strongly correlates with elevated BDNF and norepinephrine levels, advanced tumor stage, and shorter OS in patients with ovarian cancer. This interaction can be targeted with pan-TRK inhibitors such as entrectinib and larotrectinib. Both drugs are showing promising results in multiple phase II trials, including ovarian cancer and breast cancer patients. Furthermore, a TRKB-specific inhibitor was developed (ANA-12), but has not been subjected to any clinical trials in cancer so far (46–49). Our analysis indicated that *BDNF* is a prominent gene (with an absolute weight > 3) in 10 TCs but not in TC121, suggesting that TC121 may indicate a distinct process unrelated to BDNF.

The significance of sensory innervation in HGSOC was evidenced by the co- localization of TRPV1, a marker for sensory neurons, and β-III tubulin, a general neuronal marker, in immunofluorescent staining of histological sections from 75 patients (50). Additionally, a murine model study employing neural tracing identified sensory neurons originating from local dorsal root ganglia and jugular–nodose ganglia, with axons extending into the TME (50). A transgenic murine model lacking nociceptors demonstrated that this specific subtype of sensory neurons was involved in tumor progression (51). Another study showed that reducing the release of Calcitonin gene-related peptide from tumor-innervating nociceptors could be a strategy to alleviate this effect of nociceptors by improving anti- tumour immunity of cytotoxic CD8+ T cells in a melanoma model bearing mice(52). This indicates that the signal from TC121 may represent an indirect influence on tumor cells via interactions with immune cells and the promotion of an immune suppressive TME. Furthermore, in cell lines derived from $Trp53^{-/-}$ $Pten^{-/-}$ murine HGSOC, the influence of nociceptors was characterized by the release of substance P (SP), their primary neuropeptide. SP is an alternative splicing product of the preprotachykinin A gene (*TAC1*) and binds to the receptor neurokinin 1 (NK1R), encoded by the *TACR1* gene. NK1R expression was confirmed in murine HGSOC cell line, and SP enhanced cellular proliferation in NK1R-positive murine HGSOC cancer cells *in vitro* (51). Our analysis identified *TAC1* and *TACR1* as prominent genes in 15 and 2 TCs, respectively, yet not in TC121, and none of these TCs were associated with patient survival. Currently, there are no drugs specifically targeting tumor innervation in (ovarian) cancer (53). Interestingly, the NK1R antagonist aprepitant effectively inhibited the metastasis-promoting effects of neural substance P in human breast and mammary cancer bearing mice (54), demonstrating the feasibility of such an approach.

Strategies to disrupt neuronal signaling and neurotransmitter release in neurons target key elements of excitatory neurotransmission, such as calcium flux and vesicle formation. Drugs like ifenprodil and lamotrigine, commonly used to treat neuronal disorders, block glutamate release and subsequent neuronal signaling. Additionally, the vesicular monoamine transporter (VMAT) inhibitor reserpine prevents synaptic vesicle formation (55,56). In vitro studies with HGSOC cell lines have demonstrated that ifenprodil significantly inhibits tumour proliferation, while reserpine induces apoptosis in cancer cells (57,58). These approaches hold promise for inhibiting neuronal signaling and interactions in the TME. Therefore, it is essential that the mechanisms driving this nerve growth, the specifics of how nerves within the TME interact with ovarian cancer cells, and how they impact the survival of patients with HGSOC are further elucidated.

Altogether, the present study uncovered a clinically relevant TC linked to synaptic signaling not previously identified in HGSOC. This TC may represent a novel cancer cell- extrinsic mechanism within the TME, illustrating how cancer cells and nerve cells interact to promote enhanced proliferation. A deeper understanding of the molecular aspects of tumor innervation could pave the way for novel drug targets for patients with HGSOC.

Data availability

Microarray expression data was collected from three public data repositories: Gene Expression Omnibus with accession number GPL570 (generated with Affymetrix HG-U133 Plus 2.0), CCLE (generated with Affymetrix HG-U133 Plus 2.0, file CCLE_Expression.Arrays_2013-03-18.tar.gz) available at *https://portals.broadinstitute.org/ccle/data* and GDSC (generated with Affymetrix HG-U219) available at *https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-3610/*. Pre-processed and normalized RNA-seq data was collected from TCGA using the Broad GDAC Firehose portal (*https://gdac.broadinstitute.org/*). Spatially resolved samples were sourced from 10xGenomics and GEO. The datasets generated during and/or analyzed during the current study are available in the website: *http://transcriptional-landscape-ovarian.opendatainscience.net*. The accession numbers of the studies included in this analysis are mentioned in Supplementary Table S2.

Additional information

Code Availability

The complete set of codes utilized in this study is available at the github repository: *https://github.com/arkajyotibhattacharya/TranscriptionalLandscapeOvarianCancer*.

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Author contribution

A. Bhattacharya: Data curation, methodology, data analysis, data interpretation, writing. T. S. Stutvoet: Data curation, methodology, data analysis, data interpretation, writing. M. Perla: Data interpretation, writing. S. Loipfinger: Data analysis, data interpretation, writing. M. Jalving: Data interpretation, writing. A. K. L. Reyners: Data interpretation, writing. P. D. Vermeer: Data interpretation, writing. R. Drapkin: Data interpretation, writing. M. de Bruyn: Data interpretation, writing. E. G. E. de Vries: Data interpretation, writing. S. de Jong: Conceptualization, data

interpretation, writing. R. S. N. Fehrmann: Conceptualization, data curation, methodology, data analysis, data interpretation, writing. All were involved in the final decision to submit the manuscript.

Additional files

Supplementary figures tables and methods

References

1. Lheureux S, Gourley C, Vergote I, Oza AM (2019) **Epithelial ovarian cancer** *Lancet* **393**:1240–53

2. (2019) **NCCN clinical practice guidelines in oncology - ovarian cancer, including fallopian tube cancer and primary peritoneal cancer**

3. Wright AA, Bohlke K, Armstrong DK, Bookman MA, Cliby WA, Coleman RL, et al. (2016) **Neoadjuvant chemotherapy for newly diagnosed, advanced ovarian cancer: Society of Gynecologic Oncology and American Society of Clinical Oncology Clinical Practice Guideline** *J Clin Oncol* **34**:3460–73

4. Corrado G, Salutari V, Palluzzi E, Distefano MG, Scambia G, Ferrandina G. (2017) **Optimizing treatment in recurrent epithelial ovarian cancer** *Expert Rev Anticancer Ther* **17**:1147–58

5. Cibula D, J. Balmaña (2016) **PARP inhibitors in ovarian cancer** *Ann Oncol* **27**:i40–44

6. Wang H, Xu T, Zheng L, Li G. (2018) **Angiogenesis inhibitors for the treatment of ovarian cancer** *Int J Gynecol Cancer* **28**:903–14

7. Wu SG, Wang J, Sun JY, He ZY, Zhang WW, Zhou J. (2019) **Real-world impact of survival by period of diagnosis in epithelial ovarian cancer between 1990 and 2014** *Front Oncol* **9**:639

8. Tewari KS, Burger RA, Enserro D, Norquist BM, Swisher EM, Brady MF, et al. (2019) **Final overall survival of a randomized trial of Bevacizumab for primary treatment of ovarian cancer** *J Clin Oncol* **37**:2317–28

9. Bell D, Berchuck A, Birrer M, Chien J, Cramer DW, Dao F, et al. (2011) **Integrated genomic analyses of ovarian carcinoma** *Nature* **474**:609–15

10. Tothill RW, Tinker AV, George J, Brown R, Fox SB, Lade S, et al. (2008) **Novel molecular subtypes of serous and endometrioid ovarian cancer linked to clinical outcome** *Clin Cancer Res* **14**:5198–208

11. Verhaak RGW, Tamayo P, Yang JY, Hubbard D, Zhang H, Creighton CJ, et al. (2013) **Prognostically relevant gene signatures of high-grade serous ovarian carcinoma** *J Clin Investig* **123**:517–25

12. Chen C, Grennan K, Badner J, Zhang D, Gershon E, Jin L, et al. (2011) **Removing batch effects in analysis of expression microarray data: an evaluation of six batch adjustment methods** *PLoS One* **6**:e17238

13. Kong W, Vanderburg CR, Gunshin H, Rogers JT, Huang X. (2008) **A review of independent component analysis application to microarray gene expression data** *BioTechniques* **45**:501–20

14. Chiappetta P, Roubaud MC, Torrsani B. (2004) **Blind source separation and the analysis of microarray data** *J Comput Biol* **11**:1090–109

15. Biton A, Bernard-Pierrot I, Lou Y, Krucker C, Chapeaublanc E, Rubio-Pérez C, et al. (2014) **Independent component analysis uncovers the landscape of the bladder tumor transcriptome and reveals insights into luminal and basal subtypes** *Cell Rep* **9**:1235–45

16. Clough E, Barrett T. (2016) **Statistical genomics, methods and protocols** *Methods Mol Biol* **1418**:93–110

17. Fehrmann RSN, Karjalainen JM, Krajewska M, Westra H-J, Maloney D, Simeonov A, et al. (2015) **Gene expression analysis identifies global gene dosage sensitivity in cancer** *Nat Genet* **47**:115–25

18. Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, et al. (2012) **The cancer cell line encyclopedia enables predictive modelling of anticancer drug sensitivity** *Nature* **483**:603–7

19. Yang W, Soares J, Greninger P, Edelman EJ, Lightfoot H, Forbes S, et al. (2013) **Genomics of drug sensitivity in Cancer (GDSC): a resource for therapeutic biomarker discovery in cancer cells** *Nucleic Acids Res* **41**:D955–61

20. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al. (2013) **NCBI GEO: archive for functional genomics data sets—update** *Nucleic Acids Res* **41**:D991–5

21. Bhattacharya A, Bense RD, Urzúa-Traslaviña CG, de Vries EGE, van Vugt MATM, Fehrmann RSN (2020) **Transcriptional effects of copy number alterations in a large set of human cancers** *Nat Commun* **11**:715

22. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. (2005) **Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles** *Proc Natl Acad Sci* **102**:15545–50

23. Köhler S, Carmody L, Vasilevsky N, Jacobsen JOB, Danis D, Gourdine J-P, et al. (2019) **Expansion of the human phenotype ontology (HPO) knowledge base and resources** *Nucleic Acids Res* **47**:D1018–D1027

24. Urzúa-Traslaviña CG, Leeuwenburgh VC, Bhattacharya A, Loipfinger S, van Vugt MATM, de Vries EGE, et al. (2021) **Improving gene function predictions using independent transcriptional components** *Nat Commun* **12**:1464

25. Canozo FJG, Zuo Z, Martin JF, Samee MdAH (2022) **Cell-type modeling in spatial transcriptomics data elucidates spatially variable colocalization and communication between cell-types in mouse brain** *Cell Syst* **13**:58–70

26. Bolton KL, Chen D, de la Fuente RIC, Fu Z, Murali R, Kbel M, et al. (2022) **Molecular subclasses of clear cell ovarian carcinoma and their impact on disease behavior and outcomes** *Clin Cancer Res* **28**:4947–56

27. Huang RY, Chen GB, Matsumura N, Lai HC, Mori S, Li J, et al. (2012) **Histotype-specific copy-number alterations in ovarian cancer** *BMC Méd Genom* **5**:47

28. Jongsma APM, Piek JMJ, Zweemer RP, Verheijen RHM, Gebbinck JWTK, Kamp GJ van, et al. (2002) **Molecular evidence for putative tumour suppressor genes on chromosome 13q specific to BRCA1 related ovarian and fallopian tube cancer** *Mol Pathol* **55**:305

29. Ko J, Fuccillo MV, Malenka RC, Südhof TC (2009) **LRRTM2 functions as a neurexin ligand in promoting excitatory synapse formation** *Neuron* **64**:791–8

30. de Wit J, Sylwestrak E, O'Sullivan ML, Otto S, Tiglio K, Savas JN, et al. (2009) **LRRTM2 interacts with neurexin1 and regulates excitatory synapse formation** *Neuron* **64**:799–806

31. Ried T, Rudy B, Miera EV-S de, Lau D, Ward DC, Sen K. (1993) **Localization of a highly conserved human potassium channel gene (NGK2-KV4; KCNC1) to chromosome 11p15** *Genomics* **15**:405–11

32. Willis M, Trieb M, Leitner I, Wietzorrek G, Marksteiner J, Knaus H-G. (2017) **Small-conductance calcium-activated potassium type 2 channels (SK2, KCa2.2) in human brain** *Brain Struct Funct* **222**:973–9

33. Bourdeau ML, Laplante I, Laurent CE, Lacaille JC (2011) **KChIP1 modulation of Kv4.3-mediated A-type K+ currents and repetitive firing in hippocampal interneurons** *Neuroscience* **176**:173–87

34. Ehmsen JT, Liu Y, Wang Y, Paladugu N, Johnson AE, Rothstein JD, et al. (2016) **The astrocytic transporter SLC7A10 (Asc-1) mediates glycinergic inhibition of spinal cord motor neurons** *Sci Rep* **6**:35592

35. Nath A, Cosgrove PA, Mirsafian H, Christie EL, Pflieger L, Copeland B, et al. (2021) **Evolution of core archetypal phenotypes in progressive high grade serous ovarian cancer** *Nat Commun* **12**:3039

36. Hanahan D, Weinberg RA (2011) **Hallmarks of cancer: the next generation** *Cell* **144**:646–74

37. Reavis HD, Chen HI, Drapkin R. (2020) **Tumor Innervation: Cancer Has Some Nerve** *Trends Cancer* **6**:1059–67

38. Gysler SM, Drapkin R. (2021) **Tumor innervation: peripheral nerves take control of the tumor microenvironment** *J Clin Investig* **131**:e147276

39. Radin DP, Tsirka SE (2020) **Interactions between tumor cells, neurons, and microglia in the glioma microenvironment** *Int J Mol Sci* **21**:8476

40. Venkatesh HS, Morishita W, Geraghty AC, Silverbush D, Gillespie SM, Arzt M, et al. (2019) **Electrical and synaptic integration of glioma into neural circuits** *Nature* **573**:539–45

41. Preter KD, Vandesompele J, Heimann P, Yigit N, Beckman S, Schramm A, et al. (2006) **Human fetal neuroblast and neuroblastoma transcriptome analysis confirms neuroblast origin and highlights neuroblastoma candidate genes** *Genome Biol* **7**:R84

42. Mark B, Lai S-L, Zarin AA, Manning L, Pollington HQ, Litwin-Kumar A, et al. (2021) **A developmental framework linking neurogenesis and circuit formation in the Drosophila CNS** *eLife* **10**:e67510

43. Allen JK, Armaiz-Pena GN, Nagaraja AS, Sadaoui NC, Ortiz T, Dood R, et al. (2018) **Sustained adrenergic signaling promotes intratumoral innervation through BDNF induction** *Cancer Res* **78**:3233–3242

44. Rains SL, Amaya CN, Bryan BA (2017) **Beta-adrenergic receptors are expressed across diverse cancers** *Oncoscience* **4**:95–105

45. Eng JWL, Kokolus KM, Reed CB, Hylander BL, Ma WW, Repasky EA (2014) **A nervous tumor microenvironment: the impact of adrenergic stress on cancer cells, immunosuppression, and immunotherapeutic response** *Cancer Immunol Immunother* **63**:1115–28

46. Drilon A, Siena S, S-HI Ou, Patel M, Ahn MJ, Lee J, et al. (2017) **Safety and antitumor activity of the multitargeted Pan-TRK, ROS1, and ALK inhibitor entrectinib: combined results from two phase I trials (ALKA-372-001 and STARTRK-1)** *Cancer Discov* **7**:400–9

47. Ardini E, Menichincheri M, Banfi P, Bosotti R, Ponti CD, Pulci R, et al. (2016) **Entrectinib, a pan–TRK, ROS1, and ALK inhibitor with activity in multiple molecularly defined cancer indications** *Mol Cancer Ther* **15**:628–39

48. Drilon A, Laetsch TW, Kummar S, DuBois SG, Lassen UN, Demetri GD, et al. (2018) **Efficacy of larotrectinib in TRK fusion–positive cancers in adults and children** *N Engl J Med* **378**:731–9

49. Burris HA, Shaw AT, Bauer TM, Farago AF, Doebele RC, Smith S, et al. (2015) **Abstract 4529: Pharmacokinetics (PK) of LOXO-101 during the first-in-human phase I study in patients with advanced solid tumors: Interim update** *Cancer Res* **75**:4529–4529

50. Barr JL, Kruse A, Restaino AC, Tulina N, Stuckelberger S, Vermeer SJ, et al. (2021) **Intra-tumoral nerve-tracing in a novel syngeneic model of high-grade serous ovarian carcinoma** *Cells* **10**:3491

51. Restaino AC, Walz A, Vermeer SJ, Barr J, Kovács A, Fettig RR, et al. (2023) **Functional neuronal circuits promote disease progression in cancer** *Sci Adv* **9**:eade4443

52. Balood M, Ahmadi M, Eichwald T, Ahmadi A, Majdoubi A, Roversi K, et al. (2022) **Nociceptor neurons affect cancer immunosurveillance** *Nature* **611**:405–12

53. Li X, Peng X, Yang S, Wei S, Fan Q, Liu J, et al. (2022) **Targeting tumor innervation: premises, promises, and challenges** *Cell Death Discov* **8**:131

54. Padmanaban V, Keller I, Seltzer ES, Ostendorf BN, Kerner Z, Tavazoie SF (2024) **Neuronal substance-P drives breast cancer growth and metastasis via an extracellular RNA-TLR7 axis** *bioRxiv*

55. Williams K. (2001) **Ifenprodil, a novel NMDA receptor antagonist: site and mechanism of action** *Curr Drug Targets* **2**:285–98

56. Reid JG, Gitlin MJ, Altshuler LL (2013) **Lamotrigine in psychiatric disorders** *J Clin Psychiatry* **74**:675–84

57. Ramamoorthy MD, Kumar A, Ayyavu M, Dhiraviam KN (2019) **Reserpine induces apoptosis and cell cycle arrest in hormone independent prostate cancer cells through mitochondrial membrane potential failure** *Anti-Cancer Agents Med Chem* **18**:1313–22

58. North WG, Liu F, Tian R, Abbasi H, Akerman B. (2015) **NMDA receptors are expressed in human ovarian cancer tissues and human ovarian cancer cell lines** *Clin Pharmacol: Adv Appl* **7**:111–7

Author information

Arkajyoti Bhattacharya[#]
Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands
ORCID iD: 0000-0003-1479-0739

[#]Contributed equally

Thijs S Stutvoet[#]
Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

[#]Contributed equally

Mirela Perla[#]
Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

[#]Contributed equally

Stefan Loipfinger
Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

Mathilde Jalving
Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

Anna KL Reyners
Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

Paola D Vermeer
Cancer Biology and Immunotherapies Group, Sanford Research, Sioux Falls, United States

Ronny Drapkin
Penn Ovarian Cancer Research Center and Basser Center for BRCA, University of Pennsylvania, Perelman School of Medicine, Philadelphia, United States
ORCID iD: 0000-0002-6912-6977

Marco de Bruyn
Department of Obstetrics and Gynecology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

Elisabeth GE de Vries
Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

Steven de Jong
Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

Rudolf SN Fehrmann
Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

For correspondence: r.s.n.fehrmann@umcg.nl

Editors

Reviewing Editor
Katherine Lawler
University of Cambridge, Cambridge, United Kingdom

Senior Editor
Tony Ng
King's College London, London, United Kingdom

Reviewer #1 (Public review):

Summary:

This manuscript explores the transcriptional landscape of high-grade serous ovarian cancer (HGSOC) using consensus-independent component analysis (c-ICA) to identify transcriptional components (TCs) associated with patient outcomes. The study analyzes 678 HGSOC transcriptomes, supplemented with 447 transcriptomes from other ovarian cancer types and noncancerous tissues. By identifying 374 TCs, the authors aim to uncover subtle transcriptional patterns that could serve as novel drug targets. Notably, a transcriptional component linked to synaptic signaling was associated with shorter overall survival (OS) in patients, suggesting a potential role for neuronal interactions in the tumor microenvironment. Given notable weaknesses like lack of validation cohort or validation using other platforms (other than the 11 samples with ST), the data is considered highly descriptive and preliminary.

The study reveals significant findings by identifying a transcriptional component (TC121) associated with synaptic signaling, which is linked to shorter survival in patients with high-grade serous ovarian cancer, highlighting the potential role of neurons in the tumor microenvironment. However, the evidence could be strengthened by experimental validation to confirm the functional roles of key genes within TC121 and further exploration of its spatial aspects, including deeper analysis of neuronal and synaptic and other neuronal gene expression.

Strengths:

Innovative Methodology:
The use of c-ICA to dissect bulk transcriptomes into independent components is a novel approach that allows for the identification of subtle transcriptional patterns that may be overshadowed in traditional analyses.

Comprehensive Data Integration:
The study integrates a large dataset from multiple public repositories, enhancing the robustness of the findings. The inclusion of spatially resolved transcriptomes adds a valuable dimension to the analysis.

Clinical Relevance:
The identification of a synaptic signaling-related TC associated with poor prognosis highlights a potential new avenue for therapeutic intervention, emphasizing the role of the tumor microenvironment in cancer progression.

Weaknesses:

Mechanistic Insights:
While the study identifies TCs associated with survival, it provides limited mechanistic insights into how these components influence cancer progression. Further experimental validation is necessary to elucidate the underlying biological processes.

Generalizability:
The findings are primarily based on transcriptomic data from HGSOC. It remains unclear how these results apply to other subtypes of ovarian cancer or different cancer types.

Innovative Methodology:
Requires more validation using different platforms (IHC) to validate the performance of this bulk derived data. Also, the lack of control on data quality is a concern.

Clinical Application:
Although the study suggests potential drug targets, the translation of these findings into clinical practice is not addressed. Probably given lack of some QA/QC procedures it'll be hard to translate these results. Future studies should focus on validating these targets in clinical settings.

https://doi.org/10.7554/eLife.101369.2.sa2

Reviewer #2 (Public review):

Summary:

Consensus-independent component analysis and closely related methods have previously been used to reveal components of transcriptomic data which are not captured by principal component or gene-gene coexpression analyses.

Here, the authors asked whether applying consensus-independent component analysis (c-ICA) to published high-grade serous ovarian cancer (HGSOC) microarray-based transcriptomes would reveal subtle transcriptional patterns which are not captured by existing molecular omics classifications of HGSOC.

Statistical associations of these (hitherto masked) transcriptional components with prognostic outcomes in HGSOC would lead to additional insights into underlying mechanisms and, coupled with corroborating evidence from spatial transcriptomics, are proposed for further investigation.

This approach is complementary to existing transcriptomics classifications of HGSOC.

The authors have previously applied the same approach in colorectal carcinoma (for example, Knapen et al. (2024) Commun. Med).

Strengths:

Overall, this study describes a solid data-driven description of c-ICA-derived transcriptional components that the authors identified in HGSOC microarray transcriptomics data, supported by detailed methods and supplementary documentation.

The biological interpretation of transcriptional components is convincing based on (data-driven) permutation analysis and a suite of analyses of association with copy-number, gene sets, and prognostic outcomes.
The resulting annotated transcriptional components have been made available in a searchable online format.

For the highlighted transcriptional component which has been annotated as related to synaptic signalling, the detection of the transcriptional component among 11 published spatial transcriptomics samples from ovarian cancers is compelling and supports the need for further mechanistic follow-up.

Further comments:

This revised version includes a suite of comparisons between the c-ICA-derived components and existing published transcriptomic/genomic-based classifications of ovarian cancers.
Newly described components will require experimental validation, as acknowledged by the authors.

Here, the authors primarily interpret the c-ICA transcriptional components as a deconvolution of bulk transcriptomics due to the presence of cells from tumour cells and the tumour microenvironment.
In this revised version, the authors additionally investigate their TC scores in single cells from a published HGSOC single-cell RNAseq dataset, highlighting examples of TC scores within and between cell types.

c-ICA is not explicitly a deconvolution method with respect to cell types: the transcriptional components do not necessarily correspond to distinct cell types, and may reflect differential dysregulation within a cell type. This application of c-ICA for the purpose of data-driven deconvolution of cell populations is distinct from other deconvolution methods which explicitly use a prior cell signature matrix.

https://doi.org/10.7554/eLife.101369.2.sa1

Author response:

The following is the authors' response to the original reviews

> ***eLife Assessment***
>
> *This valuable study uses consensus-independent component analysis to highlight transcriptional components (TC) in high-grade serous ovarian cancers (HGSOC). The study presents a convincing preliminary finding by identifying a TC linked to synaptic signaling that is associated with shorter overall survival in HGSOC patients, highlighting the potential role of neuronal interactions in the tumour microenvironment. This finding is corroborated by comparing spatially resolved transcriptomics in a small-scale study; a weakness is in being descriptive, non-mechanistic, and requiring experimental validation."*

We sincerely thank the editors for their valuable and constructive feedback. We are grateful for the recognition of our findings and the importance of identifying transcriptional components in high-grade serous ovarian cancers.

We acknowledge the editors' observation regarding the descriptive nature of our study and its limited mechanistic depth. We agree that additional experimental validation would further strengthen our conclusions. We are planning and executing the experiments for a future study to provide mechanistic insights into the associations found in this study. In addition, recent reviews focused on the emerging field of cancer neuroscience emphasize the early stages the field is in, specifically in terms of a mechanistic understanding of the contributions of tumor-infiltrating nerves in tumor initiation and progression (Amit et al., 2024; Hwang et al., 2024). Nonetheless, we wish to emphasize that emerging mechanistic preclinical studies have demonstrated the influence of tumour-infiltrating nerves on disease progression (Allen et al., 2018; Balood et al., 2022; Darragh et al., 2024; Globig et al., 2023; Jin et al., 2022; Restaino et al., 2023; Zahalka et al., 2017). Several of these studies include contributions from our co-authors and feature *in vitro* and *in vivo* research on head and neck squamous cell carcinoma as well as high-grade serous ovarian carcinoma samples. This study further strengthens the preclinical work by showing in patient data, the potential relevance of neuronal signaling on disease outcome.

For instance, Restiano et al. (2023) demonstrated that substance P, released from tumour-infiltrating nociceptors, potentiates MAP kinase signaling in cancer cells, thereby driving disease progression. Crucially, this effect was shown to be reversible *in vivo* by blocking the substance P receptor (Restaino et al., 2023). These findings offer compelling evidence of the role of tumour innervation in cancer biology.

Our current study in tumor samples of patients with high-grade serous ovarian cancer identifies a transcriptional component that is enriched for genes for which the protein is located in the synapse. We believe that the previously published mechanistic insights support our findings and suggest that this transcriptional component could serve as a valuable screening tool to identify innervated tumours based on bulk transcriptomes. Clinically, this information is highly relevant, as patients with innervated tumours may benefit from alternate therapeutic strategies targeting these innervations.

> ***Reviewer #1 (Public review)***
>
> *This manuscript explores the transcriptional landscape of high-grade serous ovarian cancer (HGSOC) using consensus-independent component analysis (c-ICA) to identify transcriptional components (TCs) associated with patient outcomes. The study analyzes 678 HGSOC transcriptomes, supplemented with 447 transcriptomes from other ovarian cancer types and noncancerous tissues. By identifying 374 TCs, the authors aim to uncover subtle transcriptional patterns that could serve as novel drug targets. Notably, a transcriptional component linked to synaptic signaling was associated with shorter overall survival (OS) in patients, suggesting a potential role for neuronal interactions in the tumour microenvironment. Given notable weaknesses like lack of validation cohort or validation using another platform (other than the 11 samples with ST), the data is considered highly descriptive and preliminary.*
>
> *Strengths:*
>
> *(1) Innovative Methodology:*
>
> *The use of c-ICA to dissect bulk transcriptomes into independent components is a novel approach that allows for the identification of subtle transcriptional patterns that may be overshadowed in traditional analyses.*

We thank the reviewer for recognizing the strengths and novelty of our study. We appreciate the positive feedback on using consensus-independent component analysis (c-ICA) to decompose bulk transcriptomes, which allowed us to detect subtle transcriptional signals often overlooked in traditional analyses.

> *(2) Comprehensive Data Integration:*
>
> *The study integrates a large dataset from multiple public repositories, enhancing the robustness of the findings. The inclusion of spatially resolved transcriptomes adds a valuable dimension to the analysis.*

We thank the reviewer for recognizing the robustness of our study through comprehensive data integration. We appreciate the acknowledgment of our efforts to leverage a large, multi-source dataset, as well as the additional insights gained from spatially resolved transcriptomes. We consider this integrative approach enhances the depth of our analysis and contributes to a more nuanced understanding of the tumour microenvironment.

> *(3) Clinical Relevance:*
>
> *The identification of a synaptic signaling-related TC associated with poor prognosis highlights a potential new avenue for therapeutic intervention, emphasizing the role of the tumour microenvironment in cancer progression.*

We appreciate the recognition of the clinical implications of our findings. The identification of a synaptic signaling-related transcriptional component associated with poor prognosis underscores the potential for novel therapeutic targets within the tumour microenvironment. We agree that this insight could open new avenues for intervention and further highlights the role of neuronal interactions in cancer progression.

> *Weaknesses:*
>
> *(1) Mechanistic Insights:*
>
> *While the study identifies TCs associated with survival, it provides limited mechanistic insights into how these components influence cancer progression. Further experimental validation is necessary to elucidate the underlying biological processes.*

We acknowledge the point regarding the limited mechanistic insights provided in our study. We agree that further experimental validation would significantly enhance our understanding of how the biological processes captured by these transcriptional components influence cancer progression. We are planning and executing the experiments for a future study to provide mechanistic insights into the associations found in this study.

Our analyses were performed on publicly available bulk and spatial resolved expression profiles. To investigate the mechanistic insights in future studies, we plan to integrate spatial transcriptomic data with immunohistochemical analysis of the same tumour samples to validate our findings. Additionally, we have initiated efforts to set up *in vitro* co-cultures of neurons and ovarian cancer cells. These co-cultures will enable us to investigate how synaptic signaling impacts ovarian cancer cell behavior.

> *(2) Generalizability:*
>
> *The findings are primarily based on transcriptomic data from HGSOC. It remains unclear how these results apply to other subtypes of ovarian cancer or different cancer types.*

To respond to this remark, we utilized survival data from Bolton et al. (2022) and TCGA to investigate associations between TC activity scores and overall survival of patients with ovarian clear cell carcinoma, the second most common subtype of epithelial ovarian cancer, and other cancer types respectively. However, we acknowledge the limitations of TCGA survival data, as highlighted in the referenced article (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8726696/). Additionally, as shown in Figure 5, we provided evidence of TC121 activity across various cancer types, suggesting broader relevance. For the results of the analyses mentioned above, please refer to our response to remark 1.3 of the recommendation section (page 4).

> *(3) Innovative Methodology:*
>
> *Requires more validation using different platforms (IHC) to validate the performance of this bulk-derived data. Also, the lack of control over data quality is a concern.*

We acknowledge the value of validating our results with alternative platforms such as IHC. We are planning and executing the experiments for a future study to provide mechanistic insights into the associations found in this study.

We implemented regarding data quality control, the following measures to ensure the reliability of our analysis:

Bulk Transcriptional Profiles: To assess data quality, we conducted principal component analysis (PCA) on the sample Pearson product-moment correlation matrix. The first principal component (PCqc), which explains approximately 80-90% of the variance, was used to distinguish technical variability from biological signals (Bhattacharya et al., 2020). Samples with a correlation coefficient below 0.8 relative to PCqc were identified as outliers and excluded. Additionally, MD5 hash values were generated for each CEL file to identify and remove duplicate samples. Expression values were standardized to a mean of zero and a variance of one for each gene to minimize probeset- or gene-specific variability across datasets (GEO, CCLE, GDSC, and TCGA).

Spatial Transcriptional Profiles: PCA was also applied to spatial transcriptomic data for quality control. Only samples with consistent loading factor signs for the first principal component across all individual spot profiles were retained. Samples failing this criterion were excluded from further analyses.

> *(4) Clinical Application:*
>
> *Although the study suggests potential drug targets, the translation of these findings into clinical practice is not addressed. Probably given the lack of some QA/QC procedures it'll be hard to translate these results. Future studies should focus on validating these targets in clinical settings."*

Regarding clinical applications, we acknowledge the importance of further exploring strategies targeting synaptic signaling and neurotransmitter release in the tumour microenvironment (TME). As partially discussed in the first version of the manuscript, drugs such as ifenprodil and lamotrigine—commonly used to treat neuronal disorders—can block glutamate release, thereby inhibiting subsequent synaptic signaling. Additionally, the vesicular monoamine transporter (VMAT) inhibitor reserpine blocks the formation of synaptic vesicles (Reid et al., 2013; Williams et al., 2001). Previous in vitro studies with HGSOC cell lines demonstrated that ifenprodil significantly reduced cancer cell proliferation, while reserpine triggered apoptosis in cancer cells (North et al., 2015; Ramamoorthy et al., 2019). The findings highlight the potential of such approaches to disrupt synaptic neurotransmission in the TME.

To address potential translation of our findings into clinical practice more comprehensively, we have included additional details in the manuscript:

Section discussion, page 16, lines 338-341:

“This interaction can be targeted with pan-TRK inhibitors such as entrectinib and larotrectinib. Both drugs are showing promising results in multiple phase II trials, including ovarian cancer and breast cancer patients. Furthermore, a TRKB-specific inhibitor was developed (ANA-12), but has not been subjected to any clinical trials in cancer so far (Ardini et al., 2016; Burris et al., 2015; Drilon et al., 2018, 2017).”

On page 17, lines 361-374:

“Strategies to disrupt neuronal signaling and neurotransmitter release in neurons target key elements of excitatory neurotransmission, such as calcium flux and vesicle formation. Drugs like ifenprodil and lamotrigine, commonly used to treat neuronal disorders, block glutamate release and subsequent neuronal signaling. Additionally, the vesicular monoamine transporter (VMAT) inhibitor reserpine prevents synaptic vesicle formation (Reid et al., 2013; Williams, 2001). In vitro studies with HGSOC cell lines have demonstrated that ifenprodil significantly inhibits tumour proliferation, while reserpine induces apoptosis in cancer cells (North et al., 2015; Ramamoorthy et al., 2019). These approaches hold promise for inhibiting neuronal signaling and interactions in the TME.”

> ***Reviewer #2 (Public review):***
>
> *Summary:*
>
> *Consensus-independent component analysis and closely related methods have previously been used to reveal components of transcriptomic data that are not captured by principal component or gene-gene coexpression analyses.*
>
> *Here, the authors asked whether applying consensus-independent component analysis (c-ICA) to published high-grade serous ovarian cancer (HGSOC) microarray-based transcriptomes would reveal subtle transcriptional patterns that are not captured by existing molecular omics classifications of HGSOC.*
>
> *Statistical associations of these (hitherto masked) transcriptional components with prognostic outcomes in HGSOC could lead to additional insights into underlying mechanisms and, coupled with corroborating evidence from spatial transcriptomics, are proposed for further investigation.*
>
> *This approach is complementary to existing transcriptomics classifications of HGSOC.*
>
> *The authors have previously applied the same approach in colorectal carcinoma (Knapen et al. (2024) Commun. Med).*
>
> *Strengths:*
>
> *(1) Overall, this study describes a solid data-driven description of c-ICA-derived transcriptional components that the authors identified in HGSOC microarray transcriptomics data, supported by detailed methods and supplementary documentation.*

We thank the reviewer for acknowledging the strength of our data-driven approach and the use of consensus-independent component analysis (c-ICA) to identify transcriptional components within HGSOC microarray data. We aimed to provide comprehensive methodological detail and supplementary documentation to support the reproducibility and

robustness of our findings. We believe this approach allows for the identification of subtle transcriptional signals that might have been overlooked by traditional analysis methods.

> *(2) The biological interpretation of transcriptional components is convincing based on (data-driven) permutation analysis and a suite of analyses of association with copy-number, gene sets, and prognostic outcomes.*

We appreciate the positive feedback on the biological interpretation of our transcriptional components. We are pleased that our approach, which includes data-driven permutation testing and analyses of associations with copy-number alterations, gene sets, and prognostic outcomes, was found to be convincing. These analyses were integral to enhancing our findings' robustness and biological relevance.

> *(3) The resulting annotated transcriptional components have been made available in a searchable online format.*

Thank you for this important positive remark.

> *(4) For the highlighted transcriptional component which has been annotated as related to synaptic signalling, the detection of the transcriptional component among 11 published spatial transcriptomics samples from ovarian cancers appears to support this preliminary finding and requires further mechanistic follow-up.*

Thank you for acknowledging the accessibility of our annotated transcriptional components. We prioritized making these data available in a searchable online format to facilitate further research and enable the community to explore and validate our findings.

> *Weaknesses:*
>
> *(1) This study has not explicitly compared the c-ICA transcriptional components to the existing reported transcriptional landscape and classifications for ovarian cancers (e.g. Smith et al Nat Comms 2023; TCGA Nature 2011; Engqvist et al Sci Rep 2020) which would enable a further assessment of the additional contribution of c-ICA - whether the cICA approach captured entirely complementary components, or whether some components are correlated with the existing reported ovarian transcriptomic classifications.*

We acknowledge the reviewer's insightful suggestion to compare our c-ICA-derived transcriptional components with previously reported ovarian cancer classifications, such as those from Smith et al. (2023), TCGA (2011), and Engqvist et al. (2020). To address this, we incorporated analyses comparing the activity scores of our transcriptional components with these published landscapes and classifications, particularly focusing on any associations with overall survival. Additionally, we evaluated correlations between gene signatures from a subset of these studies and our identified TCs, enhancing our understanding of the unique contributions of the c-ICA approach. Please refer to our response to remark 10 for the results of these analyses.

> *(2) Here, the authors primarily interpret the c-ICA transcriptional components as a deconvolution of bulk transcriptomics due to the presence of cells from tumour cells and the tumour microenvironment.*
>
> *However, c-ICA is not explicitly a deconvolution method with respect to cell types: the transcriptional components do not necessarily correspond to distinct cell types, and may reflect differential dysregulation within a cell type. This application of c-ICA for the*

> *purpose of data-driven deconvolution of cell populations is distinct from other deconvolution methods that explicitly use a prior cell signature matrix."*

We acknowledge that c-ICA, unlike traditional deconvolution methods, is not specifically designed for cell-type deconvolution and does not rely on a predefined cell signature matrix. While we explored the transcriptional components in the context of tumour and microenvironmental interactions, we agree that these components may not correspond directly to distinct cell types but rather reflect complex patterns of dysregulation, potentially within individual cell populations.

Our goal with c-ICA was to uncover hidden transcriptional patterns possibly influenced by cellular heterogeneity. However, we recognize these patterns may also arise from regulatory processes within a single cell type. To investigate further, we used single-cell transcriptional data (~60,000 cell-types annotated profiles from GSE158722) and projected our transcriptional components onto these profiles to obtain activity scores, allowing us to assess each TC's behavior across diverse cellular contexts after removing the first principal component to minimize background effects. Please refer to our response to remark 2.2 in the recommendations to the authors (page 14) for the results of this analysis.

References

Allen JK, Armaiz-Pena GN, Nagaraja AS, Sadaoui NC, Ortiz T, Dood R, Ozcan M, Herder DM, Haemerrle M, Gharpure KM, Rupaimoole R, Previs R, Wu SY, Pradeep S, Xu X, Han HD, Zand B, Dalton HJ, Taylor M, Hu W, Bottsford-Miller J, Moreno-Smith M, Kang Y, Mangala LS, Rodriguez-Aguayo C, Sehgal V, Spaeth EL, Ram PT, Wong ST, Marini FC, Lopez-Berestein G, Cole SW, Lutgendorf SK, diBiasi M, Sood AK. 2018. Sustained adrenergic signaling promotes intratumoral innervation through BDNF induction. Cancer Res 78 (12):3233-3242.

Ardini E, Menichincheri M, Banfi P, Bosotti R, Ponti CD, Pulci R, Ballinari D, Ciomei M, Texido G, Degrassi A, Avanzi N, Amboldi N, Saccardo MB, Casero D, Orsini P, Bandiera T, Mologni L, Anderson D, Wei G, Harris J, Vernier J-M, Li G, Felder E, Donati D, Isacchi A, Pesenti E, Magnaghi P, Galvani A. 2016. Entrectinib, a Pan–TRK, ROS1, and ALK Inhibitor with activity in multiple molecularly defined cancer Indications. Mol Cancer Ther 15:628–639.

Balood M, Ahmadi M, Eichwald T, Ahmadi A, Majdoubi A, Roversi Karine, Roversi Katiane, Lucido CT, Restaino AC, Huang S, Ji L, Huang K-C, Semerena E, Thomas SC, Trevino AE, Merrison H, Parrin A, Doyle B, Vermeer DW, Spanos WC, Williamson CS, Seehus CR, Foster SL, Dai H, Shu CJ, Rangachari M, Thibodeau J, Rincon SVD, Drapkin R, Rafei M, Ghasemlou N, Vermeer PD, Woolf CJ, Talbot S. 2022. Nociceptor neurons affect cancer immunosurveillance. Nature 611:405–412.

Bhattacharya A, Bense RD, Urzúa-Traslaviña CG, Vries EGE de, Vugt MATM van, Fehrmann RSN. 2020. Transcriptional effects of copy number alterations in a large set of human cancers. Nat Commun 11:715.

Burris HA, Shaw AT, Bauer TM, Farago AF, Doebele RC, Smith S, Nanda N, Cruickshank S, Low JA, Brose MS. 2015. Abstract 4529: Pharmacokinetics (PK) of LOXO-101 during the first-in-human Phase I study in patients with advanced solid tumors: Interim update. Cancer Res 75:4529–4529.

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