Prognostic pathways in early stage ovarian cancer – can gene expression transcend histological subtype?

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Ovarian cancer, like many other solid malignancies, presents a paradox. Things change, and yet they stay the same: our understanding of fundamental disease biology has improved dramatically in the past decade [1], yet the large majority of patients still present with advanced disease, and overall survival still remains poor [2].

One critical change has been the realisation that ovarian cancer is a series of separate diseases, driven by distinct mutational processes [1]. The commonest histological subtype, high grade serous carcinoma, is marked by universal mutation in *TP53* [3], chromosomal instability [4] and rapid dissemination around the peritoneal cavity, with many cases arising in the secretory cells of the distal fallopian tube rather than the ovary itself [5]. By contrast, clear cell and low-grade endometrioid carcinomas are *TP53* wild-type but often contain mutations in *ARID1A,* a component of the SWI/SNF chromatin re-modelling complex [6], and *PIK3CA* [7]. Many mucinous ovarian cancers, with frequent *KRAS* mutations, represent metastases from gastrointestinal tumours [8, 9], whilst low grade serous carcinomas, arising on the background of borderline/low malignant potential tumours, harbour mutations in *KRAS* and *BRAF* [10]. Improved immunohistochemical analyses now allow for more accurate classification of tumours [11], and pathological re-examination of archival specimens frequently leads to re-classification [12]. Critically, patterns of expression are consistent within anyone ovarian cancer subtype, and do not alter with stage [13].

Gene expression studies have also been revealing. Early array data indicated that the individual subtypes of ovarian cancer had very distinct patterns of gene expression [14, 15] that correlated with their different tissues of origin [16]. More recently, large consortium studies have indicated that, within cohorts of predominantly high grade serous tumours, there are distinct expression subgroups, with markedly different prognoses [4, 17]. Exactly how these gene expression subgroups link to specific mutation patterns, however, remains unclear.

Only around 20% patients are diagnosed with stage I disease (confined to the ovary with or without cyst rupture and/or positive cytology in peritoneal washings). Outcome for these patients is generally good, with 80% still alive five years following diagnosis [18]. Two large randomised trials, ICON1 and ACTION, indicated that there is a significant improvement in overall survival when platinum-based chemotherapy is given following initial surgery [19, 20]. This improvement remained after long-term follow up, although debate remains as to whether patients with low grade stage IA/B disease [21] and those who undergo extensive staging surgery [22] require adjuvant chemotherapy.

It is against this background that Carula et al present their analysis of a series of stage I ovarian cancers [23]. Using frozen specimens collected at surgery between 1992 and 2011 in three large Italian centres, the authors analysed gene expression in stage I ovarian cancers of all histological subtypes, and describe a gene expression signature (ISC, the 'Integrated Signature Classifier') of 26 individual genes and miRNA that defines a poor prognosis group. The genes include those involved in cell cycle regulation (e.g. *CCND1/2/3, CDK4/6*), Hedgehog signalling (e.g. *GLI1*) and activin/inhibin signalling (e.g. *ACVR1/2B*). Key miRNA included *let-7e*, *miR-34a* and *miR-145*. The original discovery sample set consisted of 34 tumours, 17 grade 1 and 17 grade 3. Further refinement was performed on a training set of 157 tumours that also included the original 34 discovery samples. An ISC score, ranging from 0 – 1, was given to each sample, depending upon how well the gene expression of that tumour correlated with the 26-gene poor prognosis signature. Separate Receiver Operating Characteristic (ROC) curves for progression-free and overall survival were used to define classifiers of high and low risk. Finally, these parameters were applied to a validation cohort of 46 tumours.

Overall, this is an admirable piece of work – the authors have utilised large, well-annotated sample sets and have undertaken extensive analyses using methods that are clearly described, and raw expression data are publically available. The results are simultaneously impressive and intriguing. Impressive because, in both univariate and multivariate analyses, the ISC classifiers were extremely powerful predictors of both progression-free and overall survival in the validation cohort. Intriguing because the authors appear to have identified patterns of gene expression that predict outcome across multiple histological subtypes that, as stated above, represent different diseases with distinct oncogenic drivers. Moreover, the risk classifiers worked independently of chemotherapy treatment.

There are some caveats and quirks in the analysis that cannot be ignored. The samples were collected over a 20 year period and were not subject to full pathological review using current methodology and consistent diagnostic criteria. Patients were also not managed with uniform chemotherapy protocols or follow up schedules, so progression-free survival data will be less robust than in a prospective clinical trial. It is not clear whether all patients underwent optimal staging as defined by Trimbos et al in the ACTION study [22]. Sample set B is stated to harbour no *TP53* mutations yet contains 32 high grade serous tumours, where *TP53* mutations are universal [3, 24]. There is also some circularity in the sample utilisation and analysis – all 34 discovery samples were re-analysed in the test set, whilst the decision to use grade (1 vs 3) to define prognostic gene expression signatures was partially based upon an initial multivariate analysis of all 203 tumours in the discovery, test and validation cohorts.

Despite these reservations, the data are impressive and do suggest that there are patterns of gene expression that are prognostically important. The presence of cell cycle genes in the ISC is perhaps not unexpected – in ovarian high grade serous tumours, amplification of *CCNE1* is a poor prognosis feature, associated with primary platinum resistance [25], whilst *CCNE1* amplification and increased *CCND1* expression are both negative features in breast cancer [26, 27], suggesting that aberrant G1/S checkpoint control is a general poor prognostic marker. Similarly, there are data to suggest that Hedgehog signalling is a marker of poor outcome across multiple tumours [28-30]. Data on activin/inhibin signalling are sparser, but again results suggest that upregulation of this pathway is a poor prognosis feature in other malignancies [31, 32]. Thus, there do appear to be certain pathways that are universal markers of poor outcome. It would be interesting to see if the ISC signature applies to more advanced ovarian cancer, and the availability of large publically available data sets such as TCGA would make such an analysis relatively simple. Certainly, further validation in an entirely independent sample set of early ovarian cancers would be most reassuring.

The critical question is how to utilise these data. Do we yet have confidence to apply the ISC prospectively, to recommend no chemotherapy to some women with early stage disease who would currently be offered platinum? The answer must be ‘not yet’. However, the ability to simplify gene expression assessment using platforms such as Nanostring and Taqman [33] means that rapid and sensitive assessment for prospective stratification in clinical trials is imminent.

Overall, the results remind us that, although classification of tumours based upon DNA mutations is extremely important, gene expression analyses remain vital and can provide valuable information that will ultimately be utilised to guide treatment decisions.

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