Editorial

miRNA based signature for predicting epithelial ovarian cancer relapse-progression: a step forward to prime time clinical adoption?

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Epithelial ovarian cancer (EOC) is an extremely genomically heterogeneous disease (1) with a unique clinical phenotype: high rate of chemosensitivity but also high risk of relapse, also after adequate primary treatment (2). Even so, the development of predictive tools to stratify relapse risk can be helpful in identifying high risk populations, which represent 70% of advanced stage disease patients, and in tailoring following treatment. The Cancer Genome Atlas (TCGA) project aimed to unravel this genomic complexity, providing gene expression profiles to discriminate prognosis among patients (3,4). More recently, a meta-analytic approach merging all the gene expression signatures for EOC increased the prognostic accuracy of the model, but no clinically relevant prognostic differences among groups were identified (5).

In this context, in the last years an effort has been made to scrutinize the non-coding transcriptome portion (ncRNA) composed of several entities and diverse roles (6). Among ncRNAs, microRNA (miRNA) is the most highly studied due to its key role in gene expression regulation. miRNAs are short nucleotide (21–25 nucleotides) sequences that regulate gene expression through two main mechanisms: inhibiting translation and inducing degradation of transcripts. Their hairpin loop tertiary structure makes them resistant to degradation, extremely stable in the cytosol, nucleus, and even as circulating cell-free molecules (7). Thus, it is a promising category as a cancer biomarker (8).

Several reports describe their key role in several cancer types (9). miRNAs' role has been investigated also in EOC, demonstrating relevant prognostic (10,11) and biologic correlation (12). The commented paper provides an important step forward in this direction.

Bagnoli and colleagues (13) report a multi institutional research to test miRNA expression in EOC with the aim of identifying a signature associated to relapse-progression. Samples with full clinical data from treatment naïve patients were used, analyzing miRNA expression either from formalin-fixed paraffin-embedded (FFPE) and fresh frozen (FF) samples. A 179 sample training set (OC179) was obtained from available tissue of a randomized clinical trial (14), and a 263 sample validation set (OC263) was retrieved from archival tissue from two oncologic centers. Further, in silico validation was performed with the TCGA dataset composed of 452 samples (OC452). All together, the reported sample size is the largest in the literature that tests miRNA expression in EOC.

The authors obtained a 35 miRNAs signature (MiROvaR) that successfully segregated low and high risk of relapse-progression patients. The observed median difference in progression free survival (PFS) between the low and high risk groups was 20 months. This study is an important step forward in addressing the role of miRNA as prognostic biomarker in EOC. The strength of the study is that it analyzed for miRNA expression 894 samples across cohort of patients from different clinical centers.

Despite training set and validation set were obtained

with different array platforms, thus limiting the analysis to 384 of the 2,000 miRNA known to data, the authors have identified a minimal common signature of 35 miRNAs whose expression correlate with prognosis that may represent the "core" of miRNA deregulation in bad prognosis EOC patients.

Interesting to note that the authors claim that this "core" is independent from histological features and it might have a prognostic significance. Indeed, while TCGA cohort is characterized by high grade serous ovarian cancer only, the OC179 and the OC263 cohorts are characterized by different histological subtypes and tumor grades. This is the second study to date (15) showing that the prognostic role of miRNA in EOC is shared across different histological subtypes, making this class of non-coding RNA very attractive in the clinic as prognostic biomarkers. Although this study is an important step forward in the identification of molecular portraits with prognostic relevance in EOC, there are some minor points that should be discussed in detail and that limit the final results of the study.

Technology and biostatistics

Several miRNA panels were used to identify differentially expressed miRNAs through microarray technology: different platforms were used, among which 385 probes were commonly present (Agilent miR-Base 17; Agilent miR-Base 10; Illumina miR-Base 12). This approach excluded from possible comparison more than 80% of predicted miRNA sequences (2,000+) (16), missing to analyze part of the miRNA landscape and theoretically reducing the detection capability of the research. Also, this underestimation is not counting recent evidence of numerous newly discovered miRNAs in human genome. This evidence was provided by recent deep sequencing technologies, yet was not available at the time of the study (17).

Nonetheless, given the multiple variables in using different platforms, the risk of undergoing important batch effects is present. This risk is also increased by different tumor fixation materials used that can affect the quality of the tested material. Batch effect risks can potentially be tackled if addressed in the project design (18). On this regard, the authors proposed approaches for comparing miRNA microarray data from different platforms (19), for normalizing microarray data from different tissue fixation materials (20), and for correcting through bioinformatic analysis the possible batch effect in microarray analysis (21).

Population

The observed population has intrinsic heterogeneity. The training and first validation set yielded cases of either early and late stage of disease, and of mixed histology, whereas TCGA validation set was composed of late stage high grade serous EOC. This difference raises some concerns on the appropriateness of comparing intrinsically different types of disease (22). For example, comparing the training set population, early stage EOCs were significantly more present in the low risk group (see appendix, pp 8). Questions should be raised whether a similar signature could be detectable repeating the analysis selecting Type II and high grade serous EOCs in the training set, as the authors presented in the manuscript as a sub-analysis for OC263 population.

Nevertheless, the identified MiROvaR signature still performed in the more homogeneous population dataset, such as OC452 (from TCGA). Namely, median PFS difference between high and low risk groups was 20 months for OC179, 22 months in OC263, and 4 months in OC452.

Prediction power

As shown in the multivariate logistic regression analysis, the MiROvaR model appears able to independently discriminate the clinical outcome in all the tested populations, outperforming the classic clinical classifiers (stage of disease and residual disease after surgery). However, the ability of a biomarker assay is mainly measured over its accuracy in classifying high and low risk population. Overall, the ROC curves as a measure of MiROvaR model classifier capability generated a limited area under the curve in the different datasets with the best performance obtained in OC263, with an area under the curve (AUC) of 0.72±0.01 (representing standard deviation). This performance was not confirmed among datasets, with the lowest discrimination ability in OC452 validation set (AUC: 0.58±0.02). As such, at this level the test is still not helpful to guide clinical decision making, especially in homogeneous populations, such as TCGA dataset.

The reasons for the different performance of the test go beyond the scope of this comment, however it is possible to question whether the integration of a broader set of non-coding biomarkers (23) or synchronous gene expression analyses (3) could have better described the highly heterogeneous EOC population (24).

Biology

One problem related to miRNAs expression and its correlation to biology is that same miRNAs are independently regulating different pathways and so far we are not able to fully understand the meaning of miRs expression with no other transcriptional data. Of extreme interest would be to corroborate the MiROvaR signature with correlations with the gene expression profiling of the samples, gaining insight on the molecular basis that underlies EOC biology.

For what we already know, consistent literature shows that OC miRNA prognostic signatures' are deeply related to a deregulated activation of biologic checkpoints related to hallmarks of cancer. As relevant examples, miR-506 is known to play a key role as a suppressor to tumor proliferation (25) and reducing chemosensitivity (26), and its loss leads to activation of epithelial-to-mesenchymal transition (EMT) pathway (27); otherwise, miR200 family has relevant role in controlling the EMT (28) and has been linked to EOC in several reports (10,29). Yet, biologic correlation is still on the verge of understanding, it needs to be deeply investigated and multi-platform assays are needed to unravel EOC.

In conclusion, in a period of cancer research in which the advent of next generation sequencing is driving the main conclusions for selecting prognostic and diagnostic biomarkers, this paper demonstrates once more the importance of expression studies, in particular on the non-coding area of the genome to provide information of tumor progression and relapse. As the authors themselves correctly pointed out, this study requires further prospective validation to before entering into the clinical routine.

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Footnote

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