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EDITORIAL

Molecular Subtypes of High-Grade Serous Ovarian Cancer: The Holy Grail?

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With the development of robust genomic platforms and extensive genetic profiling of tumors, the identification of previously unrecognized cancer subtypes has become a reality. Molecular subtypes can reflect important biology, developmental origins, and most importantly have clinical utility. While genomic subtyping efforts have rapidly produced results for some cancers, the identification of molecular subtypes has been difficult for high-grade serous ovarian cancer (HSOC). What makes subtyping so difficult? The Cancer Genome Atlas (TCGA) (1) showed that in HSOC, hundreds of genes are affected by recurrent focal copy number and promoter methylation events, as well as by a small number of recurrent somatic short variant mutations. These extensive genetic abnormalities are likely due to a profound abnormality in DNA repair, resulting in genomic chaos and, in addition to the recurrent driver events, a large numbers of passenger events. It is predictable that HSOC would be genetically plastic with rapid evolution during the disease course, with extensive heterogeneity at the time of initial diagnosis. This would make the identification of specific tumor subtypes particularly challenging. In the face of such complexity, patients can be grouped based on combinations of genomic or epigenetic events in an almost arbitrary number of ways.

So how can high-throughput transcriptomic data help in identifying clinically relevant subtypes? First, it can identify groups of patients whose disparate genomic events have similar expression footprints. Second, it can help to prioritize alterations with strong expression phenotype over ones with only small effect on gene expression. Unsupervised clustering of transcriptome data organizes tumors into discrete groups based on these two criteria, and it is important to acknowledge that this process will almost always succeed in identifying clusters discovery or even random data (2).

In HSOC, the first study to report subtypes was the Australian Ovarian Cancer Study (AOCS) (3). This unsupervised microarray-based effort analyzed a cohort of tumors of mixed histology, mixed tumor grade, mixed sampling locations, and variable amounts of stroma. The Cancer Genome Atlas (TCGA) later reported largely overlapping subtypes and titled these "immunoreactive," "differentiated," "proliferative," and "mesenchymal" but was unable to show any difference in clinical outcome between the subtypes. Independent of these efforts, other contemporaneous large array–based studies of high-grade advanced stage serous ovarian cancers generated prognostic signatures, but could not describe any molecular subtypes (4,5). Thus, the robustness of these subtypes has remained controversial.

In this issue of the Journal, Konecny and colleagues (6) present more evidence for the existence and survival association of four HSOC molecular subtypes as proposed by TCGA, in a new microarray dataset of 174 patients with clinical follow-up at the Mayo clinic. Consistent with a recent meta-analysis by Verhaak et al. (7), the authors convincingly demonstrated that patients classified as "immunoreactive" have on average best prognosis, whereas the "mesenchymal" subtype is associated with poor outcome, with an adjusted hazard ratio (HR) comparing these two groups of 1.84 (95% confidence interval [CI] = 1.15 to 2.94, $P = .01$). A difference in patient survival reinforces that subtypes exist, because differences in overall survival are expected to originate from key biological distinctions. The authors also developed a novel subtyping system for HSOC based on their Mayo cohort expression data. Robust clustering utilizing the 1850 genes with highest variability in the Mayo cohort was observed for subgroupings into two, three, and four different subtypes, but only a subgrouping into four subtypes, very similar to the TCGA subtypes, produced a classification system with prognostic relevance in this discovery cohort (adjusted HR for immunoreactive vs mesenchymal of 2.45 [95% CI = 1.43 to 4.18], $P = .001$). Sixty-eight percent of patients from the Mayo cohort were classified equally when comparing the TCGA and the Mayo subtypes. Among the genes differentially expressed in the immunoreactive subtypes of both classification systems were *B7-H1* and *IRF7*, and the authors speculate that immune modulatory antibodies may show clinical efficacy in this subtype. Reproducibility and prognostic relevance of the subtypes was then demonstrated in a microarray cohort published by Bonome et al (4). Compared with the TCGA subtypes, 56% of patients were classified into the corresponding Mayo subtypes. Survival association of the Mayo subtypes was slightly higher compared with TCGA subtypes in the Bonome validation cohort.

While these results are encouraging, clinical translation of HSOC subtypes is likely still far away. Inferring from the more extensive literature on transcriptome subtyping of breast cancer, even for well-established subtypes, there is a lot of work between initial clustering (8) and defining a robust single-patient tool such as PAM50 (9) that is externally validated (10,11). The present and previous studies make clear how difficult it is in HSOC to unambiguously assign single patients to subtypes. Verhaak and colleagues showed that most tumors exhibited properties of multiple TCGA subtypes, and that every possible combination of subtypes existed within at least one TCGA patient. Konecny and colleagues

confirmed this finding in the Mayo and TCGA cohorts, in which 42% and 82%, respectively, of all patients were assigned to at least two subtypes. Complexity of subtypes might arise within a molecularly homogenous tumor, when a tumor displays characteristics from multiple subtypes, or when a single tumor consists of multiple subclones with distinct genotypes and phenotypes. In a comparative deep-sequencing study, Lohr et al. (12) estimated that 95% of ovarian cancer tumors display clonal heterogeneity (more than one subclone), and many had four or more subclones. Thus, it is unclear whether the observation that most ovarian tumors exhibit properties of multiple subtypes occurs within or between subclones of a tumor. While the fact that combinations of subtypes are common does not necessarily prohibit diagnostic usage, such a complexity certainly represents a challenge for the necessary prospective validation studies. One approach to simplification is to define a supervised score based on signatures of intrinsic subtypes, such as the PAM50 Risk of Recurrence (9) for breast cancer. In HSOC, such a score may turn out to be related to several validated immune response–associated prognostic signatures (13) or the suboptimal debulking score recently published in this journal (14).

For molecular subtyping to be useful, it should have clinical utility, meaning it guides clinicians in treatment choices. No molecular signature or subtype of ovarian cancer has yet had such influence. The treatment of ovarian cancer has limited alternatives, and the up-front therapy has not substantially changed since the GOG111 approval of Taxol nearly two decades ago. It is not clear that any of the proposed tumor subtypes will be targetable by available small-molecule inhibitors, and none identify actionable prognostic groups such as refractory (progression or persistent disease to initial chemotherapy) or suboptimally debulked cases. If these patient subgroups could be predicted, they could be triaged to phase II trials or treated with neoadjuvant therapy, respectively.

The present classification systems may help future research efforts by grouping patients into more homogenous subpopulations and supplementing prognostic and predictive models. However, if ovarian cancer subtypes do not result in clinical translation, one may wonder whether unsupervised clustering of expression data alone is still the best tool to identify clinically relevant subtypes. If the prediction of Lohr et al. is correct and subclones are the norm in HSOC, then only early driver events will be effectively treatable: later events are more likely to be subclone specific, and targeting one subclone will only allow other subclones to take its place (15). We are not aware of any work that establishes whether clustering of cancer expression data is inherently biased towards late events, but drastic expression changes are certainly more likely tolerated after certain hallmarks of cancer (16) are acquired. Future subtyping efforts might need to consider a more supervised approach that takes into account order of events in tumorogenesis and known biology of promising treatment options. We look forward to the outcome.

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