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European Association of Urology

Letter to the Editor

Re: Tuan Zea Tan, Mathieu Rouanne, Kien Thiam Tan, Ruby Yun-Ju Huang, Jean-Paul Thiery. Molecular Subtypes of Urothelial Bladder Cancer: Results from a Meta-cohort Analysis of 2411 Tumors. Eur Urol 2019;75:423–32

Tan and colleagues [1] combined publicly available microarray data sets for gene expression in bladder cancer to redefine molecular subtypes. Here we present three arguments why the merging of data sets and the derived tumour classification are problematic.

First, data from different cohorts and different technological platforms or protocols are subject to various biases, such as clinical and biological characteristics, sampling conditions, and technical variations. Adjustment of batch effects before combining data sets supposes that sources of biases are well identified, properly characterized on a per sample basis, and do not strongly overlap [2]. These conditions are only rarely verified in practice. Adjusting data while violating these conditions will corrupt the signal. The ComBat adjustment described in Supplementary Figure 2 of Tan et al. [1] does not address the problem that technical biases are entwined with clinical and biological cohort differences. When faced with numerous and poorly characterized source of biases, combining data should be avoided.

Second, the authors derive a nearest-centroid classifier used to classify external RNA sequencing data. The classifier could have been internally applied to the original contributing data sets. Sample-by-sample reclassification concordance would then either be high for all cohorts, varying between cohorts, or low for all cohorts. This would have ensured internal validity by answering the question: Did batch effects and ComBat cause changes in classification? We reclassified three quantile-normalized centered data sets in isolation using the published centroids [1], which resulted in subtype proportions that clearly deviated from those shown in Supplementary Figure 3D [1]. In our opinion, internal validation should have been systematically performed and the reclassification concordance of each data set should have been presented.

Third, the final subtypes are influenced by technical factors. As seen in Supplementary Figure 3D [1], the distribution of formalin-fixed, paraffin-embedded (FFPE) samples is skewed (2×6 χ^2 test of independence, $p < 2.2e-16$), especially for the "neural" subtype, with 77% FFPE samples, and the "Lum" subtype, with only 7%. The FFPE-low "Lum" subtype was instead enriched for Illumina-HT12 platforms (89%, compared to 20–69% in the other subtypes; χ^2 $p < 2.2e-16$). Two FFPE studies contributed significantly to the "neural" subtype: GSE39016 and GSE57933. Neither study used selection criteria that explain an enrichment of "neural" tumours. In fact, the cohorts were described as just "bladder carcinoma" and "muscle-invasive and/or node-positive bladder cancer". In our experience, molecular subtype proportions are stable between studies despite different patient populations. Thus, we do not consider de facto enrichment of truly "neural" tumours in these studies to be a likely explanation. It seems more likely that remaining batch effects, or the ComBat adjustment itself, caused a difference in profile between FFPE and fresh-frozen samples in the merged data set. While this may seem innocuous, the end result is the publication of FFPE-associated molecular subtypes.

Considering our relatively good knowledge about bladder cancer subtypes, a consensus study would probably represent a slight refinement of existing classes rather than discovery of new subtype structures. Refinement of existing results puts high demands on the precision and quality of data. Results lacking such precision will not improve classification, despite being based on many samples.

Conflicts of interest: The authors have nothing to disclose.

References

[1] Tan TZ, Rouanne M, Tan KT, Huang RY, Thiery JP. Molecular subtypes of urothelial bladder cancer: results from a meta-cohort analysis of 2411 tumors. Eur Urol 2019;75:423–32.

[2] Leek JT, Scharpf RB, Bravo HC, et al. Tackling the widespread and critical impact of batch effects in high-throughput data. Nat Rev Genet 2010;11:733–9.

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