Commentary

Epi-drivers and cancer-testis genes

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Wang *et al*. (1) reported new candidate cancer drivers by studying cancer-testis expression patterns in large cohorts of normal and cancer samples. The authors assumed that few cancer can be fully explained by the mutation drivers (mut-drivers) and thus they focused on studying epigenetic drivers (epi-drivers); genes that are altered by epigenetic mechanisms and confer selective growth advantage (2). The authors reasoned that since epi-drivers are aberrantly expressed in cancer due to underlying epigenetic alterations, it is possible to search for epi-drivers by using transcriptomics data. This is a very tempting idea since little is known about epi-drivers and their role in cancer is relatively poorly understood as compared to mut-drivers. However, epigenetics mechanisms alone do not fully explain altered gene expression, which can be caused by multiple factors such as mutations in promoter regions, DNA copy number alterations, mutations of the up-stream transcription factor or changes in signaling. For example in our recent transcriptome study of genes de-regulated and activated across multiple cancer types (3) we opted for not calling the broadly de-regulated genes as "epi-drivers". Interestingly, Wang *et al*. regarded *MEIOB* (meiosis specific with OB domains) to be an epi-driver, while also noting that its expression is correlated with (and probably driven by) arm level and focal DNA copy number alterations (CNAs). While *MEIOB* is a reported cancer driver (4,5) that is differentially expressed in cancer, there is no epigenetic data to regard *MEIOB* as an epi-driver.

Wang *et al*. did in fact attempt to associate the differential expression of cancer testis genes with underlying epigenetic changes by integrating DNA methylation data. The first analysis is presented an "enrichment" of demethylation sites within testis specific regulatory elements, which, as authors note, is "consistent with conventional knowledge of *CT* gene activation" and is of limited novelty to the field. In second, more detailed analysis, the authors tested their *Extremely highly Expressed CT* genes (EECTGs) for promoter demethylation. *EECTG*s are a term introduced by the authors for cancer-testis gene that show "extreme expression" according to their methodology. In their analysis, they confirmed two EECTGs (*RHOXF1* and *VCX3B*) to be reactivated by promoter demethylation, while finding no such demethylation for other tested EECTGs (*LIN28B*, *MEIOB* and *SPATA22*). Wang *et al*. suggested that EECTGs are potential epi-drivers, but this is not supported by their results. One can guess a fraction of EECTGs are reactivated by demethylation, because it is a well-known mechanism of cancer-testis gene re-activation, but in general EECTGs should not be proposed to be epi-drivers without supporting epigenetic data.

It is also not clear if cancer-testis expression pattern—the main focus of the paper—helps to prioritize the search of epi-driver candidates. First, many true epi-drivers may not show testis-specific expression. Second, epigenetic drivers are not limited to gene expression changes and can act by increasing C-to-T mutations, genome instability or activation of repetitive elements (6). In general, we believe that identification of epi-driver should be primarily based on epigenomic data whereas gene expression analyses can only indicate the mechanism of action of an epi-driver, such as aberrant gene expression or other ways mentioned above.

We would also like to comment on the choice to select/prioritize targets solely based on "extreme expression" in cancers without considering the expression in adjacent

normal tissues. The authors stated that: "*Because adjacent cancer tissues* (we assume the author meant "cancer-adjacent normal tissue") *usually do not express CT genes, it was challenging to determine activated samples individually*." Our opinion differs and we propose that normal tissue should be used as a "control" where CT genes should not be expressed. This would allow for discovery of highly cancer specific genes. In other words, we would prioritize cancer specific expression over "extreme expression" in cancer. In fact, Organ-Specific Controls data is available from TCGA. However, we see a merit in "extreme expression" criterion, as the genes highly expressed in cancer can potentially be easier to target.

In addition, in supplementary methods the authors stated: "*In our LUAD validation, because the expression of MEIOB of sample 130717001 approaches the extremely-high expression criteria and its co-factor SPATA22 is validated, we consider it as a validated EECTP and include it in the further functional assay*", which makes us believe that the main focus gene of the study, *MEIOB*, was chosen primarily based on prior knowledge and reports (4,5) rather than strictly on the proposed methodology. Unfortunately, this contradicts the methodology as a way to identify new potential cancer drivers.

Wang *et al*. started from the assumption that few cancers can be fully explained by driver mutations and thus epi-drivers may provide an explanation for the unexplained. While we believe that there is a lot of potential in studying epi-drivers, the tremendous progress in the field of mutational cancer drivers should not be overlooked. The oncogenic pathway can be affected by mutations in any of the multiple genes in the pathway. Thus the frequencies of pathway alterations are much higher than single mut-drivers. In a pan-cancer TCGA paper, Ciriello *at al*. (7) showed that driver genes can be affected by mutation, copy number alteration or change at DNA methylation, and that while frequency of alteration of a single gene is often low (long tail distribution) the frequency of a pathway alteration (alteration of any gene in a pathway) is much higher, up to 100% depending on cancer type. In fact, Wang *et al*. reported that the frequency of activation of EECTGs was also quite low (maximum 7%), which is much lower than mutation frequencies of some mut-drivers like TP53, PIK3CA or PTEN. In our opinion, this shows that there are no perfect targets or biomarker that drive or are expressed in all tumors. Rather, various alterations such as mutations, copy number, transcriptional or epigenetic changes are present in a fraction of cancers and their frequencies follow long tail distribution, with many alterations present only in a small fraction of tumors.

The authors suggested that discovery of new cancer testis genes can help finding new biomarkers and therapeutic targets. However, they also noted that the clinical experiments aiming for developing anticancer vaccines targeting well-known cancer testis antigens have consistently failed. We believe that the well-known and most frequently expressed cancer testis antigens (e.g., MAGEA3 or PRAME) are more likely to become effective targets than novel, but probably rarely activated cancer testis genes. Thus the main challenge remains to translate the existing knowledge into improved clinical outcomes. As authors noted, previous trials may have failed because of low frequency of CT activation, thus more personalized approach of testing individual tumors for CT expression can help improve the efficacy of the vaccines.

Alternatively, there is a competing approach based on targeting neo-antigens, also called tumor-specific mutant antigens or private antigens. Interestingly, recent reports show that both driver and passenger mutations can be targeted as long as the expression of the mutated gene produces an immunogenic protein product (8). This is in contrast to the long-standing assumption that driver genes are optimal therapeutic targets. Neo-antigens are a very promising and active field of research that greatly benefits from large mutational studies. In comparison, the research on cancer testis antigens has been slowing down. However, targeting neo-antigens involves exome sequencing and custom design, which requires more time and resources. If the challenges of targeting cancer testis antigens are overcome, CTAs can be targeted by more generic vaccines and used for cancers with low mutational load (9).

Another point raised by the authors is the presence of the cancer-testis non-coding RNAs (CT-ncRNAs). This is largely unexplored field as cancer testis genes were previously mostly considered as protein coding genes (cancer-testis antigens, CTAs). Previously we have observed the activation of non-coding RNAs in liver cancer that were normally expressed in testis and placenta (10), so we were glad to see more research in this space. We hope that more studies of the ncRNAs expression can further explain the uncanny link between cancer and testis biology and bring new idea for RNA level therapies that will hopefully be more successful than protein level approaches used for cancer testis genes so far.

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Footnote

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