Review of: "Loss of polycomb repressive complex 1 activity and chromosomal instability drive uveal melanoma progression"

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Does progression of uveal melanoma depend on loss of polycomb repressive complex 1 activity and chromosomal instability and is risk a continuous feature that depends on the admixture of cells with different molecular lesions?

Uveal melanoma (UM) is a rare cancer and it is very different under many aspects from its cutaneous counterpart [1,2]. Unfortunately, UM has not benefitted from the dramatic improvement in melanoma therapy since it only weakly responds to targeted therapies [3] and to immunotherapy [4]. Survival after diagnosis of metastatic UM has not much changed over decades [5]. UM is perhaps the cancer with the most accurate prognostic procedures: gene expression profiling [6], copy number alterations [7] and DNA methylation analysis [8] allow for a very reliable classification according to a risk that cannot significantly be lowered through therapy. There is, however, some silver lining, such as T-cell redirection [9], anti-LAG3 immune checkpoint blockers [10–13] and drugs targeting the co-driving YAP/TAZ pathway [14]. Yet deepening our understanding of UM is still needed in order to uncover unthought of therapy targets.
The article “Loss of polycomb repressive complex 1 activity and chromosomal instability drive uveal melanoma progression” [15] by Mathiue F. Bakhoum ad co-workers applies single cell transcriptomics (sc-transcriptomics) to six primary UMs in order to detect tumor heterogeneity. In accordance with a recent study [11] ad a case report [16] that applied the same technique, the authors convincingly show that UM heterogeneity is much higher than previously expected. Low heterogeneity was expected since whatever is analyzed, low and high-risk UM yield very different patterns with high concordance within the risk class. Sc-transcriptomics identifies and confirms the known molecular classes, the two classes defined by gene expression profiling based prognostic procedures (GEP class 1 and 2) [17] widely applied in the clinics [6], the classes commonly defined by cytogenetics (monosomy of chromosome 3, chr8q and chr6p gain and their combinations) [7], the three classes revealed by somatic mutation screening (BAP1, SF3B1, none of the two) [18] as well as the four classes identified by integrative genomics [8]. But importantly, sc-transcriptomics shows that these classes co-exist in five of 6 cases analyzed that are distinguished by the differential admixture of the classes. UM progression appears to be a continuum in the transition from low to high risk.

The paper also investigates into the consequences of BAP1 mutations, a tumor suppressor gene [19]. In high risk UM, one of the two copies is lost through monosomy of chr3 and the other copy undergoes mutations that abolish its function as a deubiquitinase [20], nuclear localization or expression of the protein through missense, frameshift or splice site mutations [21]. Unexpectedly, BAP1 mutated UM showed lower levels of ubiquitination of the lysine residue 119 in histone 2A (H2AK119) and Bakhoum and coworkers identifed the polycomb repressive complex 1 (PRC1) as a major factor of tumor progression. PRC1 mediates the ubiquitination of the H2AK119 and is therefore in direct competition with BAP1. Yet the components of the PRC1 complex are apparently silenced during UM progression and its inhibition by a drug induces features of high-risk UM in a low risk cell line. The authors suggest that reduced expression of PRC1 induces a progression prone chromatin modification, transcriptional signatures, inflammatory response programs and aneuploidy. The latter is based on evidence that inhibition of PRC1 induces chromosome instability (CIN) in cell lines, yet it is not clear how this relates to the relatively few chromosomal alterations found in high risk UM. Perhaps PRC1 expression loss determines a big bang [22] from which only a low number of combinations of copy number alterations (CNA) emerge as viable clones. Yet the question arises why not more bystander CNAs occur in high risk UM. Chr3 monosomy and chr8q gain are clearly associated with high risk in UM and other CNAs are frequent, yet CIN awaits to be established for UM.

The paper yields deep insight into the mechanisms of UM progression but the work has some limitations. The analysis was performed on just six UM samples. Four were classified as high risk (gene expression class 2 signature) and the remaining two were classified as low risk (GEP class 1A and 1B). There is some doubt concerning the selection of cases: it is unclear why UM1 (GEP2, BAP1 mutated, CNA = NA, no metastasis, 7 months follow-up), certainly not a typical case with very short follow-up, has been included. UM06 was classified as GEP class 1A despite chr3 monosomy, chr8q gain, BAP1 truncation mutation. To little surprise, this cancer actually developed metastases and sc-transcriptomics showed a prevalence of GEP-2 features. The misclassification evidently occurred at the level of bulk transcriptomics showing the limits of this technique for prognostication. The main part of the paper is based on the analysis of just two cell lines and only one *bona fide* low risk case. Given the complex nature of regulation of chromatin compaction and gene transcription and their effects on the metastatic potential it would have been useful to validate the data on a panel of cell lines rather than on just two of them. This is particularly important for UM since UM cell lines only partially reflect the situation in human tumors. Most of the cell lines, in contrast to high risk UM tumors, show chr3 disomy or isodisomy and primary cultures of UM with chr3 monosomy most often end up by generating disomic cell lines (our unpublished observation that also indicates the presence of different populations in a single UM). As a matter of fact, the 92.1 cell line is near tetraploid with three copies of chromosome 3 that contain wild type sequences of BAP1 [22], MP38 shows loss of chr3p and uniparental disomy of chr3, no gain of chr8q, deletion mutation in BAP1 [23]. The findings reported by Bakhoum and colleagues would certainly have gained weight if they had been confirmed in a larger panel of UM cell lines.

The role of BAP1 mutation, perhaps together with chr3 monosomy the molecular event with the highest impact on metastatic risk, remains unclear and its relation to PRC1 activity to be defined. The claim that UM risk classes constitute a continuum determined by the clonal composition of more or less cells with high risk features appears well underpinned by sc-transcriptomics that yield composite risk features for 5 of 6 UM tested. However, it is unclear how this continuum can give rise to so exceptionally clear-cut distinction obtained by bulk transcriptomics, methylomics and CNA analysis. Bulk techniques are expected to loose tiny admixtures yet the continuum of different ratios of low and high risk cells should yield a continuum also at the bulk level. It appears therefore a little early to completely dismiss the hypothesis of two independent progression paths of respectively low and high risk. Extension of these analyses to many more samples will likely bring clarity.

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