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PROSTATE CANCER

Analysis of progression through time and space

A new study shows that paired evaluation of single cells and cell clusters in peripheral blood samples (PBS) and bone marrow aspirates (BMA) from patients with prostate cancer offers new possibilities to characterize disease progression and aid treatment planning. The methodology enables genomic and proteomic analysis on a single-cell level and provides an uncomplicated liquid-biopsy approach for monitoring metastasis.

"The spatiotemporal evolution of prostate cancer in patients is critically important for our understanding of the biology of this disease and our ability to provide scientific input into future treatment strategies," explains Peter Kuhn from the University of Southern California, Los Angeles, USA, corresponding author of the study. "Our goal is to provide the scientific evidence that supports the use of temporally resolved liquid biopsies of blood and bone marrow as a data continuum for the clinical management of the disease continuum."

The research team extended their high-definition single-cell analysis platform for use with BMA and analysed prospectively collected, matched PBS and BMA from 141 men with prostate cancer of different stages: 52 men with biochemical relapse after definitive therapy but without metastases (group 1); 26 men with newly diagnosed metastatic, hormone-naive (castration-sensitive) disease (group 2); and 63 men with metastatic castration-resistant disease (mCRPC; group 3). Immunohistochemical assessment consisted of cytokeratin 19 and pancytokeratin staining to mark cells of epithelial (therefore, presumably tumour) origin, CD45 staining to enable exclusion of leukocytes and androgen receptor (AR) staining.

For PBS, more samples from group 1 than group 2 contained ≥1 tumour cell (24% versus 18%, respectively), but the mean concentration of cells was higher in group 2. Men in group 3 had the highest prevalence of tumour cells in PBS (33%). For BMA, no samples in group 1, 26% in group 2 and 39% in group 3 were positive for tumour cells. The researchers also analysed bone marrow core biopsy samples and found no tumour cells in group 1, and decreased prevalence in core biopsies compared with BMA in group 2 and group 3 (13% and 30%, respectively). As cell clusters might be more relevant to metastasis than single cells, the team then evaluated cluster presence in PBS and BMA and found increasing prevalence with increasing disease stage; clusters were more abundant and larger in BMA than in PBS.

Investigation of AR staining showed a positive correlation between AR expression and cluster size in PBS and BMA in men with mCRPC, but not in those who had not received hormonal treatments (group 1 and group 2). In addition, manual classification of AR expression in 10 patients with matched PBS and BMA with ≥1 tumour cell showed that the proportions of cells that were AR^+ and AR^- were similar for PBS and BMA, indicating that tumour cells in PBS might be adequate surrogates for cells in metastatic deposits. However, when the team evaluated whole-genome copy number variation in 3 of these 10 patients they found differing clonal patterns and distribution between PBS and BMA, suggesting high genomic variation in and between patients.

Finally, the group performed survival analysis for men with mCRPC (progression rates during follow-up periods for group 1 and group 2 were too small). Presence of tumour cells in PBS, and also in BMA, from these patients was prognostic for decreased progression-free and overall survival. In those with tumour-cell-positive BMA, a high proportion of cell clusters indicated the shortest progression-free and overall survival.

"We now have data supporting that prostate cancer's primary spread to the bone likely uses blood as a key intermediary, allowing us to characterize the cancer across its temporal evolution using both PBS and BMA," highlights Kuhn. "Our method shows that cells from both compartments are prognostic and can be genomically characterized. The findings are a milestone supporting the next steps for deep genomic and proteomic characterization of single cells across the spatiotemporal continuum in prostate cancer."

Clemens Thoma

ORIGINAL ARTICLE Kuhn, P. *et al.* Paired high-content analysis of prostate cancer cells in bone marrow and blood characterizes increased androgen receptor expression in tumor cell clusters. *Clin. Cancer. Res.* http://dx.doi.org/10.1158/1078-0432.CCR-16-1355 (2016)