


1. BIOLOGY AND PRECLINICAL

# TRANSCRIPTOMIC PROFILING OF CIRCULATING TUMOR CELLS (CTCS) IN MULTIPLE MYELOMA REVEALS EGRESSION-ASSOCIATED PROFILE RELATED TO CTC BURDEN

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**Background.** CTCs have emerged as an important prognostic factor in newly diagnosed (ND) MM. However, the mechanisms of plasma cell (PC) egression from the bone marrow (BM) to peripheral blood (PB) remain poorly understood. Transcriptomic comparisons of BM plasma cells (BMPCs) and CTCs have been limited by low CTC levels precluding successful isolation of sufficient number of PCs. In this work, we address this gap by providing insight from the transcriptional profiling of CTCs across various levels of CTCs, including very low infiltration.

**Methods.** PB samples from 54 NDMM/primary plasma cell leukemia patients were analyzed by next-generation flow cytometry (EuroFlow protocol; median LOD=0.0004) and stratified into logarithmic groups: 9 patients (100–10%), 9 (10–1%), 16 (1–0.1%), 9 (0.1–0.01%), and 11 (0.01–0.001%). Paired CTCs and BMPCs were sorted either by FACS or combined MACS/FACS to obtain sufficient cells from low-CTC cases. Differential expression was analyzed with DESeq2. To derive a CTC-like score, top 50 genes upregulated in CTCs were weighted by log2FC and –log10(padj). Genomic and transcriptomic data from 635 NDMM patients in the CoMMpass dataset were used to assess associations of the CTC-like score with progression-free survival (PFS), and cytogenetic features by linear regression. All p-values were Benjamini-Hochberg adjusted.

**Results.** We identified 2990 differentially expressed genes (DEGs), including 124 up- and 53 downregulated with absolute log2FC>1 in CTCs compared to their BM counterparts. Proliferation-related genes and signatures (e.g., PR score; Zhan et al., 2006) were lower in CTCs ($p<0.001$) and showed lower expression of genes from translation-related pathways, suggesting more quiescent state. Notably, key BM retention genes CXCR4 and CD138 had lower expression in CTCs (log2FC -0.83 and -0.49, respectively). CTC-upregulated genes included FLNA, TAGLN2, CD44 or EMP3, suggesting altered cytoskeletal dynamics and motility. Additionally, all 3 genes of the ANXA2-S100A10-AHNAK complex were upregulated in CTCs, linked to extracellular matrix remodeling and cytoskeletal dynamics. Importantly, the greatest expression differences of DEGs between BMPCs and CTCs were observed in samples with low CTC burden. As CTC burden increased, these differences progressively decreased, reflecting greater variability between BMPC samples across logarithmic CTC burden groups compared with the relatively lower variability observed in CTC samples. To investigate genomic alterations linked to CTC burden, we computed CTC-like score for CoMMpass samples. Higher scores were associated with gain/amp 1q, t(14;16), del 13q, and inversely with hyperdiploidy. These alterations were previously linked to elevated CTC numbers in CoMMpass and other datasets using CTC enumeration. Moreover, stratifying patients by median CTC-like score, the group with higher levels showed worse PFS (26 vs 42 months, $p=0.001$).

**Conclusions.** Using a paired BMPC-CTC transcriptomic dataset (N=54), we observed clear differences between the two populations that became more pronounced as CTC burden decreased. Compared with BM counterparts, CTCs exhibited reduced PR profile, and DEGs suggested altered cytoskeletal dynamics, extracellular matrix remodeling, and reduced BM homing as potential mechanisms underlying egress from the BM. Additionally, the derived CTC transcriptional score was positively associated with t(14;16), 1q gain, del 13q, and worse prognosis in CoMMpass dataset.

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