

Circulating Tumor Cells (CTCs) for Comprehensive and Multiregional Non-Invasive Genetic Characterization of Multiple Myeloma (MM)

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Introduction

Genetic characterization is becoming relevant to predict risk of progression in smoldering MM and is fundamental to estimate survival in active MM.¹⁻³ Thus, patients undergo multiple bone marrow (BM) aspirates for genetic screening that beyond painful, may not be fully representative due to patchy BM involvement, spatial genomic heterogeneity, or extramedullary disease.⁴ Accordingly, cell-free DNA has been investigated and showed high concordance with BM aspirates, but information is typically restricted to a few recurrent mutations since comprehensive genetic characterization (e.g., whole-exome sequencing, WES) is applicable to <25% of MM patients (those with ≥10% tumor DNA).⁵⁻⁷ By contrast, CTCs are detectable in virtually all smoldering and active MM patients and their numbers are prognostically relevant,⁸⁻¹⁰ but their applicability for non-invasive genetic characterization of MM has been poorly investigated.

Objective

To compare the genetic landscape of CTCs vs matched BM clonal plasma cells (PCs) and extramedullary (EM) plasmacytomas, and validate standardized assays for CTCs' detection, isolation and genetic characterization.

Methods

We used EuroFlow next-generation flow (NGF) cytometry¹⁰ to detect and isolate peripheral blood (PB) CTCs and matched BM clonal PCs from 51 MM patients (35 at diagnosis and 16 at relapse). In 8 cases, clonal PCs from EM plasmacytomas were also FACSorted. PB T cells were always used as matched germline control. In the training set, we performed custom WES (preceded by triplicates of whole-genome amplification)

in matched CTCs, BM and EM clonal PCs from the 8 patients with all three spatially distributed clones. Only those mutations present in 2/3 libraries analyzed per sample were considered positive. In the validation set, we compared mutations, copy number alterations (CNA) and translocations present in CTCs and BM clonal PCs using the Chromium Exome Solution for low DNA-input (n=8), and solely CNA using the Affymetrix CytoScan HD platform (n=35). Read mapping, variant and structural calling were performed with the *Multisample Exome* (DREAMgenics) and *longranger* (10X Genomics) pipelines. The *Chromosome Analysis Suite software* (Affymetrix) was used to analyze CNA. Only those mutations with $\geq 10\%$ variant allele frequency (VAF) and CNA larger than 1Mb were considered.

Results

In the training set, 212/246 (86%) and 256/295 (87%) of total mutations present in BM and EM clonal PCs, respectively, were detectable on CTCs. All MM recurrent mutations (e.g., *BRAF*) found in BM or EM clonal PCs were present in CTCs. Furthermore, up to 50 mutations were present in CTCs while undetectable in BM clonal PCs (n=52) or EM plasmacytomas (n=8). Together with 47 mutations detected in EM plasmacytomas and in CTCs but absent in BM clonal PCs, these results provide unprecedented evidence of spatial heterogeneity in MM, which could be partially captured by the genetic interrogation of CTCs.

After showing that CTCs harbor most mutations present in both medullary and extramedullary disease and even unveil mutations undetectable in single BM aspirates or individual EM plasmacytomas, we sought to evaluate the performance of standardized assays suitable to screen mutations and/or CNA from low cell numbers (i.e., CTCs). WES after molecular barcoding showed that 415/497 (83.5%) of total mutations, including all mutations located in genes commonly sequenced in cfDNA, were detectable on CTCs. The cancer cell fraction of private mutations was significantly lower than shared mutations (medians of 0.07 vs 0.64; $P = 2.2 \times 10^{-16}$), suggesting sub-clonality in their respective spatial regions. On the other hand, 46/63 (73%) total CNA (31/33 [93%] at the chromosome arm-level) and 6/14 (43%) translocations present in BM clonal PCs were detectable in CTCs. Using the Cytoscan HD platform, there was 100% concordance in CNA at the chromosomal arm-level between CTCs and BM clonal PCs. All mutations in *TP53* were detectable in CTCs. Furthermore, +1q, del(1p), del(17p) or t(4;14) were always detected in CTCs whenever present in BM clonal PCs (except for one case). Conversely, such comprehensive genetic characterization unveiled innumerable CNA (e.g., *MYC* amplification) and IgH translocations not tested by routine FISH panels.

Conclusions

Using two different standardized methods, we showed in the largest series in which CTCs were genetically characterized, that these are a reliable surrogate of MM patients' genetic landscape inside and outside the BM. Because NGF is broadly used, quantification, isolation and genetic characterization of CTCs may emerge as an optimal and standardized approach for non-invasive risk-stratification of MM patients.

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