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translational research

52P MULTIPARAMETER PLOIDY PROFILING: A POWERFUL TOOL TO INVESTIGATE THE GENOMICS OF DIPLOID TUMOR POPULATIONS

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Aim: Genomic intratumor heterogeneity is an increasingly recognized phenomenon in various solid tumors. However, most methods are not designed to resolve the complexity of mixed cell populations. We have therefore developed the method of Multiparameter Ploidy Profiling (MPP). By applying this technology on tumor specimens from non-small cell lung cancer (NSCLC) we aim to decipher and genomically investigate all tumor populations (sharing same ploidy) present in the tumor specimens.

Methods: MPP involves isolation of nuclei from tissues, multiparameter flow-sorting of tumor populations by DNA content and specific tumor markers, and profiling genomes using high resolution array comparative genomic hybridization (aCGH) and next generation sequencing (NGS). As some tumors are diploid by flow cytometry, the specific lineage marker TTF1 (NSCLC: adenocarcinomas) was used to separate these from normal cells. Fluorescence-in-situ hybridization (FISH) and immunohistochemistry (IHC) were performed on sorted nuclei and on whole tissue sections to validate results.

Results: MPP allowed sorting of different tumor populations within a single tumor specimen, while excluding non-neoplastic contamination in downstream analysis with aCGH and NGS. We separated three different tumor populations from a single primary NSCLC with a TTF1 multiparameter sort. We detected significant differences in copy numbers, e.g. a CDKN2A homozygous deletion only present in the diploid tumor population. Finally, we used population-specific genomic data to infer the clonal relationship and to postulate the evolution of these tumor populations within single tumor specimens.

Conclusions: Most importantly MPP enables the separation of diploid tumor populations from diploid, benign stromal cells that can serve as reference for genomic profiling studies, where germline DNA is otherwise not available. To our knowledge, we for the first time purifiied diploid tumor populations from normal cells in a multiparameter sort and genomically characterized them. We also demonstrated that the analysis of diploid tumor populations within a single biopsy is crucial for the understanding of the clonal composition and evolution of a given tumor.

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