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Insight into CTC diversity through single-cell analysis and CDX development

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Genomic analyses of CTCs may provide unique information on tumor heterogeneity, resistance mechanism, and dominant clones with dissemination potential. Our laboratory recently conducted two studies aimed at comparing the mutational diversity of individual CTCs and matched-metastasis biopsies in patients with advanced metastatic cancer. In a small cohort of 11 patients with metastatic castration-resistant prostate cancer (mCRPC) we performed whole-exome sequencing (WES) of individual CTCs with various epithelial-to-mesenchymal transition (EMT) phenotypes and examined both shared somatic mutations between CTCs and matched-metastasis biopsies, and CTC-private (exclusively detected in CTCs) mutations. CTC-private mutations undiagnosed in matched-metastasis biopsies were identified in both epithelial and non-epithelial CTCs and affected cytoskeleton, invasion, DNA repair, and cancer-driver genes. In seven *ALK* (*Anaplastic Lymphoma Kinase*)-rearranged non-small-cell lung cancer (NSCLC) patients treated by ALK-inhibitor crizotinib, shared and CTC-private mutations were examined in individual CTCs isolated at resistance by targeted PCR and NGS. In accordance with published data on resistance mechanisms to crizotinib, the analysis of CTC-private mutations highlighted the importance of bypass signaling events that are parallel to ALK pathways in resistance to crizotinib. Results from these two studies show that sequencing of individual CTCs can reveal undiagnosed mutations in matched-metastasis and provide a unique representation of metastasis mutational content that is otherwise inaccessible. We established and characterized four NSCLC (GR-CDXL1, GR-CDXL2, GR-CDXL3, GR-CDXL4) and one prostate cancer (GRCDXP1) CTC-derived explants (CDX). Four *in-vitro* CDX-derived cell lines were established from GR-CDXL1, GRCDXL3, GR-CDXL4 and GRCDXP1 starting from CDX. All cell lines express cancer stem cell markers. WES of CDXs, CDX-derived *in-vitro* cell lines, matched-tumor biopsies and single CTCs isolated at the time of CTC implantation revealed the important mutational diversity of CTCs but a limited number of genetic alterations associated with the tumorigenic activity of CTCs. Mutational trees reconstructing the phylogenic evolution of matched-tumor biopsies, CTCs, CDX and cell lines enabled the identification of clonal and sub-clonal mutations and dominant clones associated with the metastatic potential of CTCs. Our converging findings emphasize how CTC can improve our knowledge on the genetic features of CTCs that seed metastasis and serve an unmet need for optimal therapy selection and precision medicine.