ABSTRACT

Blood samples (10 ml) from CCA patients were processed with a novel single-CTC characterization approach (1) which includes:

- CTC-enrichment using a method based on cell size and deformability (Parisorx®)
- Staining with fluorescently-labeled antibodies against epithelial and leukocyte-specific markers
- Identification of epithelial CTCs (eCTC, expressing epithelial markers) and non-conventional CTCs (ncCTCs, lacking epithelial and leukocyte markers)
- Recovery of single CTCs with the DEPArray®
- Whole Genome Amplification and quality check using Ampli1 kit and Ampli1 QC kit
- mutational profiling using Ion AmpliSeq Cancer Hotspot Panel v2 and AmpliSeq somatic pipeline for variant calling
- copy number alteration (CNA) analysis using Ampli1 LowPass kit, plus unsupervised clustering and frequency alteration analyses.

RESULTS:

- Mutational profiling of 19 CTCs (from 6 patients) were analyzed by targeted sequencing of 50 cancer genes in ductal and ampulla of vater carcinomas.
- Mutational profiling of single-CTCs from CCA patients

CONCLUSIONS:

- Our results support the possibility of using CTC molecular characterization to identify both resistance mechanisms and patient-specific targets, thus opening the way for a shift in treatment management of CCA towards an innovative and personalized therapy.

METHODS

19 CTCs (from 6 patients) were analyzed by targeted sequencing of 50 cancer-associated genes. Most CTCs presented a unique mutational profile indicating high intra-patient heterogeneity. CTCs’ heterogeneity limits the applicability of this approach in patients with few CTCs (most mutations present in only 1 CTC). Nonetheless, in 1 patient presenting 9 CTCs (panel on the left), we identified 1 mutation in KIT shared by 7/9 CTCs, indicating it as a possible treatment target for this patient.

 RESULTS

Clustering analysis of CNA profiles from 88 single CTCs (collected from 23 patients).

CONCLUSIONS

Our results support the possibility of using CTC molecular characterization to identify both resistance mechanisms and patient-specific targets, thus opening the way for a shift in treatment management of CCA towards an innovative and personalized therapy.

REFERENCES