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Molecular characterization of circulating tumor cells in cholangiocarcinoma patients: A new tool for treatment management?

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ABSTRACT

BACKGROUND:
Cholangiocarcinoma (CCA) is a highly fatal disease mainly treated with standard chemotherapy, albeit with limited efficacy. New therapeutic options are greatly needed, but the use of targeted treatments is often prevented by the impossibility to obtain tissue biopsies for molecular characterization. Here, we propose the use of circulating tumor cells (CTCs) as an alternative source of tumor material to perform molecular characterization for the identification of novel therapeutic targets.

MATERIALS AND METHODS:
Blood samples (10ml) from patients with advanced CCA were processed for CTC isolation as follows:
-CTC enrichment with Parsortix
-identification and single-cell recovery of epithelial CTCs (expressing epithelial markers) and non-conventional CTCs (lacking epithelial and leukocyte markers) using 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:
We analyzed 88 single CTCs isolated from 38 blood samples longitudinally collected from 23 patients (12 with intrahepatic, 9 with extrahepatic CCA and 2 with gallbladder cancer). CNA profiles showed a high level of both inter- and intra-patient heterogeneity, with each CTC displaying a unique profile. Intra-patient heterogeneity was further confirmed by clustering analysis as, in most cases, CTCs from the same patient clustered independently. CTC clustering was also not affected by sampling time (before/during chemotherapy), nor by the anatomical location of primary tumor. Conversely, we observed an enrichment of CTCs derived from patients non-responding to therapy (showing a PD according to RECIST criteria) in 2 of the 4 identified clusters (p=0.00041). By pair wise comparison of CNAs among clusters, we identified 2 regions more frequently altered in one cluster enriched for CTCs from non-responders: 10q22.2 and 3p11.1. The latter encodes, among others, for EPHA3, a targetable gene whose involvement in chemoresistance will be investigated by in vitro studies.

Mutational profiling of 19 CTCs (from 6 patients) also confirmed the high intra-patient heterogeneity with most mutations being present in only 1 CTC. This limits the applicability of this approach in patients with few CTCs. Nonetheless, in 1 patient presenting 9 CTCs, we identified 1 mutation in KIT shared by 7/9 CTCs, indicating it as a possible treatment target for this patient.

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.

OBJECTIVES

Premise: We developed a protocol for the detection of a novel CTC subpopulation (1) that allows an increase in CTC-positivity from 19% to 83% in CCA patients (2).

Here, we propose the use of CTCs as an alternative source of tumor material to perform molecular characterization for the identification of novel therapeutic targets in CCA treatment management.

METHODS

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 (Parsortix)
- 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 WGA kit and Ampli1 QC kit
- Targeted sequencing of 50 cancer-associated genes (Ion AmpliSeq Cancer Hotspot Panel v2 and AmpliSeq somatic pipeline for variant calling)
- Copy Number Alteration (CNA) analysis using Ampli1 LowPass kit for lowpass WGS, followed by unsupervised clustering and comparison of alteration frequencies

RESULTS

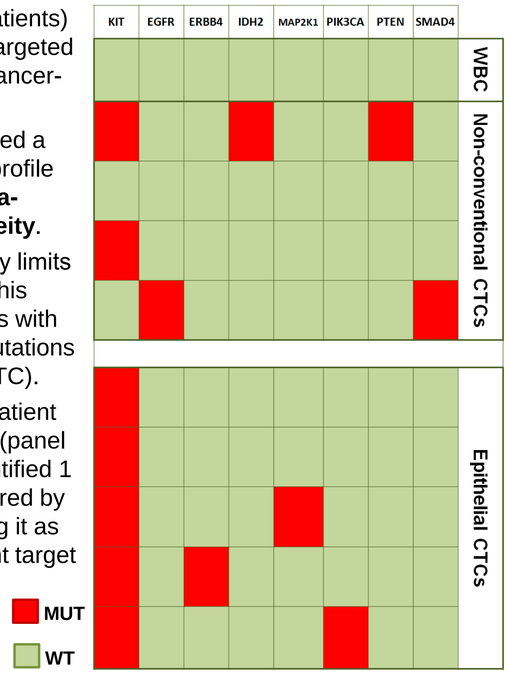
Mutational profiling of single-CTCs from CCA patients

19 CTCs (from 6 patients) were analyzed by targeted sequencing of 50 cancer-associated genes.

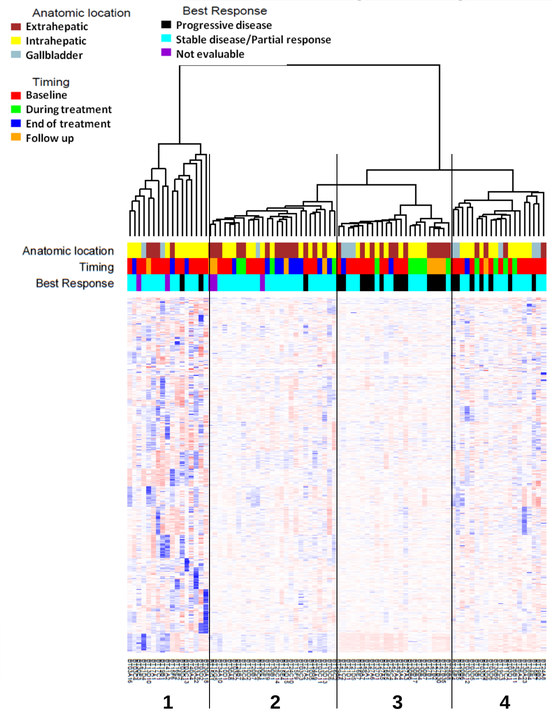
Most CTCs presented a unique mutational profile indicating **high intra-patient heterogeneity**.

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.



CNA profiling of single-CTCs from CCA patients



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

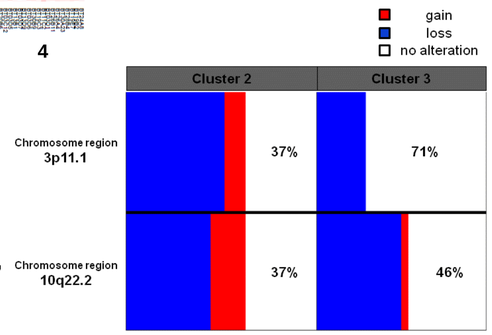
CNA analysis confirmed a high level of inter-/intra- patient heterogeneity.

Four clusters were identified and no association with anatomic location and sampling time was observed.

Conversely, we observed an enrichment of CTCs derived from non-responding patients (i.e. patients whose best response was PD) in clusters 3 and 4 (p=0.00041).

Pair wise comparison of CNAs among clusters allowed the identification of 2 chromosome regions more frequently altered in CTCs included in cluster 3 (enriched in CTCs from non-responders) than in cluster 2.

Region 3p11.1 encodes, among others, for *EPHA3,* a targetable gene possibly involved in chemoresistance (3).



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

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