Abstract:

Background:

Despite the overall decreasing incidence, colorectal cancer (CRC) still represents one of the most common malignancies with a high mortality rate. Introducing liquid biopsy into real-world clinical routine would increase individualized actionable information that physicians could leverage before and during CRC treatment. In other malignancy types, we have been observing increasing trend in using liquid biopsy, specifically circulating tumor DNA (ctDNA), in various use cases, which may bring future benefits to CRC patients as well.

Methods:

We have conducted two investigations. In both cases, the High-Definition Single Cell Assay (HDSCA) platform was used to identify and analyze circulating rare events. In the first investigation, the first generation of the HDSCA platform analyzing three markers (CK, CD45 and DAPI) was used. Subsequently, enumeration of detected rare cell subtypes was done, together with the analysis of changing levels of cells between sample collection timepoints. This data was then correlated with available clinical data, including patient´s survival - progression-free (PFS) and overall (OS). In the second investigation, two protocols of the third generation of the HDSCA platform were used. The Landscape protocol utilized vimentin and CD31 for the fourth immunofluorescence (IF) channel, while the CDX2-targeted protocol for the fourth channel utilized CDX2 as a colorectal-specific marker. Also here, the enumeration of rare events was done and changing levels of cells were analyzed. However, in comparison to the first study, the focus was on identifying and describing rare subtypes of cells identified thanks to new protocol markers.

Results:

Our first investigation has shown some actionable correlation of circulating tumor cells (CTC) levels and survival. At the 1-month time-point after the surgery, higher count of CTC-CKtotal/mL is related to worse OS ($p = 0.0492$, $HR = 1.02$) and higher levels of HD-CTCs/mL was marker for worse PFS ($p = 0.0468$, $HR = 1.03$). Similar correlation was found for CTC dynamics between timepoints. A higher increase of HD-CTC/mL levels than 49.77 between pre-resection to post-resection sample was related to worse OS ($P\_BC = 0.0270$, $p = 0.8464$, $HR = 0.99$). Also, if CTC-Apoptotic increased levels by more than 12.28 cell/mL from pre- to post-resection samples, it worsened patients´ PFS prognosis ($p = 0.0024$, $HR = 1.01$).

The second investigation was able to identify and describe additional specific rare events. First, using the Landscape protocol, two cell subtypes were categorized not just on the channel-type classification, but also based on their unique morphological features. We hypothesize that the stippled CD45/CD31 population of cells that contains some level of variation, especially in different shapes and nuclear to plasma ratio, is a population of megakaryocytes.

Additionally, we speculate that the cells of interest that show threadlike vimentin signal together with a stippled CD45/31 signal and are also characterized with a higher rate of a forming clusters, is population of circulating endothelial cells.

Another detected rare event were oncosomes. From a morphological perspective, oncosomes were found both in the contact with nearby nucleated cells (48.91% of all oncosomes) and as a secluded rare event (51.09% of all oncosomes). After processing and centrifugation, they were found in the cellular fraction of peripheral blood, and in terms of size, they could be as large as WBC or smaller (~10 μm). The phenotype of oncosome was homogeneous across the vesicle, with IF signal distributed evenly. Most oncosomes were also positive for CK signal.

Multiassay analysis, crosschecking slides processed by both advanced protocols using the same sample, also brought interesting findings. For example, the non-correlation between CD45/CD31 population from the Landscape-staining protocol and the CD45 population from the CDX2-staining protocol ($p = 0.645$, $\tau = 0.12$) implies that important marker distinguishing those populations is most likely the CD31.

Conclusions:

The HDSCA platform harbors more information for potential use in clinical routine than just simple enumeration of CK positive cells. There are nuances between the rare events that can be further analyzed and correlated with clinical data. If confirmed in a prospective study with a larger cohort and a balanced and stratified population, the HDSCA platform (and therefore liquid biopsy) can be used for treatment selection/response and prognosis in CRC. Also, we have shown that the unbiased approach of the HDSCA platform can increase the understanding of tumor pathophysiology by broadening the list of separate subtypes of cells that can influence malignant processes.

Key words: liquid biopsy, circulating tumor cell, cell-free DNA, circulating tumor DNA, CTC, cfDNA, ctDNA, colorectal cancer, CRC, HDSCA, high-definition single cell assay