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Isolation of Circulating Tumor Cells in Patients with Thyroid Cancer

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Abstract

Circulating Tumor Cells (CTCs) are considered a liquid biopsy of solid tumors, reflecting the disease complexity at any stage of cancer progression. CTCs, in fact, represent a surrogate material of the tumor which can be obtained by a minimally invasive blood draw and used for disease monitoring and orienting therapeutic decisions, but their detection and characterization are technically challenging.

Keywords: Diagnostic practice; Thyroid nodules; Thyroid carcinoma; K_3EDTA

Introduction

CTCs in thyroid cancer have not been investigated much so far [1,2]. In the past years studies on CTCs in thyroid cancer patients relied on indirect methods based on the identification in venous whole blood of mRNAs expressed by thyroid cells by RT-(q)PCR [3,4] and only more recently circulating epithelial cells were identified in the circulation of patients with thyroid carcinoma by fluorescent microscopy after red blood cell lysis and centrifugation of the blood sample but with no specificity for patients affected by carcinoma [5], thus questioning the origin of these cells and their nature. With the same method a decrease in the number of circulating epithelial cell was observed after radioiodine therapy in patients with thyroid cancer [6]. A recently proposed approach to determine the tissue origin of circulating epithelial cells in patients with thyroid cancer by the analysis of thyroid specific mRNA demonstrated that in subjects with differentiated thyroid cancer with detectable serum thyroglobulin most epithelial cells derive from the thyroid gland, but further investigations are needed to validate the clinical impact of this method [7]. At present the only reports on CTCs from thyroid cancer patients are represented by a pilot study on six patients involving a new detection method for CTCs by flow cytometry [8] and a study conducted by CellSearch (Menarini Silicon Biosystems, Italy) in metastatic patients [9].

Current diagnostic practice for thyroid nodules is based on ultrasound examination of the neck and cytological analysis of fine-needle aspiration [10], however 20% of nodules are diagnosed as indeterminate, and, within this category, only 20% to 30% of patients will have thyroid cancer [10]. Tumor-specific non-invasive markers, such as CTCs, are needed for the diagnosis and follow-up of thyroid cancer.

We aim at demonstrating the feasibility of a workflow for CTC enrichment and identification in patients affected by thyroid carcinoma.

Materials and Methods

Blood from a patient with cytological diagnosis of thyroid carcinoma (Thy5 according to the classification by the British Thyroid Association [11]) was collected before surgery in Cell-Free DNA BCT CE tubes (Streck, USA) containing the anticoagulant K_3EDTA and a cell preservative in a liquid medium.

CTC enrichment was performed by the Parsortix system (Angle, UK), a microfluidic device which traps CTCs on the basis of size and deformability and allows subsequent recovery of the cells in 200 µL PBS buffer.

Recovered cells were fixed with formaldehyde at 4% final concentration and stained with CD45 antibody conjugated to APC, pancytokeratin antibody conjugated to PE and NucBlue Fixed Cell Ready Probe (Thermo Fisher Scientific, USA), according to a previously published protocol [12]. After the staining, the sample was centrifuged at 800 g for 5 minutes, the supernatant was removed, and the cell pellet suspended in 13.5 µl of SB115 buffer (Menarini Silicon Biosystems).

The sample was loaded into the DEPArray A300K cartridge and submitted to single cell sorting by DEPArray (Menarini Silicon Biosystems) according to the manufacturer's instructions. The A300K cartridge is a single-use, microfluidic chip containing an array of individually controllable electrodes, each one with embedded sensors. Chip scanning was performed by an automated fluorescence microscope to generate an image gallery, and cells selected according to their morphology and staining pattern. After tumor cell identification, single cells or groups of few cells were moved by dielectrophoresis and recovered into 200 µl tubes.

Results

Cells were scanned by the DEPArray systems and CTCs were identified according to their morphology (round shape, round nucleus within the cytoplasm) and staining pattern: NucBlue positive, PE positive (pancytokeran positive), APC negative (CD45 negative cells).

The DEPArray allowed the identification of about 148 CTCs showing positive fluorescent signals for cytokeratins and for the nucleus and no signal for CD45.

A background of contaminating leukocytes expressing positive signals for CD45 and the nucleus and no signal for cytokeratins was visible in the sample enriched by Parsortix. Figure 1 shows the images of one CTC and one leucocyte from the same patient.

Discussion

We report the feasibility of a workflow for CTC identification

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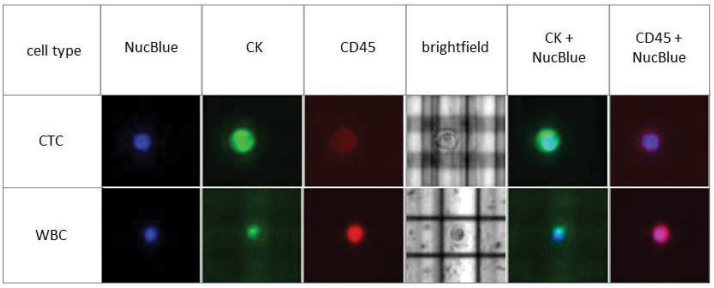

Figure 1: Shows the images of one CTC and one leucocyte from the same patient.

patients with cytological diagnosis of thyroid cancer involving an unbiased enrichment of tumor cells on the basis of size and deformability and subsequent CTC identification based on cell morphology and expression of specific markers [8]. Our method provides the possibility of recovering pure single or pooled cells by dielectrophoresis. With this approach we could identify a high number of CTCs in a single Thy5 patient.

CTCs had been detected previously in metastatic patients affected by medullary thyroid carcinoma [9] by CellSearch, but the same approach on the whole failed to identify CTCs in patients affected by differentiated thyroid carcinoma. This could be due to the main drawback of CellSearch system which is represented by the fact that cells are selected a priori on the basis of EpCAM expression. Other authors reported low or undetectable expression of EpCAM in thyroid cancer [8]. On the contrary an enrichment step based on physical properties of CTCs, such as size and deformability, enables the capture of a heterogeneous population of CTCs, more representative of the disease complexity. Moreover, our workflow allows the recovery of a population of pure CTCs for downstream analysis, which is not feasible by CellSearch or flow cytometry.

Conclusion

Our preliminary results in a patient with cytological diagnosis of thyroid cancer open the perspective of isolating single/pooled circulating tumor cells for downstream mutational analysis.

By applying the same workflow to subjects in earlier stages of the disease we could provide a new approach based on the mutational analysis of CTCs as an alternative to invasive fine needle aspiration.

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