Feasibility of genetic aberrations analysis in the Circulating Tumor Cells (CTCs)

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Background

In current practice cancer tissue is taken at diagnosis to assess the presence of treatment targets. This however is suboptimal since tumor cells evolve due to genomic instability. Assessment of the genotype and phenotype of the CTCs will provide insights into which treatments would be most beneficial for the individual patient. Feasibility to detect treatment targets in CTCs has been demonstrated (Meng, Tripathy et al. 2004; de Bono, Attard et al. 2007; Rossi, Basso et al. 2010; Wang, Pfister et al. 2010). In this context, the development of a cytogenetic assay for CTCs will be crucial for successful molecular targeted therapy in cancer patients. Genetic characterization of CTCs is expected to gather new knowledges on the mechanism of metastasis and on potential targets of novel therapeutic strategies.

Rationale

The development of a cytogenetic assay for CTCs will be crucial for successful molecular targeted therapy in cancer patients. Genetic characterization of CTCs is expected to gather new knowledge on the mechanism of metastasis and on potential targets of novel therapeutic strategies.

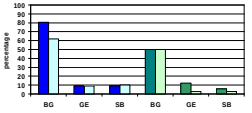
Patients and Methods

Assay optimization for analysis of genetic aberrations of CTCs was performed with cells from tumor cell lines. Tumor cell lines with known chromosomal aberrations and mosaicism were spiked into 7.5mL whole blood samples, at numbers similar to those observed *in-vivo* in cancer patients. Tumor cells were enriched by CellSearch System and off-line purified, and individually analyzed for chromosomal alterations. The procedure was next validated by using blood samples collected from cancer patients.

Development of single cell WGA protocol

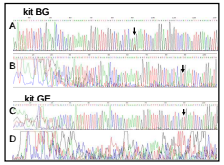
Single cell possess 6–7 pg of genomic DNA (gDNA), that is few log below the DNA amounts currently required for a cytogenetic assay.
Whole genome amplification (WGA) protocols can amplify the whole genome with a high fidelity of the genome by more than 1000-fold. This WGA procedure method results in unbiased amplification to maintain relative quantities of DNA across the entire genome.
Different protocols was compared using SurePlex DNA Amplification System (BG, BluGnome), Illustra Genomiphi (GE, GE Helthcare), and Ampli1 (SB, Silicon Biosystem) to assess the quality of single cell DNA amplification.

Efficiency of different WGA protocols



WGA performances of different protocols are valuated by PCR (BRAF specific band in agarose gel, dark blue or dark green histograms) and sequence analysis (light blue or light green histograms) in HT29 (heterozygous for the BRAF mutation, blue histograms) and MCF7 (wt for the mutation, green histograms) cell lines. Thirty replicates of a single cell (sorted by flow cytometry) for each cell line were analyzed with each protocol (BG, GE, and SB).

Feasability of sequence analysis



To compare the quality and the representation of the genes, cells derived from both wt and heterozygous tumor cell lines were used.
The figure shows sequence analysis of the heterozygous HT29 cells. An example of good quality (in A and in C) and of bad quality (in B and D), obtained with two different protocols are shown.

Feasibility of CGH array

To further address the feasibility of more complex analysis by starting from a WGA product of purified tumor cells, spiked samples were prepared at numbers similar to those observed *in-vivo* in cancer patients (200-1000 cells /7.5 ml), processed by CellSearch and further off-line purified at purity >95%.
Starting from 10 and 2 cells CGH analysis (by BAC array, 100 KB resolution) can be performed.

10 HT29 cells

2 HT29 cells

Conclusions

A robust protocol for the isolation of individual CTCs followed by DNA extraction and WGA amplification after CellSearch enrichment was established.
The number and the quality of CTCs needed to obtain an informative analysis of chromosomal aberrations were set up.
Patients samples has been collected and WGA amplified. Currently, we are optimizing the CGH array of *ex vivo* samples.

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