NGS cfDNA data as a basis for the development of qPCR diagnostic systems

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Introduction

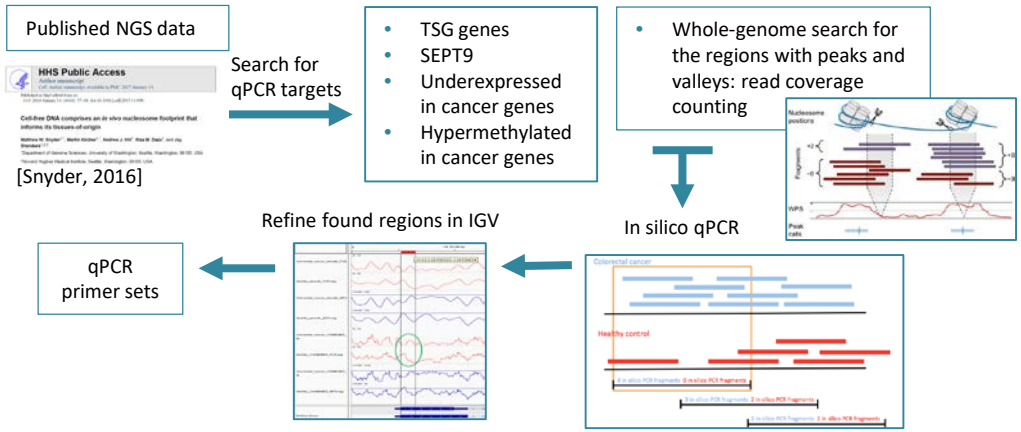
According to present knowledge circulating free DNA (cfDNA) is a powerful diagnostic tool. However, most modern diagnostic techniques are based on the expensive NGS procedures and there are only a few special methods based on cfDNA analysis which are accepted for wide clinical practice (e.g. US FDA PMA #P130001). Here we propose a new approach for leveraging cfDNA data for development of cheap methods for diagnosis of cancer.

Aims

- Develop a method for selecting the genomic regions that can serve as biomarkers based on the joint positioning of the fragments
- Find such regions specific for colorectal cancer and analyze them for their biological significance and diagnostic usefulness
- Design qPCR systems for the obtained targets and test them for diagnostic suitability on cfDNA samples from the patients with colorectal cancer.

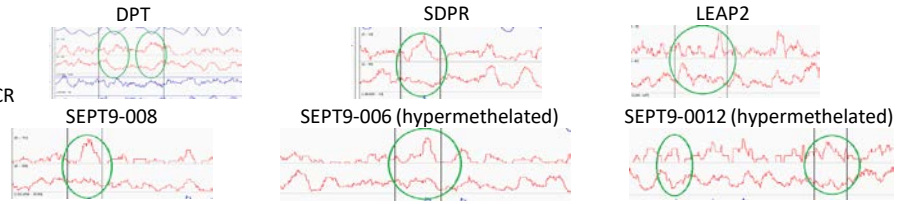
Methods

From the published NGS data (Snyder et al.) on cfDNA from healthy donors and patients with colorectal cancer, we obtained the coverage distribution by fragments of different lengths for selected genomic regions. Based on fragments joint positioning, we developed a method for selecting the characteristic and reference genome regions. The reference region was chosen as the area in which the coverage levels have the greatest positive correlation between patient samples and healthy ones. Characteristic regions were searched in the environment of transcription start site (TSS) transcripts of genes with the greatest increase in colorectal cancer expression and suppressor genes, among which KIF1B, LPAR1, APC, FN1, SEPT9 were chosen as a result of the analysis. The characteristic region was chosen as the region with the highest negative correlation between patients and healthy individuals. Then we simulated qPCR using the data from selected regions. In the end, we evaluated the results of in silico qPCR to predict the diagnostic value of the characteristic and reference fragments.



Results

- Developed an algorithm for selecting the targets that can serve as potential biomarkers based on NGS data (used colorectal cancer as a model)
- Discovered different NGS patterns for various isoforms, therefore the designed qPCR systems have to be isoform-specific
- In vitro validation has to be performed of the size selected short fraction of cDNA (around 150bp).



Conclusions: The accumulated NGS data on cfCDA can be used for the detection of the cancer-specific differentially-represented transcripts. The presented methodical algorithm can be a promising tool for the development of simple qPCR test-systems for tumor diagnostics.