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Ultrasensitive DNA sequencing using liquid biopsies enables precision medicine

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i hörsal Arvid Carlsson, Medicinaregatan 3, Göteborg, den 9 April 2020, klockan 9.00

av Gustav Johansson

Fakultetsopponent:

Prof. Dr. Michael W. Pfaffl, Technische Universität München, Tyskland

Avhandlingen baseras på följande delarbeten

I. Johansson, G., Andersson, D., Filges, S., Li, J., Muth, A., Godfrey, T.E. and Ståhlberg, A. *Considerations and quality controls when analyzing cell-free tumor DNA.* Biomolecular detection and quantification*, 2019*; 17: 100078.

II. Johansson, G., Kaltak, M., Rîmniceanu, C., Singh, A.K., Lycke, J., Malmeström, C., Hühn, M., Vaarala, O., Cardell, S. and Ståhlberg, A. *Ultrasensitive DNA Immune Repertoire Sequencing Using Unique Molecular Identifiers.* Clinical Chemistry, 2020; 10: 1-10.

III. Johansson, G., Berndsen, M., Lindskog, S., Österlund, T., Fagman, H., Muth, A. and Ståhlberg, A. *Monitoring circulating tumor-DNA during surgical treatment in patients with gastrointestinal stromal tumors.* (Manuscript), 2021.

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INSTITUTIONEN FÖR BIOMEDICIN

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Abstract

Liquid biopsies are minimally invasive and allow repetitive sampling of body fluids. Analysis of cell-free tumor DNA in liquid biopsies can be used as a biomarker for cancer. However, in most clinically relevant liquid biopsies, cell-free DNA is present at low concentrations, contains minute tumor allele frequencies, and is highly fragmented. Analysis of immune cell DNA in liquid biopsies can be profiled to examine the immune cell repertoire. However, this application requires unbiased quantification and accurate sequencing in an incredibly diverse DNA background. The overall aim of this thesis was to develop ultrasensitive sequencing approaches that enable the detection and quantification of individual molecules and single cells in these applications. We applied SiMSen-Seq, an ultrasensitive sequencing strategy based on unique molecular identifiers that enables error-free and quantitative sequencing. First, we showed that the amounts of plasma and DNA, number of targeted somatic variants, assay length, and target sequences affect the sensitivity of ctDNA analysis. We developed multiple quality control steps to evaluate a preanalytical workflow to analyze the amount of amplifiable DNA, degree of cellular contamination, and enzymatic inhibition. In patients with gastrointestinal stromal tumors, cell-free tumor DNA correlated with risk classification, treatment response, tumor size, and cell proliferation. Our data indicate that our method can be applied to monitoring treatment efficacy and identifying relapse early, especially in high-risk patients. Finally, we developed a targeted and ultrasensitive immune repertoire sequencing method to profile T-cell clonality. By studying the DNA of γδ T cells, we demonstrated that our approach is characterized by a wide dynamic range and high reproducibility and can be applied to enriched and non-enriched cells. In conclusion, we developed two flexible and simple liquid-biopsy applications that use ultrasensitive DNA sequencing to monitor cancer in patients with gastrointestinal stromal tumors and profile the immune repertoire of γδ T cells, respectively. We expect that several diagnostic applications that utilize liquid biopsies will be implemented in clinical routines in the future. Further technology development and the use of diverse types of analytes will advance this field of research. Ultimately, the development and implementation of ultrasensitive liquid biopsy-based analysis will facilitate precision medicine for more patients and improve their survival and quality of life.

Keywords: Liquid biopsy, cell-free DNA, immune repertoire, next-generation sequencing, unique molecule identifier, GIST, γδ T cell

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