1 Correlation of spatial transcriptomics RNA sequencing with PET-MRI imaging in prostate cancer samples

Partners: Lab. Panagiotis Alexiou (Vojtěch Bystrý), CEITEC MU and Lab. Vladimir Benes, EMBL

1.1 SUMMARY

Spatially resolved transcriptomics (spatial transcriptomics) is a highly novel and trending experimental method crowned in 2020 as the Method of the Year by Nature Methods[1]. We propose a CEITEC's spatial transcriptomics pilot project in which we would sequence prostate cancer (PCa) samples which were also visualized with nuclear medicine imaging and combine the data. The project will have a highly positive impact on the RNA research in CEITEC by allowing for the technological transfer of cutting edge technology expertise from EMBL. The project is also scientifically very exciting since it will allow for better insight into the correlation between PCa metabolism and its molecular mechanics.

1.2 SCIENTIFIC BACKGROUND

Spatial transcriptomics methods are a continuation of the development of RNA sequencing (RNA-seq) resolution improving single-cell RNA sequencing technology. Single-cell RNA sequencing brings much more fine-grained assessment of each cell's transcriptome than bulk RNA-seq, but it still dissociates cells from tissue. Spatial transcriptomics methods allow scientists to get cell's transcriptomic data in the positional context of those cells in a tissue. Spatial transcriptomics is the next wave after single-cell analysis, which will be particularly useful to labs studying human diseases, which often starts with single cells and spreads spatially. As such, this method might be highly beneficial to RNA research in CEITEC's molecular medicine program. This project will facilitate the establishment of this method in CEITEC, with the help of EMBL expertise and experience, on both lab preparation level and mainly data analysis level.

Positron emission tomography–magnetic resonance imaging (PET–MRI) is a hybrid nuclear medicine imaging technology that incorporates magnetic resonance imaging (MRI) - enabling soft tissue morphological imaging - and positron emission tomography (PET) - providing functional information. It is a very new technology being used in oncology for solid tumour diagnostics, tumour staging and cancer patients stratification[2]. Specifically, PSMA ligand PET-MRI were successfully used for primary prostate cancer tumour staging with good clinical impact[3].

In recent years there has been significant effort to combine nuclear medicine imaging with molecular medicine -omics methods to better describe solid tumours diagnostics[4,5,6]. The modelling usually tries to correlate overall imaging features, so called radiomics features, for individual lesions with bulk transcriptomics or genomics data.

With the combination of the PET imaging with the spatially resolved transcriptome, we can go to more detail than the overall radiomics features and superimpose the transcriptomes on the

individual imaging voxels getting a highly detailed view of how the metabolic activity translates to the molecular level RNA mechanisms.

1.3 AIMS

The project's goal is to use the spatial transcriptomics method to sequence primary PCa samples that were also visualized with PET-MRI imaging. We would then combine and correlate the transcriptomics and the imaging data in the subsequent data analytical step. The expected results of the project would be twofold:

1.3.1 Technological transfer

The project's main practical result for us would be to test and get experience with a novel laboratory method surpassing single-cell transcriptomics, developing and importing new bioinformatics analytical workflows and improving bioinformatics regarding single-cell in general.

1.3.2 Scientific goal

Find out if it is possible to superimpose the spatial transcriptomics "image" onto the nuclear medicine image to better understand the specific causal molecular effect manifesting itself in the live metabolic image. In reverse, we also want to see if it is possible to predict some overall transcriptomic behaviour based on the image and pinpoint the essential imagining features affecting the transcriptomic levels.

1.4 WORK PROGRAMME

1.4.1 The experimental approach including key techniques or technologies

We plan to use Visium Spatial Gene Expression Slide Reagent Kits from 10x genomics followed by NGS sequencing for spatially resolved transcriptomics.

The PCa samples will be provided through collaboration with the group of Prof. Dr. Lukas Kenner from the Department of Pathology of the Vienna AKH hospital, where the prior PET-MRI imaging and the Visium preparation protocol will be performed.

Because of the novelty of spatial transcriptomics, no standardize analytical workflow exists. However, some single-cell bioinformatics tools such as a CellRanger will be utilized mainly for the primary data analysis. The development and implementation of the bioinformatic data analysis will be the central part of the project.

1.4.2 What will be done by which partner and the expected synergies

All the lab work and sequencing will be performed on the side of CEITEC. Bioinformatics data analysis will be done by the employees of CEITEC CF bioinformatics with the help of EMBL. EMBL will provide tutoring and guidance for both the laboratory and data analysis part of the project.

1.4.3 The benefit of this research project to RNA research at CEITEC

As mentioned, spatially resolved transcriptomics is an emerging technology surpassing single-cell sequencing with a substantial prospective impact on RNA sequencing and research, especially in the context of molecular medicine. Therefore, we are confident the expertise and ability to perform and analyze single-cell and spatially resolved transcriptomics experiments is crucial for CEITEC to keep a competitive edge in RNA research.

1.4.4 Future collaborations or joint funding expected to result from work proposed

There are no specific collaboration or funding opportunities targeted right now. However, as a pilot project involving the newest technologies and unprecedented data combination, it is reasonable to expect high funding potential for subsequent experiments.

1.5 REFERENCES

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1.6 BUDGET

The seeding funds will be used for purchasing of the chemistry for the spatially resolved transcriptomics sequencing:

1 x Visium Gene Expression Slide & Reagents Kit, 4x rxns	6000 EUR

The plan is to apply for ~3 secondments to EMBL related to this project. One for wet lab preparation and 2 for the tutoring regarding bioinformatics analysis.