TRANSCRIPTIONAL PROFILING OF HUMAN PERIPHERAL BLOOD SAMPLES EXPOSED TO GAMMA-RAY

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Introduction

In large-scale radiation emergency scenario, it is essential to identify among an exposed population those individuals who require medical treatment and hospitalization [1]. A variety of different biological and physical tools are available to assess individual absorbed radiation doses following a radiation accident or hostile act. In recent years, transcriptional changes in blood have been identified as a promising biomarker for radiation response to support biodosimetric assessment of individual doses in radiation mass-casualty scenarios [2]. The improvement in high-throughput Next Generation Sequencing (NGS) technologies provide a powerful mean to analyse whole transcriptome in order to identify new radiosensitive genes that change expression profiles after exposure [3].

Aim

The aim of the present study is to investigate whole gene expression profiling, pathways and biological processes in human peripheral blood samples *in vitro* exposed to increasing γ-ray doses, performing bioinformatics and statistical analysis of the transcriptome data obtained through Next Generation Sequencing.

Material and Methods

Blood samples were collected from six healthy donors (age range: 27) and exposed with Gammacell (^{137}Cs) to a dose range between 0 Gy and 6 Gy of γ-ray (dose-rate 0.65 Gy/min). Total RNA was isolated 24 hours after exposure and *m*RNA sequencing was performed on the NextSeq 2000 Illumina platform. Reads generated during the Illumina sequencing process were quality checked using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Trimming of the poor-quality bases (qscore <20) was performed using the fastp software [4]. The trimmed reads will be mapped to the human reference genome (GENCODE human database version 43) and the reads quantification will be performed based on the human genome annotation. In order to identify the differentially expressed genes (DEGs), DESeq2 and EdgeR R packages will be applied. A false discovery rate (FDR) with p-adjusted ≤ 0.05 cut-off and a fold change of 1.5 as the minimum cut-off value will be used as parameters to identify differentially expressed genes.

Results

A total of 36 samples (6 donors for 6 γ-ray doses) were processed with two Illumina RNA-seq runs yielding a total of 2.5 billion sequences with an average of 69.5 million reads/sample. After the quality control, a

3% of the reads were discarded resulting in an average residual of 67.3 million sequences per sample. From the reads alignment on the human genome and transcriptome, a statistical analysis will be performed to obtain differentially expressed genes by comparing exposed and unexposed samples, separately for each exposure dose (0, 0.2, 0.5, 1, 2, 6 Gy). Preliminary data on the differentially expressed genes and on the biological pathways involved in the response to the exposure will be reported.

Conclusions

In the last decade, blood-based gene expression analysis has emerged as a new approach to provide rapid dose assessment for medical triage and treatment purposes. The use of an "-omics" approach can contribute to broaden the knowledge for the selection of radiation-responsive genes, in order to generate quantitative radiation dose reconstructions based on a blood sample. The identification of new biomarker genes would be invaluable in classifying individuals into different risk classes in order to direct them to the appropriate clinical treatment, particularly in the early triage performed by clinicians on signs and symptoms of Acute Radiation Syndrome (ARS).

Future perspectives of this study include increasing the sample size in terms of number of donors allowing to assess also inter-individual variability associated with eg gender and age [5].

References

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