BITS :: Call for Abstracts 2024 - Poster

Type	Poster
Session	Young BITS-RSG Symposium
Title	MATITE - a Multi-omic Approach To Investigate The human repeatomE
All Authors	Martina Coffa(1), Giorgio Grillo(1), Flavio Licciulli(1), Arianna Consiglio(1) e Francesca Bersani(2)

Affiliation

(1) CNR Istituto di Tecnologie Biomediche, Bari
(2) Università degli Studi di Torino, Dipartimento di Oncologia, Torino

Motivation

MATITE is a project with the objective of elucidating the biological mechanisms underlying the expression, regulation, and function of repetitive elements (REs) through combining multi-omics and biological methodologies. This approach integrates comprehensive bioinformatic analysis with the development of a specialized computational pipeline for RE mapping. The primary aims of this project include the identification of the fundamental molecular drivers governing RE expression, the involvement of specific transcriptional and epigenetic modulators in RE regulation, and the elucidation of the biological implications of RE modulation using in vitro models.
The project is focused on small cell lung cancer (SCLC) case study, notable for its neuroendocrine phenotype. The samples will be categorized based on histopathological characteristics and RE abundance. These samples will undergo genomic and transcriptional profiling, integrating the findings from a prior genome-wide multi-omic analysis. The project, coordinated by F. Bersani (University of Turin), will also include the study of established primary SCLC organoids from core biopsies or circulating tumor cells and the evaluation of RE expressions in these samples using quantitative PCR and RNA-in situ hybridization.

Methods

The first step is the literature investigation regarding the role of REs in SCLC. A database will be created comprising raw multi-omic data from public or primary and specialized repositories (e.g., SRA, TCGA). A series of reference data will be used to classify and/or categorize additional data. Reference databases like NCBI or Ensembl comprise annotated and curated collections of publicly accessible nucleotide sequences (DNA, coding and non-coding RNA) and their corresponding protein products. RepBase (https://www.girinst.org/repbase/) and Dfam (https://dfam.org/home) will be used as reference databases for REs.
The human samples for the study are obtained via informed consent from patients. All the tasks involve close collaboration between the two research units (CNR-ITB and UniTo).
Normal lung and SCLC organotypic cultures will be established as optimized in the PI’s lab. Cultures will be maintained in growth factor- and cytokine-enriched medium. As an alternative, circulating tumor cells (CTCs) will be used as starting material. All organoids will be verified by IHC using neuroendocrine-specific markers such as SYP and CHGA. Expression of selected repeats (such as HSATII, LINE1, HERVK) will be assessed in normal vs SCLC 3D models by qPCR and RNA-ISH. SCLC samples will be processed for RNA extraction followed by library preparation for RNA-Seq.
Ideally, 30 samples will be included in the transcriptome analysis. Optical genome mapping (BioNano) will be applied to up to 10 selected flash-frozen samples for the detection of structural and copy number variations in RE regions.
Omic datasets will be stored and analyzed: standard tools (e.g., FastQC, trimmomatic) will assess the quality of each dataset and filter out low-quality data. Standard processing pipelines already tested will be applied to each sequencing protocol. For instance, the RNA-Seq data pipeline includes sequence mapping (e.g., Bowtie, STAR), expression quantification (e.g., RepEnrich2), differential expression analysis (e.g., DESeq2, EdgeR), pathway analysis (e.g., STRING, DAVID) or co-expression evaluation.
The final task will be the establishment of a robust experimental pipeline for the study of repetitive elements.

Results

Recent advances in next-generation sequencing technologies have afforded new insight into the human genome, unveiling that over 50% comprises repetitive elements (REs). Despite this, the biological significance of these sequences and the molecular mechanisms governing their expression, maintenance, and expansion remain partly unknown. Notably, the highly repetitive nature of these elements needs long-range genome sequencing and innovative bioinformatic pipelines for accurate assembly. Moreover, the neuroendocrine lineage exhibits significant enrichment in RE expression, indicating an active involvement of neuronal master transcription factors in their modulation.
Since the analyses are ongoing, we expect, as results, the differential expression of specific Repeat Elements (REs) in SCLC: this could identify them as potential biomarkers.

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Corresponding Author

Name, Surname	Martina, Coffa
Email	martina.coffa@ba.itb.cnr.it
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