Contribution ID : **16** Type : **not specified**

SINGLE CELL RNA SEQUENCING UNCOVERS CHEMORESISTANCE GENES AND PATHWAYS OF NEUROBLASTOMA INTRA-TUMOR HETEROGENEITY

lunedì 28 febbraio 2022 13:20 (10)

Background and rationale. Human tumors are complex systems characterized by molecular, cellular and spatial diversities. The totality of features demonstrating differences within a tumor is termed intra-tumor heterogeneity (ITH). ITH may be one of the mechanisms at the basis of the drug resistance and relapse triggered, for example, via the selection of malignant clones. Single cell sequencing approaches coupled with advanced computational analyses have made a huge contribution to understand the molecular basis of tumor ITH. However, due to the lack of data at the single-cell level, little is known about these dynamics in tumors such as neuroblastoma (NB), one of the most common solid tumors of the childhood. NB affects the development of sympathetic nervous system and its treatment is still unsuccessful in half of the patients diagnosed with the high-risk subtype. Here we investigated the ITH of Etoposide and Cisplatin resistant NB cell lines and their parental cells through single cell RNA sequencing (scRNA-seq).

Methods. scRNA-seq was performed on 10X Genomics platform and barcode filtering, alignment of reads and UMI counting were carried out using Cell Ranger 3.0.1. Counts were imported into R for quality control (QC) and downstream analysis. Cells were excluded if fewer than 2000 distinct genes, 20,000 counts or more than 30% of reads mapping to mitochondrial genes were detected. Data were normalized, scaled, log-transformed and, in order to remove confounding sources of variation, percent of mitochondrial genes, read counts and cell cycle scores were regressed out using a regularized negative binomial model implemented in Seurat package. The most variable genes were used for dimensionality reduction and clustering analysis was carried out with the nearest neighbor algorithm. Gene set enrichment analysis of marker genes for each cluster was performed with Webgestalt R package. CIBERSORTx was used to deconvolute bulk RNA-seq datasets with scRNA-seq-derived cell clusters and resulting scores were correlated with clinical and survival data.

Results. We obtained transcriptional profiles of 1514 Etoposide-resistant vs. 2646 parent cells, and 1160 Cisplatin-resistant vs. 1674 parental cells after QC. TSNE and UMAP plots showed a clear separation of resistant and parental cells for both conditions and allowed to identify 8 distinct tumor clusters in Etoposide-resistant/parental and 7 in Cisplatin-resistant/parental cells. We found a significant enrichment (FDR ≤ 0.01) of pathways related to the DNA damage response in both drug resistant cells, suggesting that the upregulation of the DNA repair machinery may be a potential drug resistance mechanism in these cells. Besides, both parental cell lines showed cell clusters characterized by genes involved in embryonal differentiation trajectories and enrichment of neural crest development pathways, reflecting the dynamics of NB cell development. Deconvolution analysis of bulk RNA-seq data with cluster signatures, allowed the identification of specific clusters associated (logrank P ≤ 0.01) with worse/better survival.

Conclusions. In this study, we applied scRNA-seq and advanced bioinformatic pipeline to analyze the chemo resistant NB cell lines. We identified distinct cell populations characterizing Etoposide and Cisplatin resistant NB cell lines, provided insights into plausible mechanisms of chemoresistance and highlighted genes and cluster signatures associated with clinical outcomes that are potentially actionable as therapeutic targets.

Speaker recent publications

- **Bonfiglio F**, Liu X, Smillie C, Pandit A, Kurilshikov A, Bacigalupe R, Zheng T, Nim H, Garcia-Etxebarria K, Bujanda L, et al. GWAS of stool frequency provides insights into gastrointestinal motility and irritable bowel syndrome. ***Cell Genomics***. 2021.
- Eijsbouts C, Zheng T, Kennedy NA, **Bonfiglio F**, Anderson CA, Moutsianas L, Holliday J, Shi J, Shringarpure S; 23andMe Research Team, et al. Genome-wide analysis of 53,400 people with irritable bowel syndrome highlights shared genetic pathways with mood and anxiety disorders. ***Nature Genetics***. 2021.
- **Bonfiglio F**, Bruscaggin A, Guidetti F, Terzi di Bergamo L, Faderl M, Spina V, Condoluci A, Bonomini L, Forestieri G, Koch R, et al. Genetic and Phenotypic Attributes of Splenic Marginal Zone Lymphoma. ***Blood***. 2021.

Department

Primary author(s) : Dr. BONFIGLIO, Ferdinando (University of Naples Federico II); Dr. AVITABILE, Marianna (University of Naples Federico II); Mr. AIEVOLA, Vincenzo (University of Naples Federico II); Mr. CANTALUPO, Sueva (University of Naples Federico II); Mr. MAIORINO, Teresa (University of Naples Federico II); Dr. LASORSA, Vito Alessandro (CEINGE Biotecnologie Avanzate); Dr. DOMENICOTTI, Cinzia (University of Genoa); Dr. MARENGO, Barbara (University of Genoa); Dr. IZZOTTI, Alberto (University of Genoa); Dr. ZBYNĚK, Heger (Mendel University in Brno); Dr. VOJTĚCH, Adam (Mendel University in Brno); Prof. IOLASCON, Achille (University of Naples Federico II); Prof. CAPASSO, Mario (University of Naples Federico II)

Presenter(s) : Dr. BONFIGLIO, Ferdinando (University of Naples Federico II)