Time-varying differential network analysis from tumor-educated platelet transcriptome to capture mechanisms of tumor progression

Santiago Vessi,[1] Aurelia Rughetti,[2] Marco Filetti,[3] Lorenzo Farina[4] and Manuela Petti[4]

1. MSc Program in Data Science, Sapienza University of Rome, Rome, Italy
2. Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy
3. Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy
4. Department of Computer, Control, and Management Engineering Antonio Ruberti, Sapienza University of Rome, Rome, Italy

Differential network analysis has been designed to highlight network changes between conditions: in network biology, this approach can capture tissue-selective processes and identify critical signaling pathways altered during tumor transformation and progression [1]. In oncology, the most appropriate tumor surveillance strategy remains an unsolved issue. Indeed, the typical approach (based on solid biopsies and resource-demanding follow-up programs) has limitations in terms of invasiveness, time resolution and accessibility of molecular data. To date, blood-based liquid biopsies represent a promising source of biomarkers to detect and monitor cancer without invasive procedures. The latest developments in isolating and characterizing various elements in blood, including tumor-educated platelet (TEP) RNA, make it possible to identify cancer-specific genomic and transcriptomic abnormalities in blood. In particular, TEPs are circulating blood cells with a distinct tumor-driven phenotype and act as carriers and protectors of metastases. Some recent studies have shown that the TEPs transcriptome can be used for cancer diagnosis [2]. However, these works are only based on differential expression analysis.

The objective of this work is to propose Differential Network Analysis (DNA) to study longitudinal TEPs transcriptomic data. In particular, we applied the Time-varying DNA (tDNA, [3]) to investigate gene network rewiring during glioblastoma (GBM) progression.

We downloaded mRNA sequencing data of TEPs from the GEO database under accession number 156902 [4] and we selected GBM samples. Longitudinal data were available for 55 patients, although for a subset of 13 patients a complete set of measurements from time 0 to time 3 was available. Given the *K=4* time points, the tDNA approach jointly estimated *K-1=3* differential networks Δ, enabling the identification of both consistent and time-specific rewiring patterns driven by key genes. These genes were classified as either common (shared across time points) or time-specific critical nodes.

Across the three obtained differential networks, we identified 14 common and 134, 80, and 72 time-specific key genes for Δ_{0-1}, Δ_{1-2} and Δ_{2-3}, respectively. KEGG enrichment analysis of the identified nodes revealed convergence on immune and inflammatory signaling pathways, including IL-17 signaling (*adj-p=0.005*), Toll-like receptor signaling (*adj-p=0.008*), and Herpes simplex virus 1 infection (*adj-p=0.01*), largely driven by time-specific genes from each differential network. These findings highlight processes associated with tumor progression and immune modulation in glioblastoma [5]. To validate the findings obtained with tDNA, we compared them with DiffCorr approach [6], developed to compare pairs of experimental conditions. This alternative method identified 101, 120, and 102 critical nodes in the three differential networks, selected by intersecting the top 10% of genes ranked by degree, betweenness, and closeness centrality. Seventy-one genes overlapped with those identified by tDNA, including 4 classified as common hubs. However, KEGG enrichment analysis did not highlight significant pathways.

In conclusion, these results demonstrate that critical nodes identified in time-varying differential networks obtained from longitudinal TEPs transcriptomic data can detect molecular mechanisms of disease progression with potential clinical impact in tumor monitoring.

[1] Yan W *et al*. Mol Ther Nucleic Acids. 2024. doi: 10.1016/j.omtn.2024.102260.
[2] Best MG *et al*, Cancer Cell. 2015. Doi: 10.1016/j.ccell.2015.09.018.
[3] Xu T *et al*. IEEE/ACM Trans Comput Biol Bioinform. 2021. doi: 10.1109/TCBB.2019.2949039.
[4] Sol N *et al*. Cell Rep Med. 2020. doi: 10.1016/j.xcrm.2020.100101.
[5] Parajuli P & Mittal S. J Spine Neurosurg. 2013. doi: 10.4172/2325-9701.S1-004.
[6] Fukushima A. Gene. 2013. doi: 10.1016/j.gene.2012.11.028.

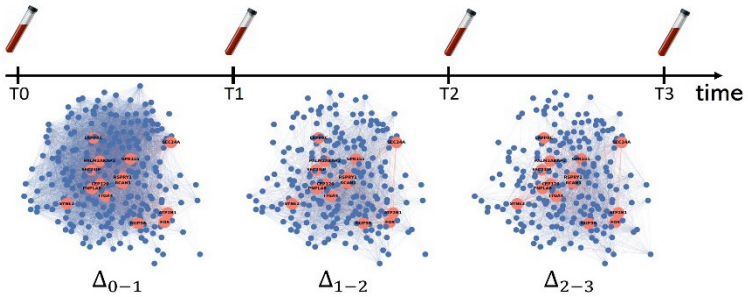

Figure 1: tDNA networks representing specific (blue) and common (salmon) critical nodes.