Transcriptome Analysis of Acute Myeloid Leukaemia Cells

Acute Myeloid Leukaemia (AML) is a blood cancer characterised by overproduction of immature white blood cells in the bone marrow. These proliferating immature cells cause disease by swamping the production of normal blood cells.

AML makes up about 1 percent of cancers, and is the second most common type of leukaemia diagnosed in adults and children. Relapse and chemotherapy resistance are the main challenges of AML care with 40 to 60 percent of adults and 30 to 40 percent of children relapsing within three years. Afterwards, relapsed patients often fail to respond to conventional chemo treatment. Together, this results in 5-year overall survival rates of only 28 percent for adults and 70 percent for children.

AML is thought to be caused by a combination of genetic alterations and aberrant gene expression patterns.

Over the last decade, genomic and transcriptomic studies into AML's causes have helped improve risk classification and treatment options of the disease. But most of these transcriptomic studies have focused on gene expression signatures at diagnosis, with only a small number investigating differential expression in relapsed and primary resistant (R/PR) AML – and of these studies, most lacked detailed knowledge about underlying genetic alterations in the form of whole-genome sequencing or whole-exome sequencing data.

Dr. Linda Holmfeldt, Principal Investigator in the department of Immunology, Genetics and Pathology at Uppsala University in Sweden, has recently been using Qlucore Omics Explorer's in transcriptomic studies to better understand the molecular characteristics of R/PR AML patients.

Holmfeldt's group analyses molecular signatures in AML cancer cells using a combination of whole genome and/or exome sequencing, RNA sequencing, mass spectrometry analysis of the proteome as well as studies of the epigenome by DNA methylation microarrays. She and her team also employ machine learning to generate hypotheses around tumour progression and/or therapy resistance.

Using these methods, Holmfeldt and colleagues are helping to build a more complete picture of DNA and RNA alterations in AML tumour cells, as well as their protein composition. Long term, by identifying these changes, the group hopes more efficient treatment alternatives can be developed for high-risk R/PR AML patients.

In the past, finding sufficient samples from R/PR cases has been difficult, says Holmfeldt.

For the recent study (published in Blood Adv January 2022), her group was able to perform transcriptome-wide RNA sequencing on 70 relapsing or primary resistant AML patients (47 adult and 23 children) with known mutational background from samples held via Uppsala Biobank and Karolinska Institute Biobank, collected between 1995 and 2016.

There were 122 samples in all, comprising 43 samples collected at diagnosis and 73 at relapse, plus six primary resistance samples. CD34-expressing bone marrow cells from five healthy donors were used as a source of normal control RNA. The RNA-sequencing yielded an average of 41 million reads per sample. The composition of genomic alterations for all AML samples had previously been analysed via whole genome or whole exome sequencing. ('*Genomic characterization of relapsed acute myeloid leukemia reveals novel putative therapeutic targets*' by Svea Stratmann *et al*; Blood Adv (2021)).

Holmfeldt and her colleagues began by extracting DNA, RNA, and protein to understand which alterations might be leukaemia-specific.

"We started out looking at the RNA transcriptome, tracking back to mutations at the DNA level," says Holmfeldt. "Around that time, our bio-informaticist left! We had a license for Qlucore Omics Explorer's and had experimented with it, so it was the perfect time to try out the tool on our RNA-sequencing data."

The group used Qlucore Omics Explorer for various types of analyses of RNA-seq data, as well as for generation of various figures used within the now published paper.

"To start with, we used Qlucore Omics Explorer for various pre-processing of the TMM-normalised [Trimmed Mean of the M-value] data, including adjusting the data for gene length, carrying out log2 transformation separately for each conducted analysis (including different sets of samples), as well as performing batch correction, " says Holmfeldt.

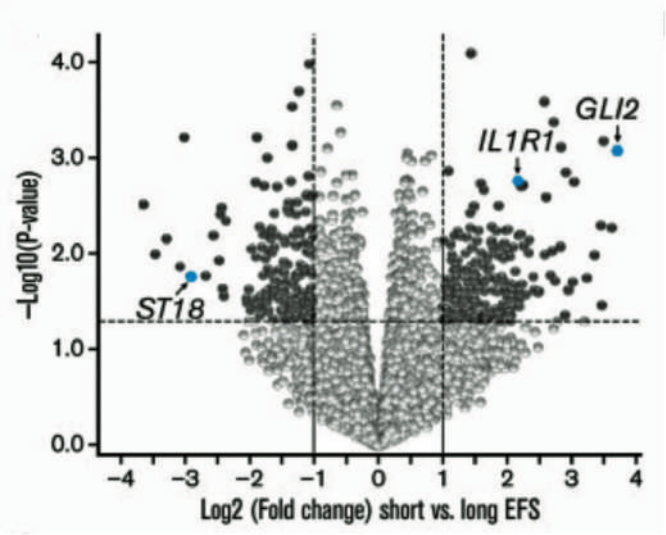
"Thereafter, we used the software for differential gene expression analysis. We carried out PCA, hierarchical clustering analysis, generated t-SNE- and Volcano plots as well as Venn diagrams," she added.

"When comparing AML samples collected at initial diagnosis with relapse samples from adult and paediatric cases, adults typically had around 400 statistically significant differently expressed genes between the two disease stages. Paediatric patients had around 200 differently expressed genes," says Holmfeldt.

The group's findings have revealed a number of novel or previously unappreciated differentially expressed genes associated with tumour progression in AML.

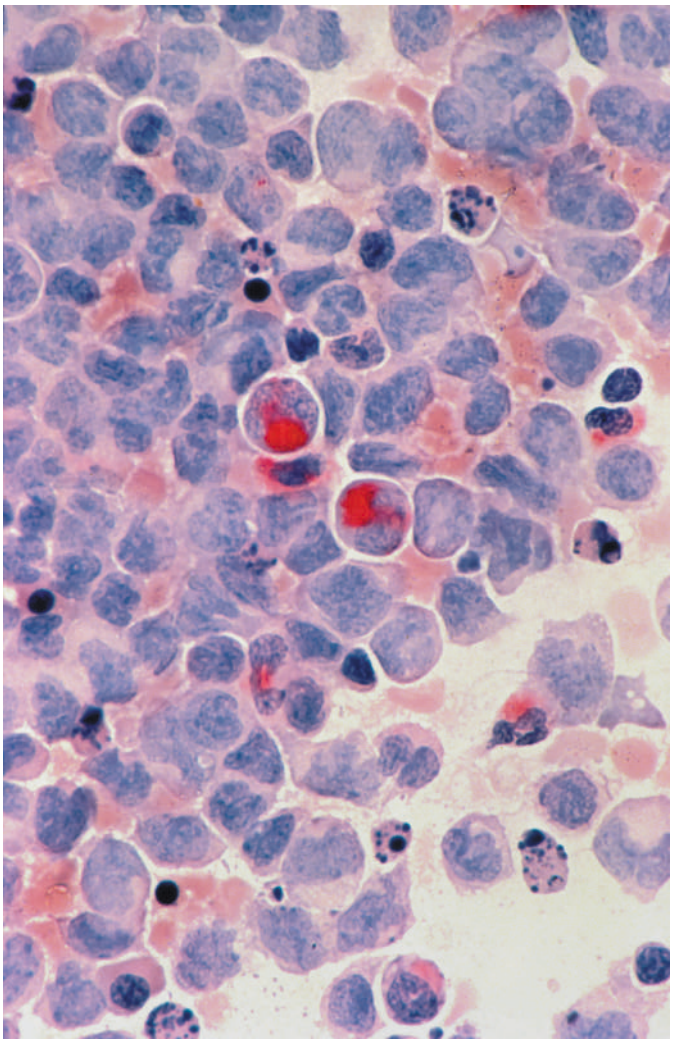
"Certain genes kept popping up in our analyses. Some pro-inflammatory ones [IL1R1 and GLI2, for example] were expressed at a much higher level in those patients who relapsed quicker. One gene inhibiting inflammation [ST18] was expressed at a much lower level, " she explains.

Altogether, the researchers found six or so genes of specific interest including CR1, DPEP1, GLI2, IL1R1, and ST18. "Within these, we identified CR1 down-regulation and DPEP1 up-regulation as specific for relapse in AML both for adults and children," says Holmfeldt.



Qlucore Volcano plot showing down-regulated ST18 (inhibitor) and up- regulated IL1R1 and GLI2 (pro-inflammatory) in AML, associated with short event-free survival.

The finding regarding GLI2, IL1R1, and ST18 was a highly significant result as it matched data from two other cohorts. These were an independent adult AML cohort from TCGA (The Cancer Genome Atlas) and an independent paediatric AML cohort from TARGET (Therapeutically Applicable Research To Generate Effective Treatments). Both cohorts showed low ST18 levels as well as high GLI2 and IL1R1 levels being associated with shorter overall survival as well as shorter event-free survival.



Finally, machine learning–based and network-based analysis also identified overexpressed CD6 and downregulated INSR as highly co-predictive genes depicting important relapse-associated characteristics among adult patients with AML.

The findings highlight the importance of a tumour-promoting inflammatory environment in leukaemia progression, as indicated by several of the differentially expressed genes.

Holmfeldt says that it also looks as if there are factors that make the leukaemia cells proliferate at slower speeds. "Chemotherapy primarily targets cells that are proliferating fast. Cancer cells that are proliferating very slowly, go under the radar, which makes them less sensitive to chemotherapy," she explains.

"Treatment-wise, our data suggest that if you can inhibit pro-inflammatory pathways, it might make the chemotherapy work better and increase survival times. By doing this you could make the leukaemia cells more responsive to treatment by changing their environment."

Together, this knowledge provides the foundation for novel personalised drug targets and has the potential to maximise the benefit of current treatments to improve cure rates in AML.

Sophisticated Analysis Without Bioinformatics Background

"Qlucore Omics Explorer is great because it allows users lacking a bioinformatics background to perform sophisticated analyses of large datasets, as well as to generate highly useful figures of publication quality," says Holmfeldt.

For the next research project, the group will be using the same cohort at the proteome level. "We now have the transcriptomes from our entire AML cohort to use as a database to map our peptides to. We might be able to pick up novel leukaemia-specific peptides that come from regions previously thought to be untranslated, " she adds.

"In parallel, we will analyse the proteomics data in Qlucore again to look at what proteins are around at diagnosis compared to relapse. We hope that our findings may be used as biomarkers or as targets for immunotherapy. For proteomics analysis, we are very lucky to have Qlucore."

REFERENCE

1. Transcriptomic analysis reveals proinflammatory signatures associated with acute myeloid leukemia progression by Svea Stratmann et al; Blood Adv. (2022) 6 (1)
2. Genomic characterization of relapsed acute myeloid leukemia reveals novel putative therapeutic targets by Svea Stratmann et al; Blood Adv (2021) 5 (3)

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