Abstract Book

Forskning och Utveckling- Klinisk kemi, 27 november 2024

Group Linda Fogelstrand

Analyser för behandlingsstyrning vid akut myeloisk leukemi

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Akut myeloisk leukemi (AML) är en form av blodcancer. I Sverige drabbas cirka 370 personer årligen, främst vuxna men även en del barn. Sjukdomen orsakas av genetiska avvikelser såsom mutationer och kromosomförändringar. Behandlingen består av cellgifter samt i vissa fall stamcellstransplantation och/eller riktade läkemedel. Prognosen påverkas starkt av genetiska faktorer och behandlingssvar. Vårt mål är att förbättra prognosen för patienter med AML genom precisionsdiagnostik och personanpassade behandlingsstrategier. I vår forskning utvecklar vi verktyg för att analysera små mängder leukemiceller, så kallad mätbar kvarvarande sjukdom (MRD), som kan användas för behandlingsstyrning. Vi ökar också kunskapen om genetik och sjukdomsförlopp vid AML.

Analys av MRD under behandlingen påverkar hur intensiv behandlingen ska vara och MRD-signaler efter behandling kan indikera ett förestående återfall, vilket möjliggör tidiga insatser och därmed bättre chanser till bot. Resultaten från vår tidigare forskning om MRD-analys med flödescytometri har implementerats i behandlingen för barn med AML och varit en starkt bidragande faktor till att fler barn nu överlever sjukdomen. Vi har också utvecklat en ny MRD-analys kallad djupsekvensering, som baseras på vilka mutationer som finns i leukemicellerna. Våra pågående projekt inkluderar retrospektiva studier av den prognostiska betydelsen av djupsekvensering av NPM1-mutationer vid AML hos vuxna och FLT3-ITD vid AML hos barn, samt av flödescytometrisk MRD-analys vid AML med RUNX1::RUNX1T1. Vi genomför också prospektiva studier med djupsekvensering av andra mutationer för tidig upptäckt av återfall hos patienter behandlade för AML. Inom det nationella samarbetet Genomic Medicine Sweden kartlägger vi genetiska förändringar vid AML. Vår nära koppling till de kliniska laboratorierna vid Klinisk kemi och Centrum för medicinsk genomik gör att vi kontinuerligt kan överföra våra resultat till vården av AML-patienter.

Group Pegah Johansson

Diagnostics for preventing severe side effects of radiation and chemotherapy in cancer patients

Group members: Shaghayegh Gharaghani, Yasaman Shamshirgaran, Anna Lyytikäinen, Hedvig Hjerpe, Simon Nyeboe, Pegah Johannson (PI)

As the cornerstones of cancer treatment, radiotherapy and chemotherapy contribute significantly to cure rates. However, the frequency of patients with severe side effects presents a challenge, particularly with an increasing number of cancer survivors. Our research focuses on reducing life-threatening, and severe side effects associated with radiation and chemotherapy in cancer treatment. We are developing diagnostic assays to predict individual patient sensitivity to these therapies. These assays are based on variation in patients' T cells to repair DNA and survive treatment in vitro. We also investigate biomarkers of treatment toxicity in hematopoietic stem cells derived from induced pluripotent stem cells to predict severe hematologic toxicity and bone marrow failure. Our group aims to use these assays, alongside patients' germline genetic profile, to predict and prevent severe treatment toxicity, with a particular focus on childhood cancer.

Current projects:

- Developing new methods to analyze DNA damage and DNA repair as a result of treatment in the patient's white blood cells.
- Identifying new biomarkers that can predict therapy sensitivity in hematopoietic stem/progenitor cells using iPSC cell models.
- Mapping the molecular mechanism behind the variation in DNA repair during radionuclide therapy.
- Evaluating our analyses through studies in different patient groups who have undergone cancer treatment, with a focus on childhood cancer.

Group Emma C. Josefsson

Platelet function and coagulation in health and disease

Emma C. Josefsson

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Platelets help stop bleeding and contribute to wound healing. In vascular injury, platelet aggregation is the last step in primary hemostasis and is preceded by platelet adhesion and a granule release reaction. To form a stable thrombus, fibrin formation in secondary hemostasis is needed. The coagulation cascade is initiated by tissue factor exposure and binding of factor VII. A subpopulation of highly activated platelets exposes phospholipids on their surface providing a negatively charged surface where coagulation factors bind and form complexes. Subsequently, thrombin is generated and mediates the conversion of fibrinogen to fibrin. The Josefsson group investigates platelet function and coagulation in health and disease. The current research projects involve the assessment of platelet granule release in the diagnostics of platelet function disorders, examine FVIII-inhibitor kinetics related to acquired hemophilia, and explore the potential of platelet- and neutrophil-released proteins as biomarkers in inflammatory disease.

Group Erik Smedler

Unravelling Neural Networks in Psychiatric Disorders and Brain Cancer: From Molecular Insights to Functional Dynamics

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Psychiatric disorders such as bipolar disorder, schizophrenia, and major depressive disorder (MDD) share common features of dysregulated neural networks. In parallel, brain cancer, particularly glioblastoma, often involves similar aberrant connectivity with healthy brain cells. Our research at the Smedler lab and conducted at the Department of Laboratory Medicine at the Sahlgrenska University Hospital, aims to elucidate the molecular underpinnings and functional dynamics of neural networks in these conditions. We focus on four main objectives. First, in bipolar disorder, we investigate the role of calcium signaling during neural development, using induced pluripotent stem cell (iPSC) and organoid models to track changes in neuronal differentiation and connectivity over time. Of note, genome-wide association studies have identified SNPs in the *CACNA1C* gene, and we utilize iPSC models to explore this variant in both healthy controls and BD patients. For schizophrenia, we are developing stem-cell-based models to study postnatal neurodevelopment, examining SCZ-risk genes in neuron-glia co-cultures to understand their role in neural maturation. BD and SCZ share significant genetic and clinical overlap, and we plan to compare iPSC-derived neurons from both disorders. In this context, deep phenotyping using clinical data is crucial, with patient responses to treatments such as lithium serving as a key stratification method. In MDD, we employ induced pluripotent stem cell (iPSC) models to unravel the molecular mechanisms of psilocybin's antidepressant effects. This includes studying its impact on synaptic density and electrophysiological properties through multi-electrode arrays and immunocytochemistry. Lastly, in glioblastoma, we focus on pacemaker cells that synchronize abnormal neural networks, using Ca^{2+} imaging, single-cell RNA sequencing, and electrophysiology to identify therapeutic targets. Our integrated approach, spanning molecular, cellular, and functional analyses, provides new insights into the shared and distinct features of neural network dysfunctions in psychiatric and cancerous conditions, offering potential pathways for future therapeutic strategies.

Keywords: *bipolar disorder (BD); calcium signaling; glioblastoma; induced pluripotent stem cells (iPSCs); major depressive disorder (MDD); neural network dysfunction; schizophrenia (SCZ)*

Group Fredrik Sterky

Att bygga en synaps – Betydelsen av adhesionsreceptorer och deras reglering

Simone Schwarzer, Berta Marcó de la Cruz, Debora Kaminski, Fredrik H. Sterky

Våra tankar, känslor och upplevelser uppstår ur informationsflödet mellan nervceller i vår hjärna, vilket sker via små kopplingar som kallas synapser. Bildandet av synapser regleras av adhesionsproteiner på nervcellens yta, som binder till motsvarande proteiner på den mottagande nervcellen. Dessa interaktioner tros inte bara vara avgörande för att synapser ska bildas, utan också för deras funktion då sammanstättningen av adhesionsproteiner i en specifik synaps bidrar till att definiera dess egenskaper. Genetiska varianter i flera av dessa adhesionsproteiner, såsom Neurexiner, har kopplats till en ökad risk för neuropsykiatriska sjukdomar, exempelvis autism och schizofreni.

Vi forskar på hur synapser bildas och omsätts i odlade mänskliga nervceller. Med hjälp av verktyg för experimentell genetik, såsom CRISPR/Cas9-teknik, kan vi specifikt modifiera eller avlägsna utvalda proteiner för att studera deras roll i synapsbildning och kartlägga de mekanismer som styr denna process. På sikt kan denna kunskap komma att utnyttjas för att motverka obalanser i hjärnans synapser som uppkommer till följd av medfödda och förvärvade sjukdomstillstånd.

Funktionella studier för precisionsmedicinsk diagnostik av medfödda metabola sjukdomar

Myra Nett, Debora Kaminski, Fredrik H. Sterky

Sjukdomar som uppstår till följd av en medfödd genetisk variant klassas ofta som sällsynta på grund deras låga prevalens, men då de är 1000-tals till antalet drabbas sammantaget en ansenlig andel av befolkningen. Många av dessa sjukdomar leder till livslånga handikapp genom att påverka hjärnans normala utveckling, till exempel de neurometabola sjukdomarna. Att kunna ställa en molekylär eller genetisk diagnos är av stor betydelse för att kunna erbjuda drabbade familjer adekvat vägledning, och diagnostikens roll kommer att bli allt viktigare i takt med att specifika behandlingar utvecklas. Teknisk utveckling har möjliggjort att en patients hela arvsmassa nu kan analyseras inom rutinsjukvården. Trots detta förblir många barn utan definitiv diagnos. Detta beror ofta på svårigheter att tolka betydelsen av en specifik variant, vilket resulterar i otillfredsställande rapporter om *'variant of unknown significance'* (VUS).

För att avgöra om en viss identifierad genetisk variant kan vara sjukdomsorsakande utför vi fördjupad molekylär diagnostik i patient-specifika cellulära modeller. I vårt forskningslaboratorium på Klinisk Kemi använder vi avancerad molekylärbiologisk metodik för att studera effekten av dessa varianter på protein- och RNA-uttryck i patientceller (oftast fibroblaster) eller andra modeller designade för att efterlikna den specifika patienten. Analysen kan inkludera metoder som RNA-sekvensering, Western blot och fluorescensmikroskopi. För experimentell genetik använder vi verktyg som lentiviral komplementering och CRISPR/Cas9-teknologi.

Utöver att förbättra den molekylära diagnostiken kan dessa funktionella analyser av patient-specifika cellulära modeller ytterligare klarlägga sjukdomsmekanismer utforska nya behandlingsmöjligheter. Vårt långsiktiga mål är att bygga upp en infrastruktur för precisionsmedicin av sällsynta medfödda sjukdomar vid Sahlgrenska.

Neurofilament light chain clearance through microglia and the implications of microglial states for biomarker interpretation

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Objectives: Neurofilament light chain (NfL) is a protein released by degenerating neurons and serves as a biomarker for various neurodegenerative diseases. In these diseases, microglial activation is a hallmark of disease onset or progression. Interestingly, recent studies suggest that NfL might not be such a stable biomarker as previously thought. Studies report that patients that received the antibiotic minocycline, which is known to inhibit microglial activation, showed elevated NfL levels. Similarly, mice receiving minocycline show an increase in NfL levels. Here, we investigate how microglia can affect NfL levels.

Methods: We use human iPSC-derived cell models differentiated to microglia, neurons, or astrocytes. To investigate the role of microglia in neurofilament light chain (NfL) clearance, we measure NfL levels in co-cultures of microglia and neurons across different conditions The uptake of Alexa-Fluor-488-labeled NfL was tracked via fluorescence microscopy.

Results: We demonstrate that the presence of microglia in neuronal cultures reduces NfL levels. In co-cultures treated with minocycline, NfL levels increase. Furthermore, by tracking labelled human recombinant NfL, we observe its uptake by microglia in both monoculture and in co-culture. Pre-treatment with minocycline significantly reduces uptake of labelled NfL. Finally, RNA sequencing analysis of microglia incubated with and without minocycline reveals changes in gene expression related to microglial functions.

Conclusion: Our data suggest that microglia might play an active role in modulating NfL levels through uptake and the inhibition of this process by minocycline may account for the elevated NfL levels observed in patients. These findings highlight the importance of considering microglial activation states when assessing and interpreting NfL levels in patients.

Deep Sequencing Is a Widely Applicable Tool for Relapse Prediction in Acute Myeloid Leukemia with Mutated NPM1

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Introduction: Analysis of measurable residual disease (MRD) is a powerful tool for assessment of treatment response and monitoring after treatment in acute myeloid leukemia (AML). For patients with mutations in *NPM1*, the most common genetic aberration in AML, MRD analysis with RT-qPCR is recommended. Since RT-qPCR is mutation-specific, many clinical labs limit *NPM1* monitoring to the most common type, although more than 90 different mutation types have been identified in exon 12. Deep sequencing is an alternative method, that covers all *NPM1* exon 12 variants in the same assay. Guidelines for its clinical use are however lacking. We here performed a retrospective analysis in a population-based cohort of AML patients to evaluate if deep sequencing MRD analysis of *NPM1* provides prognostic information.

Method: The study included 97 adult patients with *NPM1*-mutated AML in Region Västra Götaland during 2006-2016, that were treated with curative intent and achieved remission. In total 257 bone marrow samples were analyzed with deep sequencing after first cycle of chemotherapy, during consolidation and at the end of treatment. MRD positivity was defined as *NPM1* mutation at ≥0.05% variant allele frequency based on a previous comparison with RT-qPCR.

Results: MRD positivity at any time during consolidation was significantly associated with a higher risk of relapse and death: 3-year relapse-free survival 26.7%±11.4% vs 71.6%±6.2% (*p*<0.001) and overall survival 33.3%±12.2% vs 72.7%±6.0% (*p*=0.004). There was also a strong prognostic value of deep sequencing MRD status at the end of treatment. In the multivariate analysis, MRD positivity during consolidation was the strongest predictor for relapse (HR 2.54, 95% CI 1.17-5.53, *p*<0.019).

Conclusion: We conclude that MRD status by deep sequencing of *NPM1* is predictive of both relapse-free and overall survival when assessed during or after consolidation. Since deep sequencing can be used for all *NPM1* mutations, it is widely applicable and can be used in patients with rare mutations in *NPM1*.

The differential and temporal effects of a single or dual inflammatory insult on microglial activation states

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Objectives: Neuroinflammation is a key defence mechanism in the brain, primarily orchestrated by microglia, which play an essential role in responding to injury and infection. However, a prolonged or excessive response by microglia to inflammation can exacerbate neuronal damage and impair synaptic plasticity, significantly disrupting brain function and contributing to the progression of neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. Inflammation in the brain is typically a dynamic, multi-phase process, often involving multiple inflammatory triggers. Interferon gamma (IFNg), which has been found in the brains of Alzheimer's patients, acts as a "primer" of microglia, so when a secondary inflammatory trigger occurs, it can lead to an intensified microglial activation state. Here, we investigated the dual effect of IFNg and lipopolysaccharide (LPS) on the inflammatory activation cascade of microglia.

Methods and Results: We used human iPSC-derived microglia in monoculture to investigate the "priming" effect of IFNg alone or in combination with LPS. Our data revealed not only a differential effect on the gene expression of several inflammatory related markers, but we also discovered temporal changes in inflammation and morphological and motility changes in microglia exposed to either a single or a dual inflammatory insult. RNA sequencing analysis of the microglia also revealed a robust and varied effect on several different molecular functions and biological processes.

Conclusion: Taken together, these findings emphasise the importance of understanding the temporal effects, the differential response of pro-inflammatory cytokines and the diverse impact of dual inflammatory insults on the activation and response of microglia during inflammation.

A practical approach to determine assay specific cut-offs for thyroid peroxidase antibodies

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BACKGROUND: Within a study to establish reference intervals of thyroid hormones during pregnancy, thyroid peroxidase antibodies (TPOab) were measured using three commercial assays. Depending on the assay, assay specific cut-offs, and strategies to establish cut-offs, TPOab prevalence varies. We aimed to evaluate the best possible cut-offs for TPOab using the background variability and the patient population.

METHODS: 150 thyroid healthy individuals were sampled at three occasions during pregnancy, one sample for each trimester (450 samples in total). The samples were analyzed on three instrument platforms at three different hospitals. The number of TPOab positives for each method was determined based on the cut-offs provided by the manufacturer. New cut-offs were estimated based on the assays' background noise and verified using the results from the consecutive sampling regime of individuals with antibodies. True positives were expected to display gradually decreasing TPOab concentrations as pregnancy progressed. To validate the new cut-offs, the TPOab prevalence in three patient populations were determined.

RESULTS: In individuals with positive TPOab, the concentration was highest in the sample representing the first trimester. With the manufacturers cut-offs, the ratio of positive TPOab in the 450 samples was 10%, 12% and 6% with Alinity (Abbott), Centaur (Siemens) and Cobas (Roche), respectively. With the new cut-offs, there was a higher agreement between assays; the ratio of TPOab positives with Alinity, Centaur and Cobas, were 13%, 10% and 10%, respectively. The TPOab prevalence in patient results using the new cut-offs (manufacturer cut-off in brackets) were for Alinity, Centaur and Cobas, 41% (39%), 35% (41%) and 36% (31%), respectively.

CONCLUSIONS: TPOab prevalence is assay dependent. A novel approach to determine cut-offs for antibody assays based on background noise is proposed. It is suitable for commercial TPOab assays, but lot-to-lot variations and/or low dynamic range (low signal-to-noise ratio) affects the assays sensitivity.

Development of an assay to predict patient sensitivity to radiation in white blood cells.

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Radiation and Chemotherapy are the most common treatments for cancer. There is a large variability in the side effects experienced by patients even resulting in patient morbidity. However, there is no clinical assay available to evaluate sensitivity to treatment and individualize therapy. At least 80% of the sensitivity to therapy is attributable to genetic and epigenetic factors. Variations in DNA repair and cell cycle regulations appear to be the main pathways causing patient. For example, individuals with defects in DNA double-strand break (DSB) repair are hypersensitivity to radiotherapy. We hypothesize that quantifying the efficiency of DSB repair in these patient's cells could potentially serve as an indication of their hypersensitivity to treatment. Thus, we have optimized an assay capable of quantifying the two pathways involved in double-strand breaks in patient cells, namely non-homologous end joining (NHEJ) and homologous recombination (HR). To achieve this, we used transient transfection of pathway specific reporter transgenes into primary T-cells isolated from patient peripheral blood samples. These transgenes have been reported to express the GFP reporter gene upon successful repair via either NHEJ or HR. By quantification of GFP positive cells using flow cytometry, we can measure the efficiency of these repair pathways in individual patient T-cells. The percentage of cells that exhibit GFP positivity normalized for transfection efficiency provides a quantitative measure of NHEJ or HR efficiency. We have observed a considerable variability in the efficiency of NHEJ and HR among apparently healthy individuals. The assay shows high day-to day and technical reproducibility. Our data indicate that the inhibition of NHEJ kinase (DNA-PK) reduces NHEJ in primary T-cells, while inhibition of the protein involved in HR, ATM inhibitor, reduces HR confirming the specificity of the assay. This assay holds promise in hypersensitivity to treatments such as radiation.

Presence of leukemia-related basophils and mast cells with atypical immunophenotype during induction should not be interpreted as measurable residual disease in children with acute myeloid leukemia with RUNX1::RUNX1T1

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Most pediatric acute myeloid leukemia (AML) protocols utilize flow cytometry (FCM) measurable residual disease (MRD) to assess treatment response during induction. In AML with *RUNX1*::*RUNX1T1*, this is complicated by: 1) discrepancy between FCM MRD and reverse transcription quantitative PCR (RT-qPCR) MRD and 2) high basophil and mast cell levels with atypical immunophenotype interpreted as MRD in FCM analysis.

We here aimed to improve FCM MRD analysis in AML with *RUNX1*::*RUNX1T1*, focusing on basophils and mast cells.

Our study included 45 children treated with NOPHO-DBH AML 2012 protocol during 2013-2020 from Sweden, Finland, Norway, Denmark, Israel and Hong Kong, where FCM analysis included basophil markers. For comparison, 70 children with AML without *RUNX1*::*RUNXT1* from Sweden treated with the same protocol were analyzed. Clinical FCM files were enumerated for basophils (CD123+, HLA-DR-, CD33+) and mast cells (CD117++, HLA-DR-, CD33+). For gene expression profiling, the public pediatric AML dataset TARGET was used.

Children with *RUNX1*::*RUNX1T1* had elevated basophil and mast cell levels at diagnosis and day 22 after 1st induction. Often, basophils displayed an atypical immunophenotype with lower expression of CD123, CD38, CD11b, and CD13, and higher CD34 and CD117, and mast cells with lower expression of CD117. Cases with high basophils were associated with higher levels of atypical basophils and mast cells at diagnosis, and elevated mast cells at diagnosis and day 22, compared to low level cases. No difference was observed for clinical characteristics or relapse frequency. Similar results were obtained for mast cells. Compared to children with other types of AML, elevated levels and atypical immunophenotype of basophils and mast cells were unique to

AML with *RUNX1*::*RUNX1T1*. Leukemic origin of atypical cells was confirmed by FCM cell sorting and RT-qPCR of *RUNX1*::*RUNX1T1*. Gene expression profiling showed that AML with *RUNX1*::*RUNX1T1* was enriched for genes expressed by the common myeloid progenitor with eosinophil/basophil/mast cell differentiation potential.

In conclusion, children with AML with *RUNX1*::*RUNX1T1* often have increased atypical basophils and mast cells during induction treatment. These cells are leukemia-related, might reflect the cell of origin, and not associated with worse prognosis. Therefore, they should not be interpreted as MRD.

The microglial receptor AXL is involved in the antiviral defense against HSV-1 infection.

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Background
Herpes simplex virus type 1 (HSV1) is a very common virus and known for causing cold sores. In rare cases, HSV1 may enter the brain to cause a severe herpes simplex encephalitis (HSE). As the innate immune cells of the brain, microglia protect against viral infections through sensing the virus and release of the antiviral type I interferons (IFN-Is). IFN-Is induce a wide range of interferon-stimulated genes (ISGs) which act in concert to exert antiviral effects. One such ISG is the microglial receptor AXL which a phagocytic receptor known to play important roles in innate immune processes. The role of AXL in the antiviral defense in response to HSV-1 infection is, however, unknown.

Aims
Immunological control of viral infection in the brain is essential for immediate protection, but also for long-term maintenance of brain integrity. The aim of this study was to investigate in detail the role of the microglial receptor AXL during herpesvirus infection.

Methods
Here, we used human induced pluripotent stem cell (iPSC)-derived microglia and neurons. CRISPR- Cas9 technology was employed to create *AXL* knockout (KO) iPSCs.

Results
AXL KO microglia differentiated normally, morphology and marker expression were indistinguishable from controls. AXL is strongly induced in control microglia upon stimulation with IFNβ alone and by HSV-1 infection. Since HSV-1 primarily replicates in neurons, we investigated viral replication in neuron-microglia co-cultures. We found significantly increased viral replication when neurons were co-cultured with *AXL* KO microglia suggesting impaired antiviral responses. In *AXL* KO microglia, we observed decreased viral uptake, decreased viral sensing, and consequently impaired induction of *IFNb* and key *ISGs*.

Conclusion
Our findings suggest that AXL is an important mediator of the microglial response to HSV-1 infection, contributing to viral uptake, sensing, and subsequent activation of antiviral pathways.