Z3: Qualitative and quantitative proteomics for a comprehensive picture of protein dynamics in leukemia

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Summary

- Z3 will provide means to all groups within SFB 1074 to perform comprehensive proteomics in order to identify and quantify proteins and post-translational modifications (PTM) in samples with varying complexity.
- Understanding PTMs and their dynamics is essential to gain insights into leukemic processes. Z3 will provide and develop methods to comprehensively identify and quantify various modifications, phosphorylation in particular, and thus allow to generate a complete picture of cellular events under leukemic conditions.
- Most proteins undergo multiple PTMs during their lifetime, resulting in differentially modified entities, referred to as proteoforms. Gaining knowledge of the specific nature of these proteoforms and the quantitative patterns of their dynamic changes is essential in understanding cellular signalling events. Z3 will engage in the development of methods to allow proteoform characterizations within the various projects of the CRC.
- Tailored proteomic methods will be established to qualitatively and quantitatively analyze the B-cell receptor (BCR) signalling in leukemic and non-leukemic conditions. Emphasis will be given to the analysis of the BCR-complex-associated proteins CD79a and CD79b. This comprehensive proteomic analysis will allow to create a complete picture of the PTM dynamics of CD79a itself and of other B-cell signalosome components in normal and leukemic B cells.

Objectives 2nd funding period

1. Service for members of SFB1074
   - Qualitative and Quantitative proteomics
   - Establishing SILAC conditions in murine and human B cells

2. Establishing enrichment & analysis methods for various post-translational modifications

3. Establishing methods for proteoform characterization

4. Analysis of CD79 modified murine B Cells

Service for members of SFB1074

1. Qualitative and Quantitative proteomics
   - As a core project within this CRC, Z3 will mainly provide means to all groups within SFB 1074 to perform quantitative and qualitative proteomic analyses.

2. Establishing SILAC conditions in murine and human B cells
   - Z3 will help members of the CRC, who want to employ SILAC in their studies, to find suitable cell culture conditions.
   - Ensuring complete incorporation of heavy amino acids and limiting unintended metabolism of heavy amino acids.

3. Generation of super-SILAC mix
   - Various human and murine cell lines will be labeled with heavy amino acids.
   - Combining multiple cell lines will yield a super-SILAC mix.

Comprehensive PTM-analysis Platform

Due to their importance in cellular systems in general and their significant role under disease conditions, the ability to characterize a wide range of different PTMs is essential. Z3 aims to establish and improve protocols for multiple modifications, subsequently allowing projects within the CRC to use these methods to facilitate their own research.

Establishing methods for proteoform characterization

Gaining knowledge about specific PTMs is crucial in understanding cellular signalling events in general and in leukemia in particular. However, most proteins undergo multiple different modifications at different points during their life time. In most cases, the different modifications do not act on their own but in concert with other modifications harbored by the particular protein. The ability to characterize and quantify these proteoforms in a leukemic context is an interesting, yet from a technological point challenging, question.

Analysis of CD79 modified murine B Cells

In a prototypic combination of all aforementioned approaches, tailored proteomic methods will be established to qualitatively and quantitatively analyze the B-cell receptor (BCR) signalling under malignant and normal conditions. Emphasis will be given, but not restricted to the analysis of the BCR-complex-associated proteins CD79a and CD79b. The combination of bottom-up and top-down proteomics will allow to create a complete picture of the PTM dynamics of CD79a itself and of other B-cell signalosome components in normal and leukemic B cells.

Role within the Collaborative Research Centre

A2  H. J. Felting: MLL5-Interactome
A5  A. Rouhi, F. Kuchenbauer: Proteomics on Cell Model
A8  P. Gierschik: PLG2_interactome
A9  E. Hobelka: CD79 Interactomes
B3  L. Bullinger: NPM1-interactome and localization
B6  L.H. Meyer, K.M. Debatin: Phosphoproteomics of Xenograft ALLs
B8  J. Kronke: BRCC36 interactome and ubiquitome
B9  R. Siebert: ID of enhancer site binders
Z1  H. Kastler: Bioinformatics analyses