ELSEVIER

Blood 142 (2023) 3913–3914

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

201.GRANULOCYTES, MONOCYTES, AND MACROPHAGES

Characterization of TREM-1 Signaling in Human Neutrophils By Kinome Array and RNA-Seq

Frederic Ries, MD[1], *Matthias Klein, PhD*[2], *Nora Sophia Lindhauer*[1], *Sophie Többen*[3], *Florian Heidel*[4], *Markus P. Radsak*[1]

[1] Department of Hematology and Medical Oncology, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany
[2] Institute for Immunology, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany
[3] Department of Hematology and Medical Oncology, University Medical Center of the Johannes Gutenberg University, Mainz, Germany
[4] Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School (MHH), Hannover, Germany

The Triggering Receptor Expressed on Myeloid Cells (TREM)-1 is a member of the Immunoglobulin superfamily and an activating receptor mainly expressed on myeloid cells. TREM-1 ligation leads to immediate activation of polymorphonuclear neutrophils (PMN) resulting in degranulation and release of reactive oxygen species as well as various effector molecules. Beyond its role in acute and chronic inflammatory processes, TREM-1 is also involved in cancer emergence and progression probably by alteration of the tumor associated neutrophils (TAN) and macrophages (TAM). Advanced information about the TREM-1 signaling cascade may reveal novel therapeutic approaches, as various kinases can specifically be targeted with kinase inhibitors. Therefore, we investigated the protein tyrosine kinome (PTK) and serine threonine kinome (STK) of human PMN after TREM-1 activation. To gain further insights beyond the signaling cascade, we performed RNAseq to validate the TREM-1 mediated activation and to reveal the TREM-1 mediated transcriptome.

Purified PMN from healthy donors were stimulated with a monoclonal TREM-1 antibody compared to an isotype matched control for 20 minutes (kinome activity) or 60 minutes (RNAseq). For kinomics, PamChip arrays (PamGene) were used to quantify the kinome activity and upstream kinase analysis (UKA) was used to predict the kinase activity. The final data analysis and the UKA was performed by PamGene. A median final score (depending on the significance and the specificity of the kinase activity compared to the control group) > 1.2 was defined as specific kinase activity according to the manufacturer's recommendations. For transcriptomics, Novogene performed library preparation and sequencing, and data analysis was performed with CLC. Differentially expressed genes were defined as FDR adjusted p value ≤ 0.05 and fold change ≥ 2. Gene set enrichment analysis was performed with Enrichr using the libraries *Reactome 2022*, *WikiPathway 2021 Human*, and *GO Biological Process 2023* and significance was defined as adjusted p value ≤ 0.05.

We identified the activation of 31 PTKs and 38 STKs upon TREM-1 ligation. The kinase statistic revealed changes in the activation state of the kinases present in the TREM-1 treated cells compared to the untreated control group ranging from 2.4 to 5.5 (PTK) and from 1.2 to 1.6 (STK) suggesting more TREM-1 mediated activity alteration of the PTKs. Interestingly, there were no downregulated kinase activities after receptor ligation. Out of the PTK family, we identified ten Src family kinases as well as the Syk family member ZAP70 and other kinases such as BTK, already previously shown to abrogate TREM-1 mediated PMN activation.

The RNAseq after TREM-1 ligation revealed 542 differentially expressed genes from which 102 genes were upregulated and 440 genes were downregulated. Gene set enrichment analysis revealed upregulation of genes related to nuclear events (kinase and transcription factor activaton, R-HSA-198725) and cell chemotaxis (GO:0060326) validating the activation of several kinases after TREM-1 ligation. We found increased expressions of genes involved in adipogenesis (WP236) and lung fibrosis (WP3624) suitably to the observed increased activity of Src family kinases that are described to be involved in lung fibrosis. Moreover, some upregulated genes related to NGF-stimulated transcription (R-HSA-9031628) or regulation of transcription in response to stress (GO:0043618) in which the MAPK are known to be involved. However, we did not observe clearly increased MAPK activity. Besides upregulation, TREM-1 ligation led to decreased expression of several genes involved in (positive) regulation of cytokine production involved in inflammatory response (GO:1900017 and GO:1900015) and signaling by interleukins (R-HSA-449147). Taken together, the results contribute to the knowledge about TREM-1 in inflammatory disorders and reveal approaches of its role in cancer.

This study revealed various kinases possibly involved in early TREM-1 signaling of neutrophils that may be suitable targets to inhibit TREM-1 mediated cell activation. Moreover, we linked the TREM-1 mediated kinase activity to the resulting gene expression. Further studies are needed to validate the results *in vitro* and *in vivo* and and to identify suitable pharmacological inhibitors to modulate TREM-1 mediated activities in inflammatory conditions and cancer.

Disclosures Heidel: *Pfizer:* Consultancy, Honoraria; *AbbVie:* Consultancy, Honoraria; *BMS:* Consultancy, Honoraria, Research Funding; *CTI Biopharma:* Consultancy, Honoraria, Research Funding; *AOP Orphan:* Consultancy, Honoraria; *Novartis:* Consultancy, Honoraria, Research Funding; *Janssen:* Consultancy, Honoraria; *Kartos:* Consultancy, Honoraria. **Radsak:** *Corat:* Honoraria; *Cogent Biosciences:* Honoraria; *Incyte:* Honoraria; *Takeda:* Honoraria; *Bristol Myers Squibb:* Other: Travel support; *JAZZ:* Other: Travel support; *Glaxo Smith Kline:* Honoraria; *Beigene:* Honoraria; *Pfizer:* Honoraria; *Abbvie:* Honoraria; *Lilly:* Honoraria; *Otsuka:* Honoraria; *TEVA:* Honoraria; *Daiichi Sankyo:* Other: Travel support; *Novartis:* Honoraria, Research Funding; *Bristol Myers Squibb:* Honoraria; *Novartis:* Other: Travel support; *Amgen:* Other: Travel support; *Abbvie:* Other: Travel support; *Astellas:* Other: Travel support; *SOBI:* Other: Travel support; *AOP:* Other: Travel support.

https://doi.org/10.1182/blood-2023-178717