965 NFAT2 DRIVES THE CYTOSKELETAL REMODELING OF LEUKEMIA CELLS, WHICH REGULATES THEIR VULNERABILITY TO PERFORIN-MEDIATED KILLING

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Background Cytoskeletal remodeling is crucial for migration, infiltration and metastasis and overall tumor aggressiveness. In addition, cytoskeleton remodeling protects from cell membrane damage, e.g. in case of cytotoxic lymphocytes from content of their own cytotoxic granules, but also by preventing perforin/granzyme-induced lysis in case of tumor cells. Antibody dependent cellular cytotoxicity (ADCC) a major effector mechanism by which Rituximab that is routinely used for treatment of chronic lymphocytic leukemia (CLL) mediates lysis of tumor cells and thus therapeutic efficacy. We previously demonstrated that in CLL patients loss of the transcription factor NFAT2 (NFATc1) correlates with an aggressive disease course. NFAT2 further is involved in regulating cytoskeleton reorganization of immune cells. Here we investigated the role of NFAT2 with regard to susceptibility of CLL cells to perforin-induced lysis.

Methods To investigate whether and how loss of NFAT2 affects ADCC in CLL, we generated a CRISPR/Cas9 based NFAT2 knockout (KO) in MEC-1 CLL cells. Cytotoxicity assays revealed profoundly higher resistance of NFAT2 KO CLL cells to NK cell killing compared to scrambled control (SCR) CLL cells, without affecting the level of recognition as revealed by analyses of NK cell activation, degranulation and IFNy release.

Results Next, we investigated the role of NFAT2 in the Eμ-TCL1 mouse model for CLL by inducing a conditional B cell specific NFAT2 knockout. Using this model, we show that CLL cells of TCL1 NFAT2$^{-/-}$ mice are less susceptible to perforin lysis compared to that of TCL1 NFAT2$^{+/+}$ mice. When we classified CLL patients into NFAT2-low and NFAT2-high cases according to the NFAT2 transcript level, CLL cells of NFAT2-low patients showed higher resistance to perforin-mediated lysis and less perforin binding in the cell membrane, confirming our findings obtained with MEC-1 and murine CLL cells.

Finally, inhibition of CDC42, a key regulator of actin cytoskeleton remodeling, with ZCL278 resensitized MEC-1 NFAT2 KO cells to perforin mediated lysis. Similar effects were observed with the tubulin cytoskeleton stabilizing agent Paclitaxel, indicating that cytoskeletal remodeling is crucial for perforin resistance.

Conclusions Together, our results demonstrate that loss of NFAT2 allows CLL cells to evade NK cell effector function. This holds true for both, constitutive cytotoxicity and therapeutically induced ADCC and is due to lowered susceptibility to perforin-mediated membrane permeabilization, which can be overcome by inhibiting cytoskeletal rearrangement. Thus, NFAT2 loss facilitates resistance of CLL cells to immunotherapeutic treatment modalities.

Ethics Approval The study was approved by the ethics committee at the Medical Faculty of the Eberhard Karls University and the University Hospital Tuebingen (reference number 13/2007V). Human material was collected after obtaining informed consent in accordance with the Helsinki protocol.

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