CATHESPIN S ALTERATIONS INDUCE A TUMOR-PROMOTING IMMUNE MICROENVIRONMENT IN FOLLICULAR LYMPHOMA

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Background By targeted DNA sequencing of 305 diagnostic follicular lymphoma (FL) biopsies, we identified somatic mutations of Cathepsin S (CTSS) in 8% of cases (24/305), mostly clustered at Y132 (19/24) converting Y to D (16/19). Another 13% of FL had CTSS amplifications (37/286), associated with higher CTSS expression (P=0.03). CTSS is a cysteine protease that is highly expressed in endolysosomes of antigen presenting cells and malignant B-cells. CTSS is involved in proteolytical processing of antigenic peptides for presentation on MHC-II to be recognized by antigen specific CD4+ T-cells. CTSS is synthesized as an inactive zymogen, which is converted to its active form by autocatalytic cleavage of the autoinhibitory propeptide (pro-CTSS).

Materials and Methods We used CRISPR/Cas9 to introduce CTSS Y132D into Karpas422, a B-cell lymphoma cell line that harbors the FL hallmark translocation t(14;18). We purified pro-CTSS WT and Y132D and assayed the in vitro autocatalytic cleavage over time. We then tested the impact of CTSS on CD4+ T-cell activation in co-culture assays, in a previously described in vivo model which we slightly modified to reflect FL-like conditions, and in primary patient samples.

Results Single-cell derived Y132D mutant Karpas422 clones showed >3-fold higher ratios of active CTSS to pro-CTSS (N=4, P=0.0003). Immunoprecipitated CTSS Y132D had >3-fold higher in vitro substrate cleavage activity compared to CTSS wild type (WT) (N=6, P=0.001) which was mediated by an accelerated conversion from pro-CTSS to active CTSS (11 minutes for CTSS Y132D vs 17 minutes for CTSS WT; N=3, P=0.04). Molecular dynamics simulations showed that the Y132D mutation shortens the distances by ~2Å between the catalytic triad of active CTSS (C139, H278, N298) and a stretch of amino acids from the proform (L80, G81, D82, S94), which could facilitate intramolecular cleavage. The higher substrate cleavage activity of CTSS Y132D came along with a high capacity to stimulate antigen specific CD4+ T cell responses in vitro and in vivo. Additionally, CTSS overexpression could phosphory loose this high CD4+ T cell activation. Lastly, we aimed to correlate CTSS aberrations with clinical outcome in patients who received standard immunochemotherapy (R-CHOP) for advanced FL (N=51 with available CTSS mutation and gene expression data). Compared to all other patients (N=34), patients with CTSS Y132D mutations or CTSS overexpression (N=17) had longer failure free survival (P=0.012).

Conclusions Here, we provide biochemical, structural, functional and clinical evidence that aberrant CTSS activity induces a supportive immune microenvironment in FL. We propose that aberrant CTSS activity can elicit a CD4+ T-cell driven tumor-promoting immune response, which could be amplified within the microenvironment and substantially impact the biology and clinical course of the disease. Thus, aberrant CTSS activity is a promising biomarker and therapeutic target in FL and potentially also other tumors.

REFERENCES