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The Function of Progranulin in Chronic Lymphocytic Leukemia

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Chronic Lymphocytic Leukemia (CLL) is one of the most common malignant B cell diseases. Despite advances in targeted therapies, controlling CLL remains clinically challenging due to large disease heterogeneity and resistance to treatment. *In vivo*, the tumor microenvironment builds crucial supportive niches for the malignant B cells, which rapidly undergo apoptosis *ex vivo* without the support of accessory cells in co-cultures. Therefore, immunotherapy is a yet experimental but emerging approach that is expected to complement future treatment strategies in CLL. Understanding the crosstalk between the malignant B cells and accessory cells does not only increase insight into disease pathology, but also paves the way to identify new therapeutic targets. Progranulin (GRN) was recently found to be elevated in the serum of CLL patients and negatively correlated with prognosis, but its function was not clear. Based on its versatile roles in immunity, proposed growth factor capabilities, and association with other cancers, GRN appeared as an ideal new candidate to be tested as a driver of CLL and mediator within its microenvironment.

The work presented in this thesis dissects and questions the functional significance of GRN in CLL by (i) elucidating its source, (ii) systematically analyzing its effect on malignant and microenvironmental cells *in vitro,* and (iii) by investigating the consequences of GRN deficiency on CLL-like disease development in the Eμ-*TCL1* adoptive transfer mouse model *in vivo*.

(i) First, GRN was quantified in cell culture supernatants of primary human CLL cells isolated from blood samples in co-culture with different accessory cells, such as primary healthy donor blood-derived monocytes, fibroblastic stromal cell lines, and primary healthy donor bone marrow-derived mesenchymal stromal cells by enzyme-linked immunosorbent assays. These experiments revealed that GRN was not only secreted by the malignant cells themselves but also by accessory cells. Moreover, stromal cells were induced to secrete GRN by contact with CLL cells. When detecting GRN in lymph node sections from CLL patients by immunofluorescence stainings, it was predominantly located to $CD68^+$ macrophages within the stroma. In combination with GRN quantifications in the plasma of Eμ-*TCL1* adoptive transfer mouse models described below, this led to the conclusion that the elevated GRN serum levels in CLL are caused by both, secretion of CLL cells themselves, and myeloid and non-hematopoietic stromal cells, and are most likely at least in part attributed to microenvironmental crosstalk.

(ii) Subsequently, the effect of recombinant GRN on CLL and accessory cells was tested *in vitro*. There was no direct effect of GRN detectable on CLL cell viability, activation status

determined by CD86 expression levels, or CLL cell proliferation utilizing different functional assays. Next, I investigated the role of GRN in modifying phenotypes of CLL accessory cells. As of their cytoskeletal composition, mesenchymal stromal cells appeared already in an activated state when cultured *in vitro*. However, when they were co-cultured with CLL cells, they acquired a cancer-associated fibroblast-like phenotype upregulating inflammatory cytokines like CXCL8, CXCL1, and IL1B and proteases like MMP3, MMP1, and CTSK, which was determined by gene expression profiling and partly validated by protein detection. Although GRN was upregulated in these co-cultures and has been previously shown to mediate the differentiation of cancer-associated fibroblasts in other cancers, treatment of mesenchymal stromal cells with GRN did not induce significant changes in their gene expression. In addition, GRN did not alter viability or the inflammatory potential of human monocytes *in vitro*. In conclusion, against the hypothesis GRN did neither reveal any direct supporting effect on CLL cells nor did it alter CLL accessory cells towards tumor-supporting phenotypes in the conducted experiments.

(iii) To investigate the role of GRN *in vivo*, it was made use of the Eμ-*TCL1* adoptive transfer CLL mouse model, which was shown to mimic microenvironmental characteristics during CLL development, in combination with syngeneic *Grn*$^{-/-}$ mice. First, it was demonstrated that GRN is upregulated in the serum of leukemic mice after Eμ-*TCL1* adoptive transfer to wild-type mice compared to non-transplanted controls and thus recapitulates the findings in human CLL. Additionally, splenic cell compositions in *Grn*$^{-/-}$ mice compared to heterozygous controls were characterized by flow cytometry revealing no differences in the microenvironment before tumor transfer. When Eμ-*TCL1* adoptive transfer was performed, the tumor failed to engraft in *Grn*$^{-/-}$ mice in contrast to heterozygous controls which developed leukemia. However, follow-up work showed that this was attributed to a $CD8^+$ T cell-mediated rejection of *Grn*-expressing tumor cells by the GRN-deficient mice. To overcome immunologic tumor rejection, chimeric mice with deficiency of GRN in all bone marrow-derived cells and controls without GRN deficiency were generated and Eμ-*TCL1* adoptive transfer was performed. In this experimental setup, leukemia developed equally in both groups suggesting that lack of GRN in hematopoietic non-malignant cells was not essential for tumor growth. As also GRN plasma levels were not significantly different in both adoptively transferred groups, which is most likely due to the multicellular origin of GRN in CLL, further experiments are needed to clarify whether the elevated GRN levels impact on disease development in CLL. This can be addressed, for example, by crossing transgenic Eμ-*TCL1* mice with *Grn*$^{-/-}$ mice.

In conclusion, the acquired data do not provide evidence that GRN is a significant driver of CLL or crucial mediator within its microenvironment. As of knowledge today, there is thus no indication that antagonizing GRN should be tested as a novel approach in CLL therapy.