Oncogenic Mechanisms of Mutant STAT5B in Natural Killer Cells

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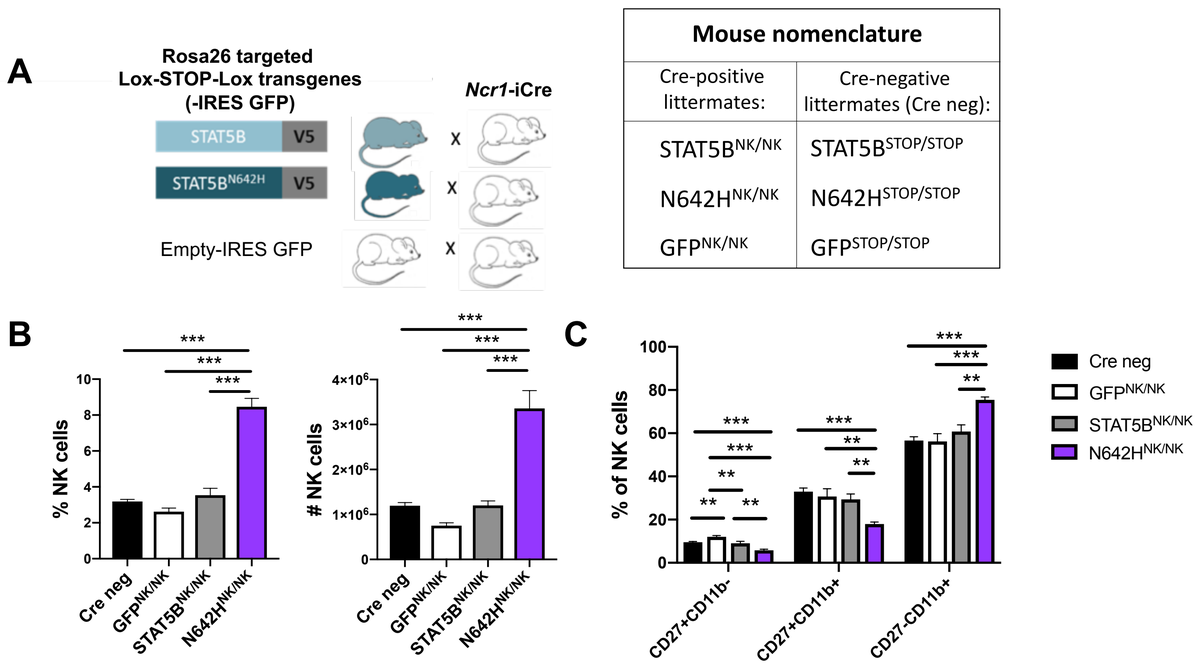
SUMMARY

STAT5B is a master regulator of development, survival and function of innate(-like) lymphocytes including natural killer (NK) cells (1-3). The gain-of-function mutation N642H of human STAT5B is associated with aggressive forms of CD56+ T (NKT) and NK cell lymphomas/leukemias (4-6). A mouse model expressing human STAT5B^{N642H} under the *Vav1* promoter develops severe CD8^{+} T cell neoplasia but no innate lymphocyte disease (7). In the absence of classical T cells, *Vav1*-driven STAT5B^{N642H} promotes aggressive innate-like NKT cell leukemia, resembling CD56+ T-cell large granular lymphocyte (T-LGL) leukemia (8). In the funded project, we aim to investigate whether STAT5B^{N642H} also acts as an oncogenic driver in NK cells and establish an NK cell leukemia model to enable the development of treatment options for currently untreatable NK cell malignancies. To avoid competition with more potently transformed cell types, we generated a novel mouse model in which STAT5B^{N642H} expression is restricted to the NK (NKp46-positive) cell lineage. NK cell-specific STAT5B^{N642H} expression in N642H$^{NK/NK}$ mice results in an increased number of NK cells in young and aged mice, but no disease symptoms have been detected up to an age of 10-13 months. These current data suggest that STAT5B^{N642H} on its own might not be able to transform NK cells, in contrast to other cell types. Ageing experiments will be continued until an age of 15-18 months to investigate an oncogenic potential of mutant STAT5B in NK cells at a later timepoint. STAT5B^{N642H}-expressing and to a lesser extent non-mutant STAT5B-expressing NK cells display a decreased expansion capacity *in vitro* that is associated with morphological changes and a potential "senescence-like" state. In parallel, NK cell cytotoxicity was impaired upon IL-2 culture *in vitro*. In case this phenomenon also takes place *in vivo*, it might provide an explanation for the absence or the delay of NK cell transformation by mutant STAT5B that might require additional hits to overcome the "oncogene-induced senescence". We aim to further explore the role of hyperactive STAT5B signaling in NK cell dysfunction. This knowledge will contribute to a better understanding of the complex regulation of NK cell activities that is valuable as NK cells are being pursued as immunotherapeutic tools.

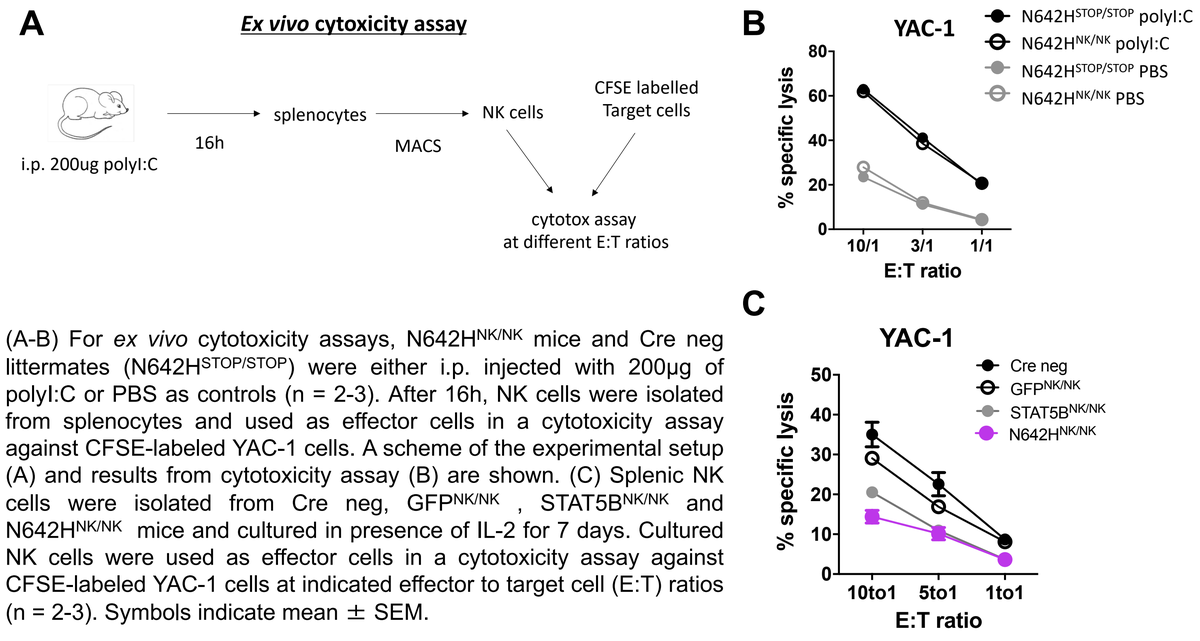
RESULTS

1. Expression of STAT5B^{N642H} in NKp46^{+} cells increases numbers and maturation of NK cells



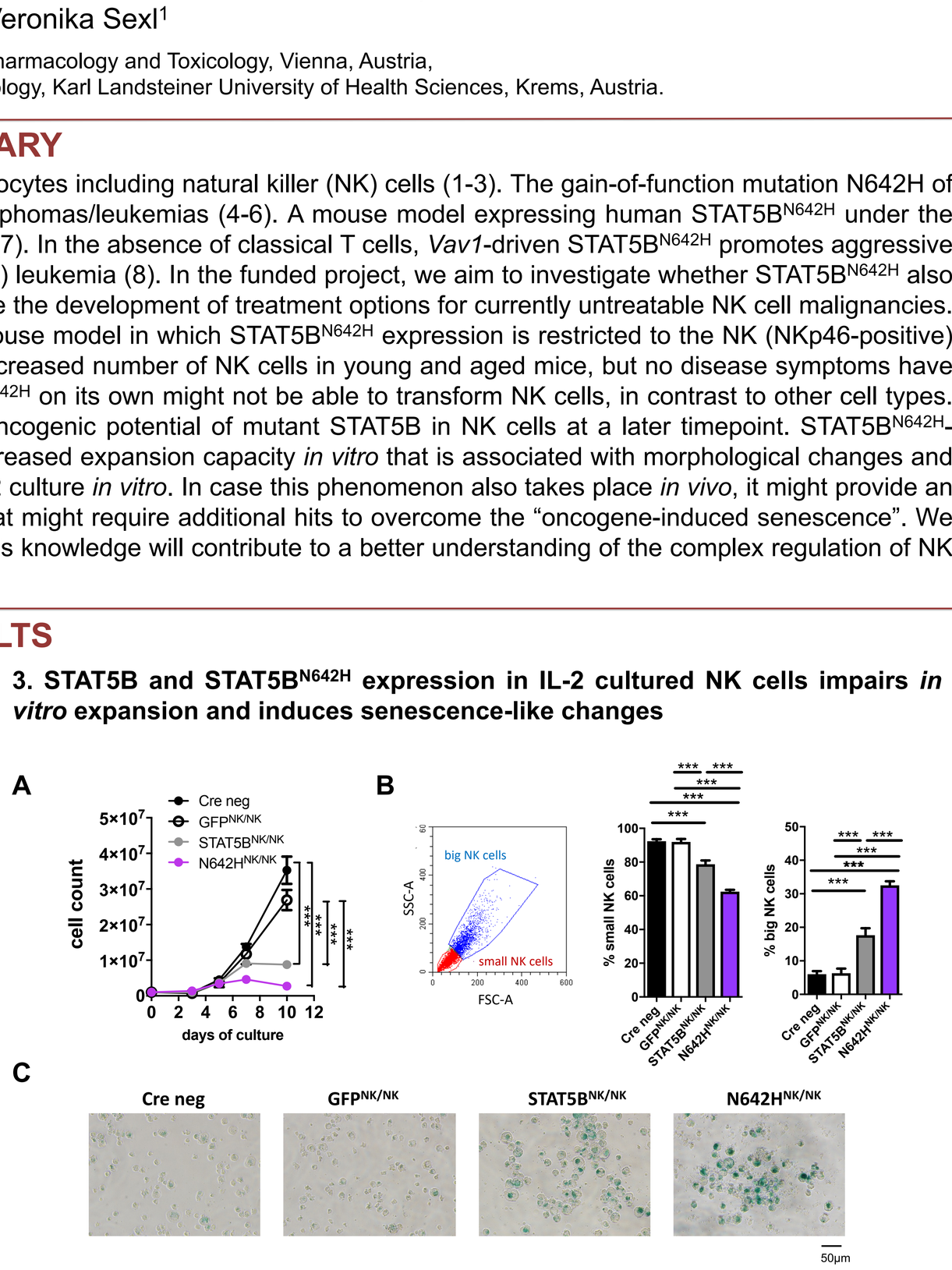
(A) Rosa 26 locus-targeted Lox-STOP-Lox transgenic mice, expressing V5 tagged non-mutant human STAT5B, STAT5B^{N642H} or empty vector IRES GFP, were crossed to *Ncr1*-iCre mice. Mouse nomenclature is indicated. (B) Percentage and numbers of splenic NK cells were analyzed in GFP$^{NK/NK}$, STAT5B$^{NK/NK}$, N642H$^{NK/NK}$ and Cre negative (neg) control mice (pool of GFP$^{STOP/STOP}$, STAT5B$^{STOP/STOP}$ and N642H$^{STOP/STOP}$ mice) by flow cytometry. (C) Splenic NK cells were analyzed for the expression of the maturation markers CD27 and CD11b. Bar graphs indicate mean ± SEM; n=10-15. *** p < 0.001, ** p < 0.01; one-way ANOVA.

2. NK cell cytotoxicity is unaffected by STAT5B^{N642H} expression *ex vivo*, while it is impaired by STAT5B and STAT5B^{N642H} expression upon IL-2 culture



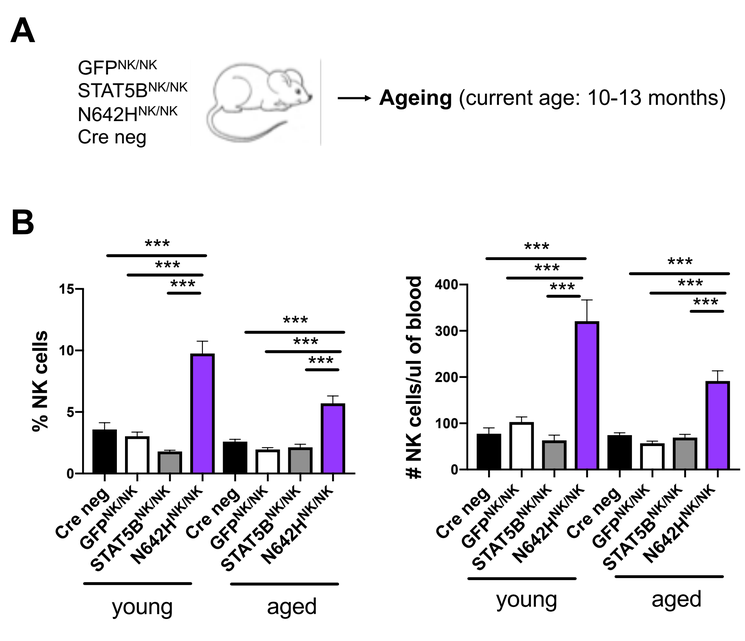
(A-B) For *ex vivo* cytotoxicity assays, N642H$^{NK/NK}$ mice and Cre neg littermates (N642H$^{STOP/STOP}$) were either i.p. injected with 200µg of polyI:C or PBS as controls (n = 2-3). After 16h, NK cells were isolated from splenocytes and used as effector cells in a cytotoxicity assay against CFSE-labeled YAC-1 cells. A scheme of the experimental setup (A) and results from cytotoxicity assay (B) are shown. (C) Splenic NK cells were isolated from Cre neg, GFP$^{NK/NK}$, STAT5B$^{NK/NK}$ and N642H$^{NK/NK}$ mice and cultured in presence of IL-2 for 7 days. Cultured NK cells were used as effector cells in a cytotoxicity assay against CFSE-labeled YAC-1 cells at indicated effector to target cell (E:T) ratios (n = 2-3). Symbols indicate mean ± SEM.

3. STAT5B and STAT5B^{N642H} expression in IL-2 cultured NK cells impairs *in vitro* expansion and induces senescence-like changes



(A) Splenic NK cells were isolated from Cre neg, GFP$^{NK/NK}$, STAT5B$^{NK/NK}$ and N642H$^{NK/NK}$ mice and cultured in presence of IL-2. *In vitro* growth of NK cells was monitored by determining absolute numbers at different timepoints during culture (n = 3-4). (B) After 7 days of culture, percentages of small and big NK cells were determined by flow cytometry (based on FSC/SSC blot, as indicated, gated on living CD3-NK1.1+ cells) (n =6). (C) After 10 days of IL-2 culture, **β**-Galactosidase (**β**-Gal) stainings of NK cells were performed. Representative images are shown. Bar graphs or symbols indicate mean ± SEM; *** p < 0.001; one-way ANOVA.

4. NK cell-specific expression of STAT5B^{N642H} increases NK cell numbers in the blood of aged mice, without signs of a malignancy.



(A) Ageing of GFP$^{NK/NK}$, STAT5B$^{NK/NK}$, N642H$^{NK/NK}$ and Cre neg control mice (n=10-12) is ongoing. No signs of a malignant NK cell disease have been detected until an age of 10-13 months. (B) Ageing mice have been bled at an age of 6-8 months and percentages and absolute numbers of NK cells were analyzed in the blood of aged compared to young (8-10-week-old) (n=5) mice by flow cytometry. Bar graphs indicate mean ± SEM; *** p < 0.001; one-way ANOVA.

CONCLUSIONS and OPEN QUESTIONS

Research question 1: Is STAT5B^{N642H} an oncogenic driver in NK cells?

- **STAT5B^{N642H} increases peripheral NK cell numbers**
- **STAT5B^{N642H} does not seem to be sufficient to transform NK cells**

Open questions:

- **Underlying mechanisms of increased NK cell numbers?**
- **What genetic alterations do NK cells require to get transformed?**

Research question 2: Does hyperactive STAT5B affect NK cell function?

- **Hyperactive STAT5B impairs expansion and cytotoxicity of cultured NK cells**

Open questions:

- **Underlying mechanisms of STAT5B-driven NK cell dysfunction *in vitro*?**
- **Is NK cell-mediated tumor surveillance affected *in vivo*?**

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