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 Vkf7 promoter develops severe CD8+ T cell neoplasia but no innate lymphocyte disease (7). In the absence of classical T cells, Vkf7-driven STAT5B(N642H) promotes aggressive innate-like NK 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 a NK cell leukemia model to enable the development of treatment options for currently untreatable NK cell malignancies.

RESULTS

1. Expression of STAT5B(N642H) in NKp46+ cells increases numbers and modification of NK cells

(A) Rosa 26 locus-targeted Lox-STOP-Lox transgenic mice, expressing V5 tagged non-mutant human STAT5B, STATB(N642H) or empty vector (RES GFP) were crossed to Ncr1-Cre mice. Mouse nomenclature is indicated: (B) Percentage and numbers of splenic NK cells were analyzed in GFFp55m, STATB(N642H), N642H(NK) and Cre negative (neg) control mice (pool of GFFp55m, STATB(N642H), N642H(NK) mouse) 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<0.05; 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) For ex vivo cytotoxicity assays, N642H(NK) and Cre neg labeled (N642H(NK)Cre) were either i.p. injected with 200μL of poly(C) or PBS as controls (n=3-23). After 16h, NK cells were isolated from splenocytes and used as effector cells in a cytotoxicity assay against CFSE-labeled target 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, GFFp55m, STATB(N642H) and N642H(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, GFFp55m, STATB(N642H) and N642H(NK) mice and cultured in presence of IL-2. (B) After 7 days of culture, percentages of small and big NK cells were determined by flow cytometry (based on FSC/SSC binned, as indicated, gated on live CD3-NK1.1+ cells) (n = 6). (C) After 10 days of IL-2 culture, β-Galactosidase (β-Gal) staining of NK cells were performed. Representative images are shown. Bar graphs and symbols indicate mean ± SEM; ** p<0.01; 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) Aging of GFFp55m, STATB(N642H) and N642H(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) Aged 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?

References


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