Investigating the immune-regulatory function of NR4A1/Nr4a1 in aggressive lymphomas

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Background

Aggressive lymphomas represent the most common type of lymphoid malignancies with a five-year survival rate of 60%. Despite effective initial treatment, one-third of all patients will experience a relapse, warranting more research to discover novel therapeutic strategies. We recently detected a significant reduction of nuclear receptor NR4A1 expression in patients with diffuse large B cell lymphomas (DLBCL) that correlated with poor cancer-specific survival.

Aim

The aim of this study is to investigate the immune-regulatory properties of NR4A1/Nr4a1 in aggressive lymphomas.

Material & Methods

(I) Kaplan Meier analysis was performed for survival and tumor formation in EµMyc Nr4a1+/+ (n=134), EµMyc Nr4a1-/- (n=84) and EµMyc Nr4a1+/-/ mice.

(II) RNA-Seq was conducted in a total of 10 tumors from EµMyc Nr4a1+/+ and EµMyc Nr4a1-/- mice (n=5 per group and genotype).

(III) Validation of the differentially expressed genes was carried out by RQ-PCR on fresh-frozen tumor specimens derived from EµMyc Nr4a1+/+ and EµMyc Nr4a1+/+ mice (n=20 per group), including those tumors used for RNA-Seq. Moreover, the same validation was performed on tumors from mice transplanted with either EµMyc Nr4a1-/- (n=14) or EµMyc Nr4a1+/+ (n=7) derived tumors.

(IV) In vivo tumor formation was induced by injection of tumor cells derived from EµMyc Nr4a1+/+ and EµMyc Nr4a1-/- mice into the tail vein of wild type (wt) C57Bl/6 (n=20 per genotype) and Fox Chase SCID Beige (n=15 per genotype) mice.

(V) In vitro cytotoxicity assay using OVA257-264 peptide-pulsed EµMyc Nr4a1+/+ and Nr4a1-/- lymphoma cells and OVA targeting OT1 CD8+ T cells with and without Ctl4a loss was performed to measure T cell-mediated lymphoma cell lysis.

Results

Loss of Nr4a1 augments tumor formation and decreases survival

Visible tumors were developed faster in EµMyc Nr4a1-/- (median 45 days) compared to EµMyc Nr4a1+/+ mice (median 107 days, p=0.001). Moreover, EµMyc Nr4a1-/- showed a decreased survival with a median of 92 days compared to EµMyc mice without Nr4a1 loss (median survival 123 days, p=0.037). Survival and tumor formation gave intermediate values for EµMyc Nr4a1+/+ (n=134) and Nr4a1+/- (n=59) mice.

Genes involved in immunoregulation are upregulated upon loss of Nr4a1

RNA-Seq was performed in a total of 10 tumors from EµMyc Nr4a1+/+ and EµMyc Nr4a1-/- mice (n=5 per genotype). By using an adjusted p-value below <0.1, 57 upregulated and 18 downregulated genes could be detected when comparing EµMyc mice with and without Nr4a1 loss. GO analyses showed that mainly genes involved in immunological processes were enriched in the EµMyc Nr4a1-/- lymphomas.

Loss of Nr4a1 accelerates lymphomagenesis in immunocompetent mice

Decreased survival and a largely similar expression pattern of these immune components were found in tumors from EµMyc Nr4a1-/-. EµMyc lymphoma cells transplanted into immunocompetent C57Bl/6 mice. In contrast to immunocompetent mice, transplanted EµMyc Nr4a1-/- lymphomas into immunodefective Fox Chase SCID Beige mice exhibited an unchanged survival when compared to transplantation of EµMyc Nr4a1+/+ lymphomas. Interestingly, we observed an altered expression of Pdp1-Pdp1-Pdp2- and Ctla4-Cd80-Cd86-axes in the immune-deficient setting. There was no expression of Pdp1 and Ctla4 and a higher expression of Pdp1, Pdp2, Cd80 and Cd86 in Nr4a1 deficient lymphomas.

Reduced lymphoma cell killing in murine lymphoma cells with Nr4a1 loss

To further investigate if the Nr4a1 expression is mandatory for active T cell-mediated anti-lymphoma immune response, we performed co-culture experiments using OVA257-264 peptide-pulsed EµMyc Nr4a1+/+ and EµMyc Nr4a1-/- lymphoma cells and OVA targeting OT1 CD8+ T cells in our coculture cytotoxicity assay. Interestingly, after 16 h and 24 h of co-incubation, no significant specific lysis compared to the Nr4a1+/+ and Nr4a1-/- setting was found.

Conclusion

Our results suggest that the loss of Nr4a1 accelerates the Myc-driven lymphomagenesis in immuno-competent mice. Further, these data indicate that Nr4a1 possesses immune regulatory function and thereby contributes to the immune evasion of aggressive lymphomas by regulating T cell-mediated anti-lymphoma immune responses. Thus, it might serve as a potential target for novel immunotherapeutic approaches to treat aggressive lymphomas.