NR4A3 suppresses lymphomagenesis by induction of apoptosis and serves as a potential drug target for lymphoma therapy

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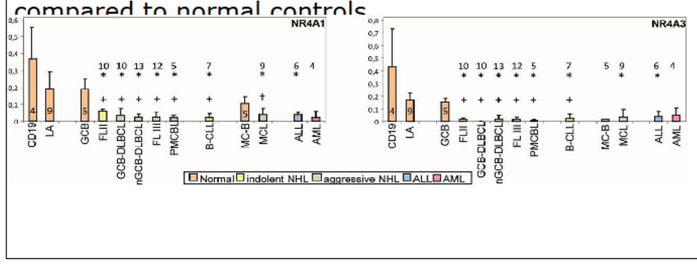
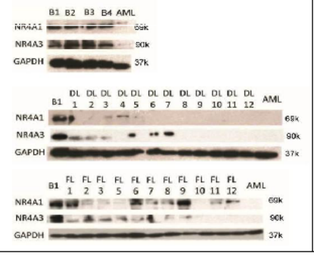
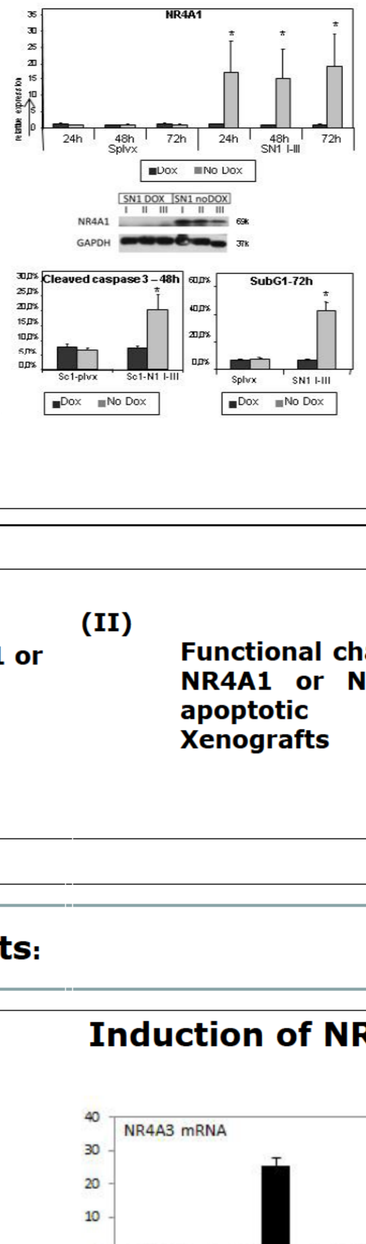
Introduction:

NR4A1 (Nur77) and NR4A3 (Nor-1) are members of the orphan nuclear hormone receptor family referred to as Nur77 family. Both nuclear orphan receptors function as critical tumour suppressors as observed by the rapid development of AML in the NR4A1 and NR4A3 double knock out mouse. Down regulation of NR4A1 and NR4A3 is a common feature in leukemic blasts from human AML patients, irrespective of karyotype. We investigated the expression of the NR4A nuclear orphan receptors in the most common types of indolent and aggressive lymphomas and showed that NR4A1 and NR4A3 expression was significantly down-regulated in chronic lymphocytic B-cell leukemia (CLL, 71%), in follicular lymphoma (FL, 70%), and in diffuse large B-cell lymphoma (DLBCL, 74%) compared to normal controls.

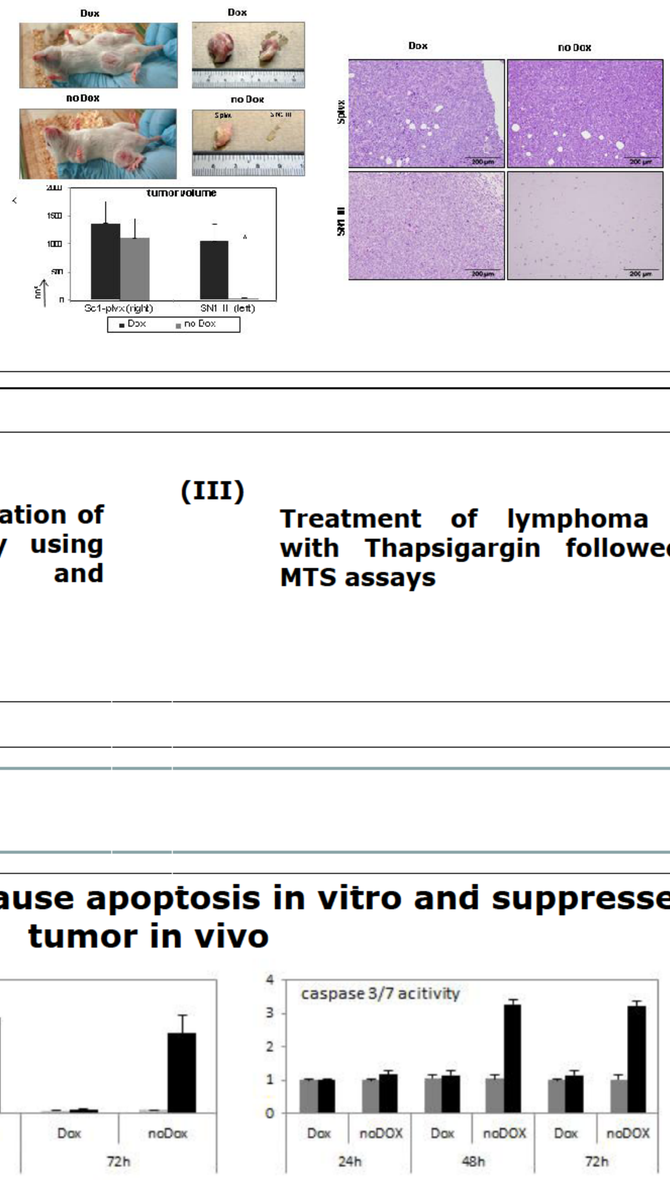




To investigate the function of NR4A1 in lymphomas, we over-expressed NR4A1 in SuDHL4 cells by using an inducible lentiviral expression system and performed apoptotic assays. Induction of NR4A1 expression led to apoptosis in a significantly higher proportion of induced SuDHL4 cells compared to their uninduced controls.



To test the tumor suppressor function of NR4A1 in vivo, the stably transduced SuDHL4 lymphoma cell lines were further investigated in a NOD/SCID/IL-2rγnull (NSG) mouse model. Induction of NR4A1 in Sc-1 cells suppressed their outgrowth in NSG mice, in contrast to vector controls and uninduced SuDHL4 cells, where massive tumor formation was observed.



Purpose:

Since the role of down-regulated NR4A3 in aggressive lymphomas is unknown, we aimed to investigate the function of NR4A3 in lymphoid malignancies.

Methods:

(I) **Transient overexpression of NR4A1 or NR4A3**

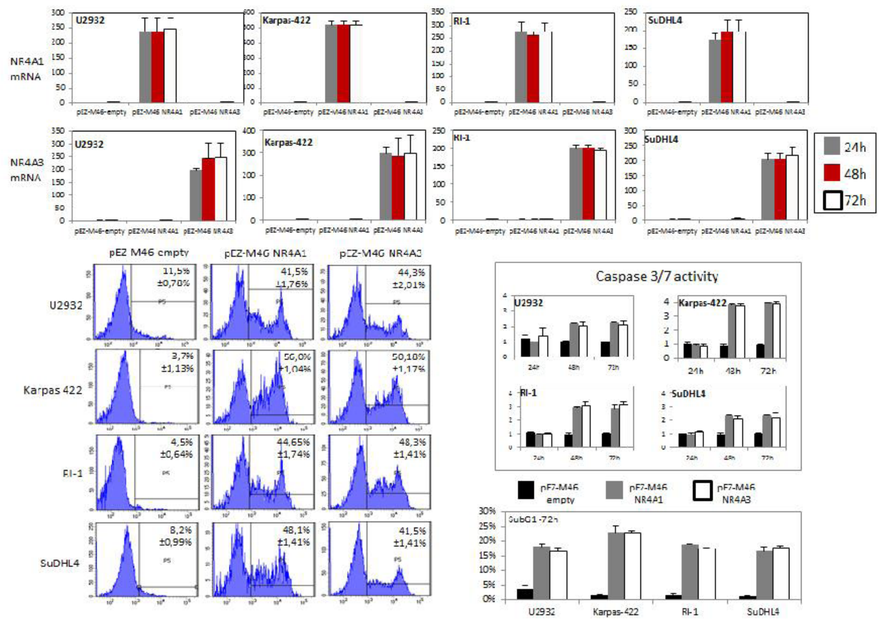
Transduction of NR4A3 in SuDHL4

(II) **Functional characterization of NR4A1 or NR4A3 by using apoptotic assays and Xenografts**

(III) **Treatment of lymphoma cells with Thapsigargin followed by MTS assays**

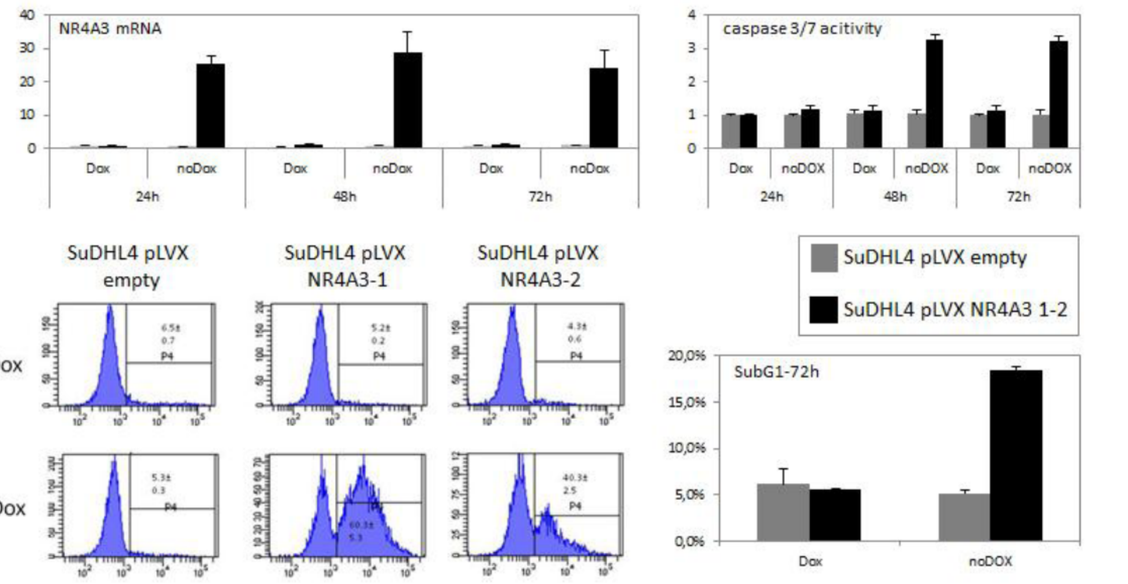
Results:

Overexpression of NR4A1 or NR4A3 in aggressive lymphoma cells



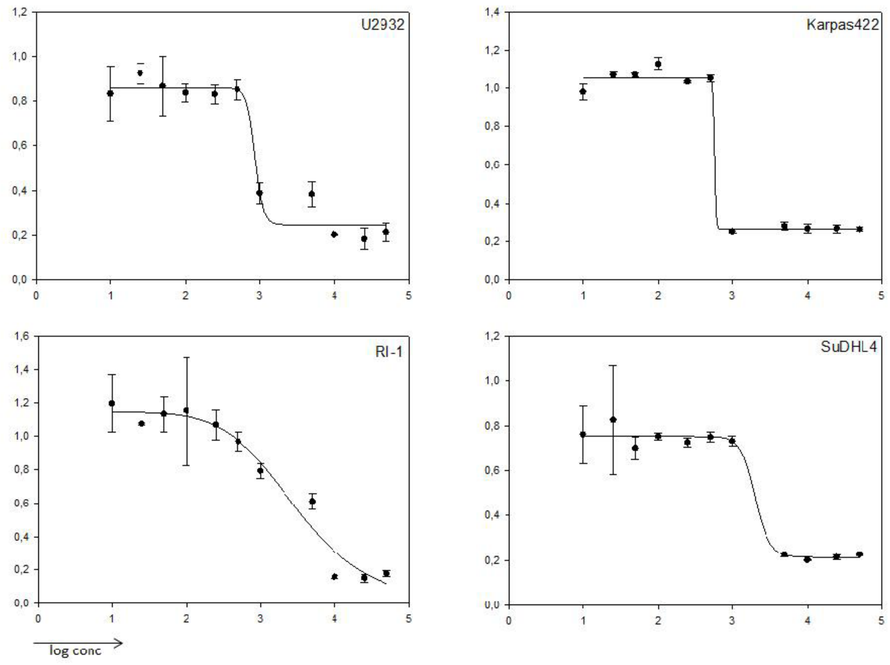
Overexpression of NR4A1 or NR4A3 expression in aggressive lymphoma cells (U2932, Karpas422, RI-1, SuDHL4) led to a significantly higher proportion of lymphoma cells undergoing apoptosis as demonstrated by DNA cleavage, Annexin V staining and increased caspase 3-7 activity suggesting a functional redundancy of NR4A1 to NR4A3 in aggressive lymphomas

Induction of NR4A3 cause apoptosis in vitro and suppresses tumor in vivo

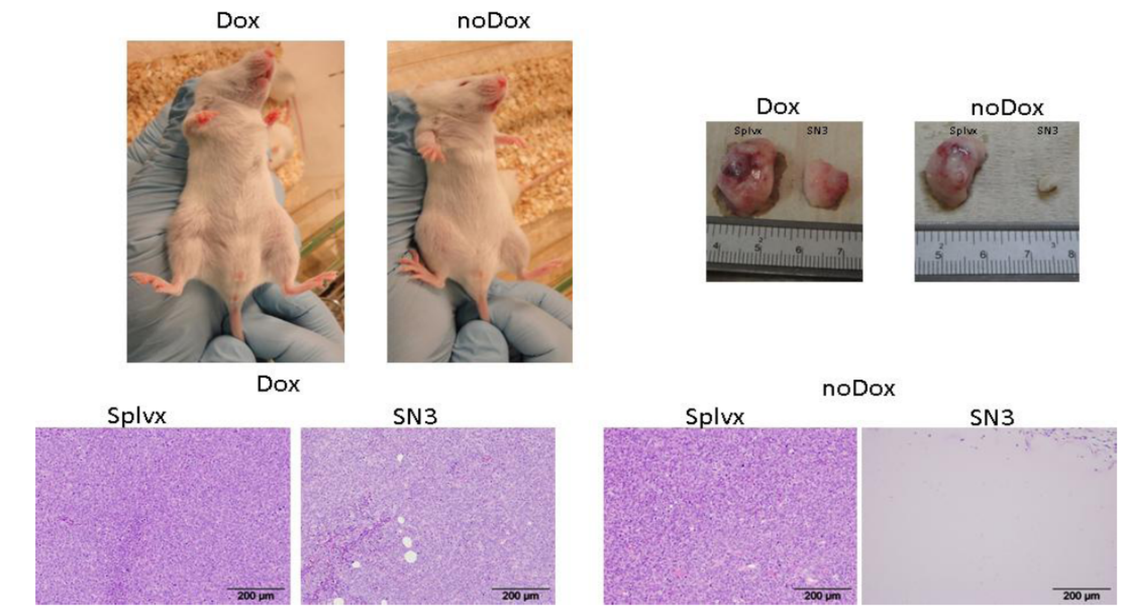


Removal of doxocycline (Dox) from the growth-media induced NR4A3 expression in all cell lines contain the NR4A3 construct (SN3 & SN3-2) but not in the vector control. NR4A3 induction causes induction of apoptosis as demonstrated by DNA cleavage (SubG1), Annexin V staining and increased caspase 3-7 activity

Treatment of lymphoma cells with Thapsigargin –a NR4A3 inducing agent- inhibited of lymphoma cell growth



To further test the effect of NR4A3 inducing agents on aggressive lymphoma cells, we treated foru lymphoma cell lines (U2932, Karpas422, RI-1 and SudHL4) with varous concentration of Thapsigargin – a NR4A3 inducing agent- and determined their cell grwoth by using MTS assay. Treatment of lymphoma cells with Thapsigargin inhibited of lymphoma cell growth in a dose dependent manner in all four lymphoma cell lines.



To test the tumor suppressor function of NR4A3 *in vivo*, the stably transduced SuDHL4 cells were further investigated in a NOD/SCID/IL-2rγnull (NSG) mouse model. Induction of NR4A3 in SuDHL4 cells suppressed their outgrowth in NSG mice, in contrast to vector controls and uninduced SuDHL4 cells, where massive tumor formation was observed.

Conclusion:

Our data suggest that NR4A3 has a pro-apoptotic function in aggressive lymphoma and define that NR4A3 together with NR4A1 function as novel tumor suppressor involved in aggressive lymphoma development. Furthermore we demonstrate that NR4A3 is functionally redundant to NR4A1 and define both receptors as novel tumor suppressor in aggressive lymphomas. Hence, NR4A3 and its agonists are promising novel targets for drug development in lymphoma therapy.

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Aggressive B-cell lymphoma

Alexander Deutsch

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