

Childhood Leukemia



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Dissection of critical cellular and molecular mechanisms of acute myeloid leukemia (AML) to develop novel therapeutic strategies

The overall goal of our research is to functionally dissect critical molecular mechanisms that drive the development and maintenance of acute myeloid leukemia (AML). Our work currently focuses on genetic alterations of epigenetic regulators such as mixed lineage leukemia (MLL) or nucleoporin-98 (NUP98) often associated with poor disease outcome. NUP98 is recurrently involved in chromosomal translocations leading to expression of fusions with multiple partner genes. We have cloned full-length cDNAs of several NUP98-fusions including NUP98-NSD1 and NUP98-MLL. In contrast to others, we found that overexpression of NUP98-NSD1 is not sufficient to transform hematopoietic stem and progenitor cells, but rapidly induces AML in mice when expressed in combination with the FLT3-ITD mutation, which is present in tumor cells from the majority of NUP98-NSD1+ patients (Thanasopoulou *et al.*, 2014). In parallel, we found that expression of transforming NUP98 fusions resulted in unprecedented alterations of the nuclear membrane (collaboration with B. Fahrenkrog, Bruxelles) (Fahrenkrog *et al.*, 2016).

In contrast to NUP98, the oncogenic potential of MLL fusion genes is well established. MLL-fusions form transcriptionally active large multi-protein complexes that bind to chromatin through adapter proteins like MENIN and LEDGF/p75. We used several *in vitro* and *in vivo* AML models to demonstrate potent anti-leukemic activities of several small molecules that inhibit critical co-factors of the MLL fusion complex such as the BET-protein BRD4 or the CBP/EP300 transcriptional co-regulators (collaboration with S. Knapp, Oxford/ Frankfurt) (Picaud *et al.*, 2015). Whether the cell of origin influences the biology of AML is an ongoing matter of debates. We established conditional transgenic mouse lines for some of the most prevalent MLL fusions ("iMLL-AF9", "iMLL-ENL") that allow to model AML from long-term repopulating hematopoietic stem cells (LT-HSC) but also more committed progenitor cells such as granulocyte-macrophage progenitors (GMP). Activation of iMLL-AF9 in LT-HSC resulted in unusually invasive clonogenic growth in methylcellulose not observed upon activation in GMP. *In vivo*, activation of iMLL-AF9 upon transplantation of LT-HSC, induced in 10–20% of the recipients an invasive and chemoresistant AML after a very short latency. Interestingly, "LT-HSC-early iMLL-AF9" AML cells expressed many genes related to adhesion, migration and epithelial-mesenchymal transition (EMT) known from progressing solid cancers. Strikingly, about 20% of a large cohort of AML patients were characterized by similar gene expression signatures: akin to the mouse model, leukemic cells from these patients expressed high levels of the transcription factors EVI1 and ERG and the EMT-regulator ZEB1 (Fig. 1). Cross-species comparison revealed >100 genes that characterized aggressiveness and poor outcome in mouse and human AML, and may represent novel biomarkers and/or origin-related therapeutic targets (Stavropoulou *et al.*, 2016).

The iMLL-ENL mouse line allowed for the first time to closely model human mixed lineage leukemia associated with this fusion. In contrast to iMLL-AF9, iMLL-ENL preferentially transformed HSC rather than more committed progenitor cells. Interestingly, transformation by iMLL-ENL was dependent of the fusion exceeding endogenous Mll mRNA levels. Importantly, *MLL-ENL* mRNA levels exceeding *MLL* in leukemic blasts were also found in patients carrying this alteration. Collectively, these experiments suggested that transformation by MLL-ENL (and most likely other MLL fusions) depends on a critical fusion dose, and is significantly influenced by cell of origin within the hematopoietic hierarchy (*submitted*).

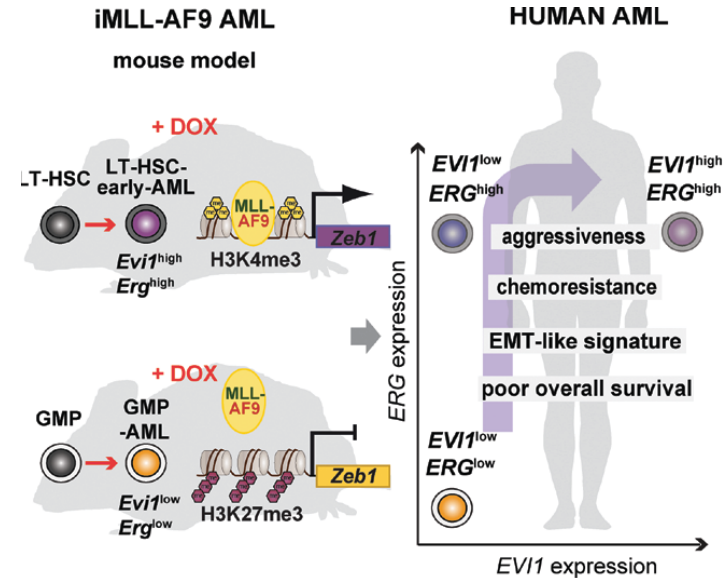


Fig. 1: Modeling of MLL-AF9-driven AML of different cellular origins in mice revealed striking similarities with the human disease. Induction of iMLL-AF9 in long-term hematopoietic stem cells (LT-HSC) resulted in a more aggressive disease than activation in granulocyte-macrophage progenitors (GMP). Invasive LT-HSC-derived AML was characterized by high expression levels of *Evi1* and *Erg*, and by an EMT-like gene expression signature with direct induction of *Zeb1*. Interestingly, high expression levels of *EVI1* and *ERG* also characterized AML patients with poor outcome suggesting a similar impact of the cellular origin on the biology of the disease in mouse and man.

Selected Publications

Thanasopoulou A, Tzankov A, and Schwaller J. (2014). Potent co-operation between the NUP98-NSD1 fusion and the FLT3-ITD mutation in acute myeloid leukemia induction. *Haematologica* 99, 1465–1471.
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Fahrenkrog B, Martinelli V, Nilles N, Fruhmenn G, Chatel G, Juge S, Sauder U, Di Giacomo D, Mecucci C, and Schwaller J. (2016). Expression of Leukemia-Associated Nup98 Fusion Proteins Generates an Aberrant Nuclear Envelope Phenotype. *PLoS One* 11, e0152321.

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