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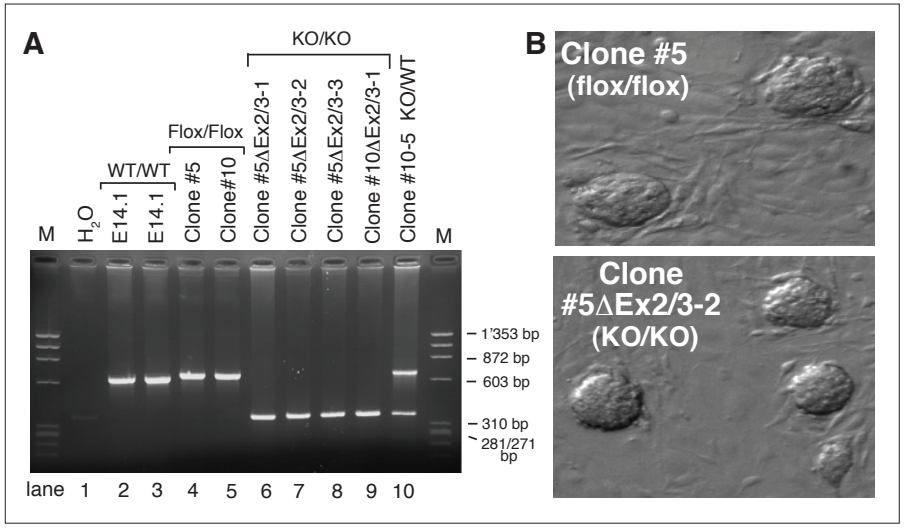
Institute of Immunology/Division of Molecular Immunology

Dissecting Phenotypic Defects in *Mixed-Lineage-Leukemia-5 (Mll5)*-Deficient Mice and Cell Lines: Towards a Molecular Understanding of Mll5 Function

Head: Hans Jörg Fehling

Gene targeting in embryonic stem (ES) cells is a key expertise of the Molecular Immunology group. We use this approach for sophisticated genetic manipulations of the mouse genome to create mouse models of human diseases, but also simply to assess unknown functions of novel genes. *Mixed-Lineage-Leukemia-5 (Mll5)* is a member of the MLL/Trithorax family of epigenetic regulators. The human gene is located in a genomic region frequently deleted in myeloid malignancies, which has led to speculation about potential tumor suppressor activities. To get a first idea about the physiological role of *Mll5*, we have generated and characterized knockout mouse mutants (Madan V. et al. 2009). These animals exhibit a variety of phenotypic abnormalities, including partial neonatal lethality, impaired fertility, retarded growth, defective lymphopoiesis, radiation sensitivity due to bone marrow failure, and numerical, functional and cell-cycle defects of specific hematopoietic stem/progenitor cell populations. The major goal of Alpaslan Tasdogan's PhD project was to obtain first insights into the molecular mechanisms underlying the described hematopoietic phenotypes. Towards this goal, Alpaslan has followed several lines of investigation. For instance, he has established stable cell lines, including embryonic stem (ES) cells, from our constitutive *Mll5*-deficient mouse mutants as well as from newly bred mice in which *Mll5* can be inactivated in an inducible or tissue-specific fashion (Fig.1). Detailed molecular and biochemical characterization of these cell lines has revealed striking molecular abnormalities which were subsequently shown to explain most of the hematopoietic defects in *Mll5*-knockout mice. In



Generation of *Mll5*$^{flox/flox}$, *Mll5*$^{KO/KO}$ and *Mll5*$^{KO/WT}$ embryonic stem (ES) cell lines.
A. Characterization of *Mll5* alleles in blastocyst-derived and Cre-recombinase-treated ES cell clones by genomic PCR. E14.1 ES cells (lanes 2 + 3) served as wild-type controls.
B. Phase contrast microscopy of a representative *Mll5*$^{flox/flox}$ ES clone (#5, derived from *Mll5*$^{flox/flox}$ mice) and a representative *Mll5*$^{KO/KO}$ clone (#5ΔEx2/3-2), documenting typical ES cell morphology.

another approach, Alpaslan has generated epitope-tagged *Mll5* alleles via gene targeting in ES cells. These genetically modified ES cells are currently used for in vitro differentiation studies and to generate corresponding knockin mouse strains. Mll5-epitope-tagged cell lines and mice will be invaluable tools for a number of molecular and biochemical analyses, including reliable quantification of Mll5 protein during specific phases of the cell cycle in synchronized cells, the detection of interacting partner proteins by co-immunoprecipitation and the identification of physiological target genes by chromatin immunoprecipitation (ChIP). The epitope-tagging experiments are done in collaboration with the laboratory of Prof. Dr. A.F. Stewart, Technical University Dresden.

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Selected Publications:

- *Luche H, Tata Nageswara R, Kumar S, Tasdogan A, Beckel F, Blum C, Martins VC, Rodewald H-R, Fehling HJ (2013): In vivo fate mapping identifies pre-TCRalpha expression as an intra- and extra-thymic, but not prethymic, marker of T lymphopoiesis. J Exp Med. 210,699-714.*
- *Teupser D, Weber O, Rao NT, Sass K, Thiery J, Fehling HJ debatin(2011): No reduction of atherosclerosis in C-reactive protein (CRP)-deficient mice. J Biol Chem. 286, 6272-9.*
- *Schlenner SM, Madan V, Busch K, Tietz A, Läufle C, Costa C, Blum C, Fehling HJ, Rodewald H-R (2010): Fate mapping reveals separate origins of T cells and myeloid lineages in the thymus. Immunity 32, 426-36.*
- *Madan B, Madan V, Weber O, Tropel P, Blum C, Kieffer E, Viville S, Fehling HJ (2009): The pluripotency-associated gene Dppa4 is dispensable for embryonic stem cell identity and germ cell development but essential for embryogenesis. Mol Cell Biol. 29, 3186-203.*
- *Madan V, Madan B, Brykczynska U, Zilbermann F, Hogeveen K, Doehner K, Doehner H, Weber O, Blum C, Rodewald HR, Sassone-Corsi P, Peters AFM, Fehling HF (2009): Impaired function of primitive hematopoietic cells in mice lacking the Mixed-Lineage-Leukemia homolog Mll5. Blood 113, 1444-54.*
- *Luche H, Weber O, Rao TN, Blum C, Fehling HJ (2007): Faithful activation of an extra-bright red fluorescent protein in "knock-in" Cre-reporter mice ideally suited for lineage tracing studies. Eur J Immunol. 37, 43-53.*