Master thesis project of **Bernd Zetsche** (2007)

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Single Copy shRNA expression in the Rosa26 locus for constitutive Bmi1 knockdown in embryonic stem cells and chimeric mice

The goal of my thesis was to generate a stable knockdown of the B-lymphoma Mo-MLV insertion region 1 (Bmi1) in wildtype (wt) and tumor-prone embryonic stem cells (ESC). Bmi1 is an oncogene of the polycomb group gene family. Bmi1 knockout mice are growth retarded and die in early adulthood. Analysis of Bmi1 mutant mice has shown it is essential for the self-renewal capability of neural stem cells. Bmi1 is overexpressed in human medulloblastoma, which is the most common malignant pediatric brain tumor. The question my research is aimed to address is whether Bmi1 is essential in medulloblastoma initiation, maintenance and/or progression.

To approach this problem and circumvent the lethality of Bmi1 knockout mice, we used RNA interference (RNAi) to stably knockdown Bmi1 expression in ES cells using shRNA constructs targeted to the *Rosa26* locus. For our knockdown approach we used two strategies to regulate Bmi1 using Cre-lox technology to engineer conditional alleles that are turned off or on respectively. The first vector expresses the shRNA constitutively until Cre recombinase deletes the shRNA allele and stops its expression. In the second vector, the shRNA expression is dormant until a DNA segment is removed by Cre, which allows the shRNA to become expressed.

To selectively knockdown Bmi1, we constructed four shRNA vectors, two sites specific for the coding sequence and two sites specific for the 3' untranslated region of Bmi1. These vectors were introduced into mouse ESC and screened for targeted insertions at *Rosa26*. By comparing alleles at the same genomic integration site we can determine the relative efficiency of each of the shRNA alleles. Knockdown efficiency of Bmi1 was determined by measuring mRNA

expression by RT-PCR and qPCR using targeted clones for all four vectors and two controls. The results demonstrated between 65%-77% knockdown relative to wildtype ESC and targeted ESC with no shRNA. We further generated chimeric embryos by microinjection into wildtype blastocysts, selected ES derived embryonic fibroblasts, and confirmed knockdown of Bmi1 mRNA in these cells. Two shRNA alleles with the greatest knockdown were used for subsequent functional validation *in vivo*.

We asked whether Bmi1 knockdown ES cells would direct a growth retardation phenotype similar to that observed in the published Bmi1 mutant. Chimeric mice for one of the shRNA alleles were weighed at daily intervals for one month and the growth of the animals was compared to the percent contribution of ESC in the chimera as determined by Q-PCR. The results showed that contribution by ESC in the chimera was inversely correlated to body weight, suggesting that Bmi1 knockdown caused the growth retardation phenotype. We are repeating this experiment using a second shRNA allele to validate these findings.