Characterization of Sirt2 using conditional RNAi in mice

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Summary

Within the past eight years, RNA interference (RNAi) has emerged as a powerful experimental tool for gene function analysis in mice. Reversible control of shRNA mediated RNAi has been achieved by using engineered promoters containing a tetracycline operator (tetO) sequences. In the presence of the tetracycline repressor (tetR), transcription is blocked through binding of the repressor to tetO. De-repression is achieved by adding the inductor doxycycline (dox), causing the release of the TetR protein and allowing shRNA transcription. To achieve spatially and temporally regulated RNAi, the tet inducible system was combined with a Cre/loxP based strategy for tissue specific activation of shRNA constructs. To this end, a loxP-flanked ‘‘promoter inactivating element’’ (PIE) placed between the proximal (PSE) and distal sequence elements (DSE) of a dox inducible promoter such that transcription is completely blocked. Activation through Cre mediated excision of the insert allows inducible gene silencing in selected tissues. In mouse ES cells, the system mediated tight regulation of shRNA expression upon Cre mediated activation and dox administration, reaching knock-down efficiencies of >80%. Unexpectedly, the system showed a limited activity in transgenic mice when applied for conditional silencing of two different targets, including Sirt2. Sirt2 is a member of the sirtuin family which has considerably gained attention for its possible role many physiological processes, including glucose homeostasis and neurodegenerative disease.

To investigate the in vivo function of Sirt2 anyhow, the unmodified dox-responsive promoter was further used for conditional RNAi in transgenic mice. Inducible shRNA expression resulted in efficient (>90 %) body-wide silencing of Sirt2. Suppression of Sirt2 during embryogenesis resulted in offspring consisting of equal ratios of wild type and transgenic pups, indicating that Sirt2 is not indispensable for development. In adult animals, glucose metabolism, insulin sensitivity and energy balance appeared to be unaffected by Sirt2 deficiency. Likewise, expression of PPARγ, a downstream target of Sirt2, was not found to be altered upon Sirt2 inhibition. Finally, Sirt2 silencing was induced in an experimental model of Parkinson disease (PD). Data from Rotarod performances to study motor behaviour did not provide any evidence for a role of Sirt2 in PD pathogenesis as suggested by previous in vitro studies. Taken together, conditional Sirt2 silencing in vivo does not support speculation concerning a central role of Sirt2 in physiological and disease processes.