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A retroviral tamoxifen-inducible human TP53ER protein activates a conserved p53 response but fails to promote senescence in mouse fibroblasts

Geboren am 6. November 1982 in Düsseldorf
Staatsexamen am 20. Mai 2009 an der Universität Heidelberg

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p53 is a transcription factor relevant for organismal homeostasis and protection from malignant transformation and it is mutated in more than half of all human cancers. Telomeres have been identified as the mitotic clocks of cells, because they shorten with each cell division in the absence of telomerase. Once they have been reduced to a critical size, they fail to cap chromosomal ends effectively, which generates high levels of genomic instability. Atm senses this kind of DNA damage and activates p53, which in turn initiates a chain of events known as DNA damage response. The DNA damage response is believed to be relevant for aging and cancer, because it determines cells with critically shortened telomeres for senescence or apoptosis resulting in a reduced regenerative potential of aged organs and tissues. However, when the response is not successfully executed, an important mechanism to prevent uncontrolled proliferation has failed. The consequence may be the development of cancer.
The aim of this study was to identify genes that are regulated by p53 during the telomere-induced DNA damage response by microarray-profiling differential gene expression. Mouse ear fibroblasts (EFs) triple deficient for telomerase, Atm and p53 were employed (TKO EFs). Originating from animals that lacked telomerase in the 4^{th} generation, TKO EFs had high levels of genomic instability, while this could not provoke a DNA damage response due to the simultaneous lack of Atm and p53. It was hypothesized that critically shortened telomeres combined with the lack of these two major genomic sentinels would generate a model system with aging- and cancer-related levels of DNA damage.
TKO EFs were infected with a retroviral hydroxytamoxifen (OHT)-inducible human TP53ER construct that was expected to mimic the function of endogenous p53 and initiate a strong DNA damage response. In mouse fibroblasts, such a response should provoke senescence. However, induction of TP53ER in TKO EFs induced neither senescence, nor a general growth arrest or a drastic reduction of cells in S-phase. TKO EFs were also not undergoing apoptosis at an increased percentage. Instead, they formed foci when left to grow in culture independent of TP53ER status indicating that they had become transformed probably due to the high levels of genomic instability. Yet freshly isolated early passage mouse embryonic fibroblasts, which were either deficient for p53 only (SKO MEFs) or for both p53 and telomerase (DKO MEFs), neither entered senescence following TP53ER induction.
Despite the lack of a distinct phenotype, induction of TP53ER led to an increase in the known p53 targets Btg2, Cyclin G1, Mdm2, p21 and Sip. In contrast, Apaf, Puma and Wip were not upregulated by TP53ER. Since reduction of TP53ER protein levels and upregulation of Mdm2 coincided, it was hypothesized that Mdm2 degraded the TP53ER protein too rapidly for it to have an effect. However, Nutlin-3a, a specific inhibitor of Mdm2, did not cause growth arrest after TP53ER induction in neither TKO nor DKO fibroblasts.
A microarray analysis of global gene expression revealed that 54 genes were regulated by TP53ER (52 up and 2 down, p-value <0.05). Among those were the nine known p53 targets Btg2, Cyclin G1, Gtse1, Mdm2, p21, Perp, Pmaip1 (Noxa), Sesn2 and Sip (Trp53inp1).

In proliferation assays following shRNA-mediated inactivation of Cyclin G1 and Eda2r, two genes that had been upregulated by TP53ER, human BJ fibroblasts slowed down in growth. However, TKO EFs and DKO MEFs did not proliferate more slowly regardless of TP53ER induction status.

Collectively, human TP53ER did not restore p53 function in p53-deficient mouse fibroblasts, while it affected the expression of several known p53 targets and various other genes that had not been previously linked to p53.