

p53 Protein Aggregation and Implications for Tumorigenesis

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SUMMARY

Mutations of the p53 tumor suppressor gene are the most common genetic alteration found in human cancers. Most of these mutations are missense mutations and it has been established that many of these p53 disease mutants not only lose their tumour suppressive functions, but also dominantly inhibit wild-type p53 and gain new functions that promote tumorigenesis. As native p53 binds DNA as a homotetramer, it is currently believed that the dominant-negative effect of p53 mutants results from the hetero-tetramerisation of mutant and wild-type proteins whereby DNA binding activity is partially or totally inhibited. Gain-of-function effects, on the other hand, are believed to result from a poorly understood mutant p53-induced conformational perturbation of heterotetrameric p53.

In this thesis I show that p53 contains a short aggregation-prone sequence stretch (or aggregation nucleus) encompassing residues 250-257 that is buried in the hydrophobic core of its DNA-binding domain. Disease mutations that destabilize the structure of this domain expose the aggregation nucleus, thereby increasing the aggregation propensity of p53 in cultured cells as well as in murine and human tumors. As both the wild type and mutant p53 sequence contain an identical aggregation nucleus, the increased aggregation of mutant p53 is able to induce the aggregation and thereby inactivation of wild-type p53 into cellular inclusions. As a result the dominant-negative activity of structural p53 disease mutants is not dependent on p53

tetramerisation, but rather can be explained by a mechanism of mutant-induced wild type p53 co-aggregation that is controlled by the aggregation nucleus encompassing residues 251-257. In addition, we found that the p53 paralogues p63 and p73 contain highly homologous aggregation nuclei, suggesting the possibility of co-aggregation of p53 mutants with p63 and p73. Accordingly, we found that co-expression of mutant p53 with p63 and p73 proteins results in the sequestration and inactivation of the p53 paralogues into cytoplasmic p53 aggregates both in cell culture as well as in murine and human tumors. The inactivation of p63 and p73 in the presence of p53 mutants has been shown before to be associated to gain of oncogenic function, but the mechanism by which this effect is evoked remained unexplained. The work presented in this thesis shows that co-aggregation of mutant p53 with p63 and p73 provides a mechanism for paralog inactivation. Again, this effect is controlled by the aggregation nucleus of p53 and is not dependent on the tetramerisation domain. In addition, the expression of aggregation-prone p53 mutants also induced significant heat shock response, upregulating the hsp70 and hsp90 chaperones, a response which is known to have antiapoptotic gain-of-function effects in cancer. Together, the findings presented in this thesis indicate an important role for protein aggregation both for dominant-negative loss-of-function effects as well as for the gain-of-function effects of p53 missense mutations. Finally, we demonstrate that aggregation-induced dominance and gain-of-function needs not to be restricted to missense mutants but can also be observed for p53 frameshift mutations. Finally, in order to analyze the impact of aggregation on the evolution of cancer we analysed patient data from somatic as well as germline p53 mutant databases. We found that p53 aggregating mutants associate with poorer therapeutic response and long-term survival. The more severe phenotypic effect of aggregating p53 mutants was also confirmed by the much lower loss-of-heterozygosity observed for aggregating mutants versus non-aggregating mutants (30% versus 92%). Taken together, our findings suggest that the inhibition of mutant p53 aggregation might be a promising strategy for the development of novel therapeutic approaches against cancer.