Tumor Suppressor p53 WT and R248Q Core Domain Aggregation at Low pH

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p53 is a nuclear tumor suppressor phosphoprotein. The major role of p53 in normal cells is to induce cell cycle arrest or apoptosis in response to cellular stress. p53 function is lost in over 50% of human cancers evidencing the role of p53 in tumorigenesis. Actually, accumulation of wild type inactive p53 has been described in various cancers. Our group has demonstrated that the core domain of the tumor suppressor protein (p53C) can form fibrillar aggregates after mild perturbation at pH 7.2. Recent studies have also shown that the p53 C-terminal and N-terminal domains can undergo amyloid aggregation in vitro. We describe here that fibrillar and amorphous aggregates occur after pressure or heat denaturation of wt p53C and oncogenic mutant R248Q at pH 7.2 or 5.0. We show that, when exposed to denaturant agents at pH 7.2 or at acidic pH, p53C forms thioflavine-T positive aggregates and we note by circular dichroism an increase of ß-sheet secondary structure. The aggregates were characterized by electron microscopy and the amyloid nature was confirmed by x-ray diffraction analysis, which showed that it is consistent with the presence of a typical cross-ß-conformation. In addition, p53 aggregates at pH 7.2 can interact with oligomer-specific antibody as shown by dot blot assay. Moreover, we observed that fibrillar and amorphous aggregates were toxic to cells by live dead assay. Taken together, our data suggest that cancer may involve the aggregation of the inactive p53 into fibrils.

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