p53 aggregation in hepatocellular carcinoma cell lines

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INTRODUCTION

P53 tumor suppressor is recurrently regarded as the “guardian of the genome”. Inactivation of p53 due to mutations is frequent in human cancers as evidenced in hepatocellular carcinomas (HCC), where 30 to 60% patients carry p53 gene mutations.Cancer may share some characteristics with amyloidogenic and prion diseases. Mutated p53 seems to have a higher propensity to form amyloid-like aggregates and to induce misfolding and coaggregation of wild-type p53 (WT p53). This would cause a dominant-negative phenomenon and gain-of function effect, corresponding to a behavior typical of a prion.This seems to be a pivotal mechanism for tumorigenesis.

OBJECTIVES

The aim of this study was to evaluate the formation of mutant p53 amyloid-like aggregates in HCC cell lines.

MATERIAL AND METHODS

For this purpose, we used co-localization immunofluorescence assays in three HCC cell lines, HepG2 (p53 WT), HUH-7 (p53 Y220C) and Hep3b (p53 null), using anti-p53, anti-amyloid aggregates and amyloid fibers aggregates antibodies. We also used western blot and immunoprecipitation assays, to analyze the presence of p53 aggregates in those three cell lines .All experiments were done, at least, three times in an independent manner. And were statistically analyzed using ANOVA.

RESULTS AND DISCUSSION

Western blot confirmed the expression of p53 in both HUH-7 and HepG2, but with different expression levels.Hep3b does not expressed p53 (confirmed by western blot). Our results showed co-localization for p53 and amyloid-like aggregates antibodies in the immunofluorescence assays for HUH-7 (p53 Y220C) cell line, but not for HepG2 (p53 WT) or Hep3B (p53 null). Immunoprecipitation with amyloid-

like aggregates antibody followed by western blot with p53 antibody showed that HUH-7 cell line have p53 in the amyloid-like aggregates, confirming our immunofluorescence results.

CONCLUSION

Our study suggests that mutated p53 has an increased ability to form amyloid-like aggregates in HCC cell lines, indicating that mutant p53 aggregation is an important target for the development of antineoplasic compounds.

Keywords: Hepatocellular carcinoma, p53 aggregation, Amyloid-like aggregates

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