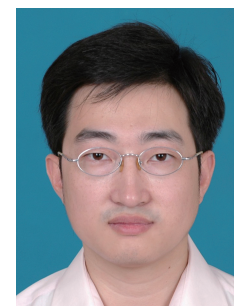


DECREASED EXPRESSION OF NEURENSIN-2 CORRELATES WITH POOR PROGNOSIS IN HEPATOCELLULAR CARCINOMA

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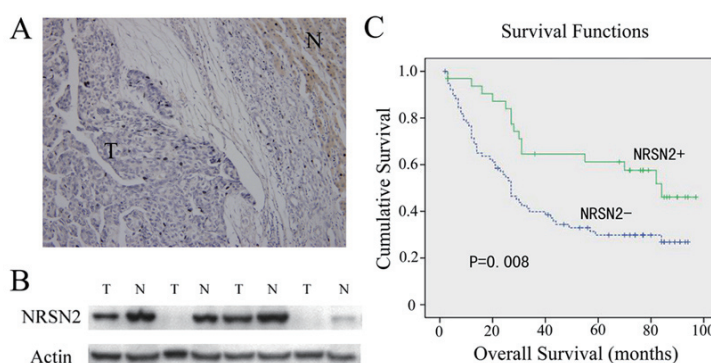
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Introduction: A recent study (Cell 135, 852-864, November 26, 2008) introduced a new, effective and high-yield approach for identifying liver tumor suppressors, by combining an integrated cancer genomic analysis, RNA interference (RNAi) technology and cancer-susceptible mouse models. This approach resulted in the functional validation of 13 tumor suppressor genes, including XPO4, DDX20, GJD4, FSTL5 and Neurensin-2 (NRSN2) etc. Interesting, the vast majority of these identified genes had not previously been linked to cancer. Therefore, more studies are needed to validate and further study the potential value of these tumor suppressors. By choosing NRSN2 as a sample randomly, we hope to provide some useful information about its role in HCC and further validate above results.

Background: NRSN2 belongs to the vesicular membrane protein (VMP) family, encodes a 21,983 Da protein composed of 204 amino acids, and shows a high sequence homology to Neurensin-1. So far, there is not definite function about NRSN2. Based on UniProt Knowledgebase, it may play a role in maintenance and/or transport of vesicles, according to its sequence similarities with Neurensin-1, and it is uncertain whether Met-1 or Met-2 is the initiator. To our knowledge, there have not been any reports about studies of NRSN2 in cancer, not to mention HCC.

Purpose: The aim of this study was to investigate the expression of Neurensin-2 (NRSN2) in HCC, its relationship to clinicopathologic parameters and its prognostic value to post-resectional survival in HCC.

Methods: In present study, a total of 110 HCC surgical resection specimens from patients with definitive clinicopathologic and follow-up data were collected at Sun Yat-sen University Cancer Center between Jan 2001 and Dec 2002. We first investigated the expression of NRSN2 by immunohistochemistry in the total of 110 HCC tissue specimens, and performed Western blotting analysis in 11 paired HCC tumor and non-tumor tissues to further validate the result of immunohistochemistry analysis. Association of NRSN2 with each clinicopathologic parameter was analyzed by Pearson chi-square test, and HCC overall survival by Kaplan-Meier curves and log-rank test. Multivariate Cox regression analyses of NRSN2 in HCC were also performed.



Results: Overall, 32 of 110 (29.1%) cases had positive expression in tumor cells, and 78 of 110 (70.9%) cases had negative expression. In cases with adjacent non-tumor tissue, we often observed a sharp contrast between infiltrative tumor areas of negative staining and the adjacent non-tumor tissue of positive staining (Fig. 1A). The immunohistochemical data were further confirmed by the results of Western analysis, in which the NRSN2 protein expression in HCC tissues were significantly down-regulated when compared with adjacent non-tumor tissues (Fig. 1B). The decreased NRSN2 expression in HCC was significantly correlated with larger tumor size ($p=0.006$). A significantly shorter overall survival time was observed in patients with negative NRSN2 expression than in those with positive expression ($p=0.008$) (Fig. 1C). Multivariate Cox regression analysis further revealed that NRSN2 expression was an independent predictor for overall survival (HR: 0.481; 95% CI: 0.270-0.854; $p=0.013$).

Conclusions: Our study indicated that NRSN2 could be a tumor suppressor gene for HCC and may be used as a candidate prognostic marker.