Inhibitor of apoptosis protein livin/BIRC7 in adenocortical tumors.

INTRODUCTION: Adrenocortical tumors (ACT) comprise frequent benign adenoma (ACA) and rare highly malignant carcinoma (ACC). Livin/ML-IAP/BIRC7 is a member of the inhibitors of apoptosis proteins family, which are involved in cancer development, progression and resistance to chemotherapy in several human malignancies, mostly through the direct inhibition of caspase-3 (Fig. 1). Aim of the study was to evaluate the expression of BIRC7/livin and its isoforms livin α and livin β in normal and neoplastic adrenal glands.

METHODS: The mRNA expression of BIRC7, its isoforms livin α and livin β, and caspase-3 was evaluated by quantitative real-time RT-PCR analysis in 84 fresh-frozen tissue samples (34 ACC, 25 ACA, and 25 normal adrenal glands=NAG) (Tab.1), including 19 paired samples of tumor and surrounding normal adrenal tissue (13 ACA and 6 ACC). The mean value of the threshold cycle was normalized with β-actin (ΔCt value). Specific primer were used to amplify 216-bp livin α and 162-bp livin β. Livin isoforms were then identified by 4x agarose gel in all paired samples.

Additionally, livin protein expression was assessed by western blot analysis (WB) in a subgroup of 15 paired samples (10 ACA and 5 ACC with surrounding normal adrenal tissue). Livin immunostaining (IHC) was evaluated in both cytoplasm and nuclei by H-score in 127 paraffin-embedded tissue sections (67 ACC, 45 ACA, 15 NAG). The antibody used for WB and IHC was livin polyclonal antibody (NB100-56145, NovusBio, 1:1000) which recognized both isoforms α and β. The relationship with several histopathological and clinical data was also evaluated.

RESULT: relative BIRC7 mRNA expression was similar between ACA (0.01±0.01) and NAG (0.01±0.02), but significantly higher in ACCs (0.06±0.12, P<0.005 vs both ACA and NAG) (Fig.2A), being more expressed in tumors than in surrounding normal tissues in paired samples (Fig.2B). Both isoforms α and β were detected in normal and tumor tissues (Fig.3), livin β being constantly higher than livin α (P=0.07 in ACC; P=0.10 in ACA; P=0.02 in NA). Comparable results were obtained with WB (Fig.4).

CONCLUSION: Our study demonstrates that BIRC7/livin is specifically over-expressed in ACC, suggesting that it may be involved in adenocortical tumorgenesis. BIRC7 could represent a novel marker for malignancy and a promising tool for targeted therapy in ACC.