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Inhibitor of apoptosis protein livin/BIRC7 in adrenocortical tumors.

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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.

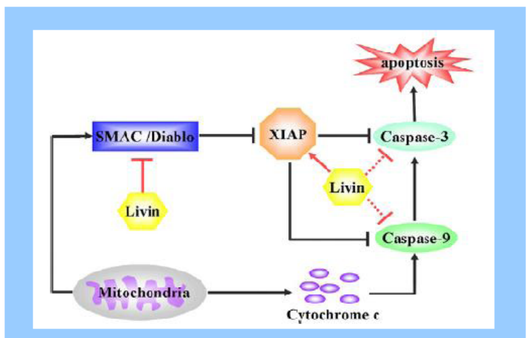

Fig. 1: *BIRC7/livin pathway. From: Liu B, et al. Cancer Letters 2007*

	ACC	ACA
N° patients	34	25
Sex F:M	18:16	16:9
Age (years) mean±SD	50±14,4	47,4±11,7
Tumor size (cm) mean±SD	9,8±5,3*	3,2±1,5
Secreting (%): • cortisol • non-cortisol	15 (44,2%) 19 (55,8%)	11 (44%) 15 (56%)
ENSAT Tumor Stage# • I-II • III • IV	13 (41%) 11 (34%) 8 (25%)	Not appl.
Ki67% mean±SD	18,8±13,6	Not appl.
Weiss score mean±SD	6±2	Not appl.

Tab.1: *Cohort of patients for mRNA analysis by RT-PCR. Not appl.: data not applicable. # two missing data. *P<0.05.*

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 policlonal 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 ACAs (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).

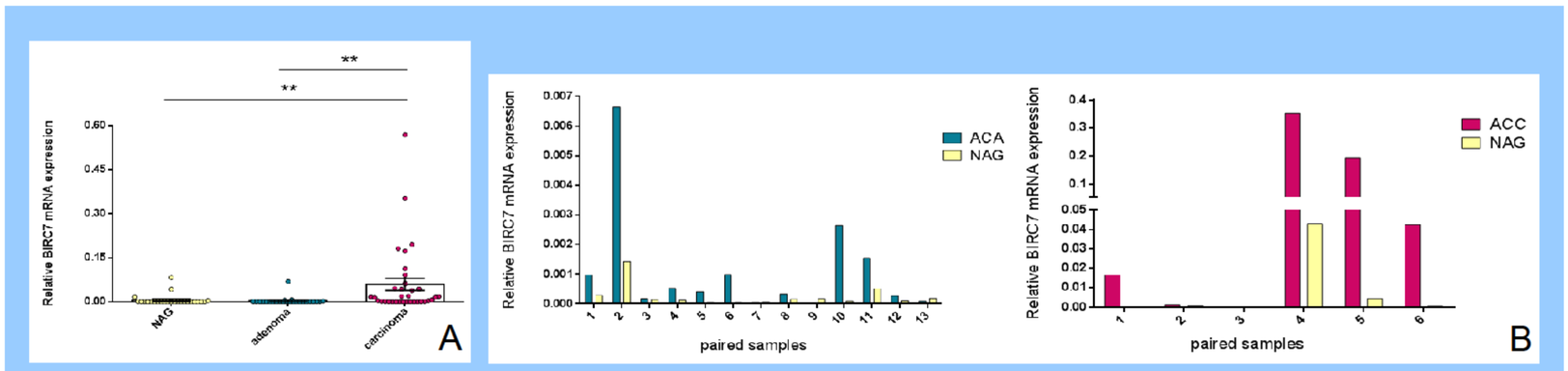

Fig. 2: *Relative BIRC7 mRNA expression levels in NAG, ACA and ACC (A) and in all paired samples of tumor (13 ACA and 6 ACC) and corresponding NAG (B). **p<0.005.*

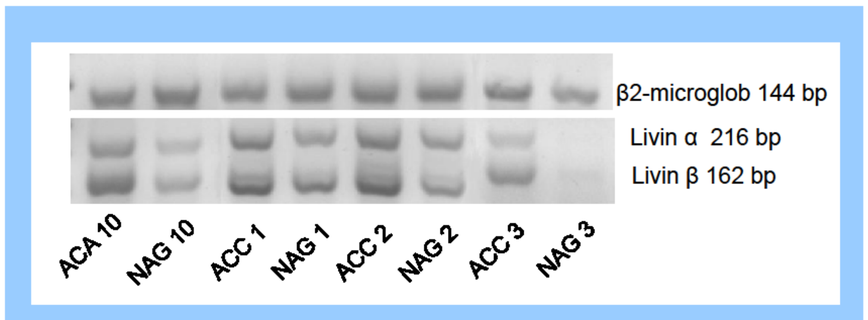

Fig. 3: *4x agarose gel for the expression of mRNA encoding livin isoforms α and β. Subgroup of paired samples of tumors (1 ACA, 3 ACC) and corresponding NAG. β2-microglobulin was used as internal standard.*

Livin protein expression in the cytoplasm was generally higher in ACC than ACA and NAG (mean±SD H-score: 1.72±0.7 vs 1.58±0.5 vs 1.33±0.6 respectively, *P=0.09*) (Fig.5A), often being stronger in the tumor than in surrounding normal adrenal tissue (Fig. 5B). At nuclear level, livin protein was highly expressed in both ACAs and ACCs, but almost absent in NAG (*P<0.001* per trend) (Fig.6). No significant correlation was observed with the histopathological and clinical data, including overall survival.
Caspase-3 mRNA level was higher in ACA than in ACC (*P=0.05*) and NAG (*P=0.03*), with a significant inverse correlation with tumor size (*P=0.005, R=0.36)*.

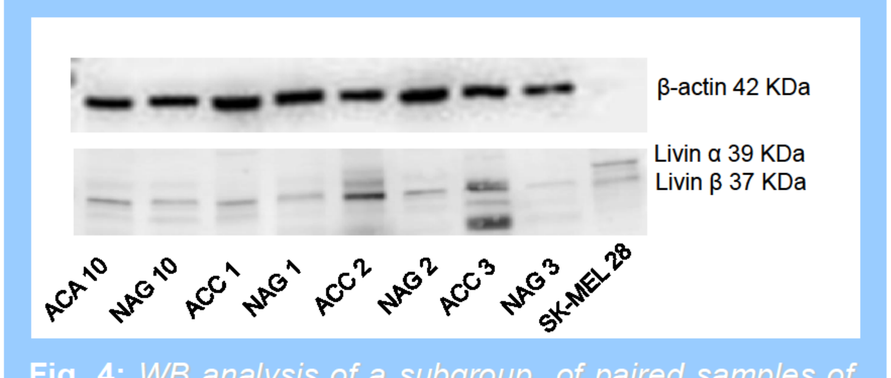

Fig. 4: *WB analysis of a subgroup of paired samples of tumors (1 ACA, 3 ACC) and corresponding NAG. SK-MEL 28 was used as positive control.*

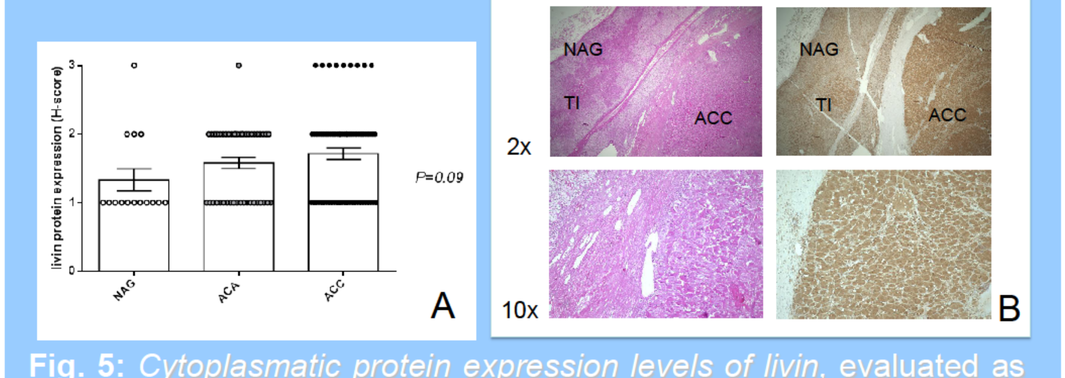

Fig. 5: *Cytoplasmatic protein expression levels of livin,* evaluated as H-score *(A). Hematoxylin and eosin stain and IHC of ACC and adjacent NAG with tumor infiltration (TI) at 2x; magnification of ACC at 10x (B).*

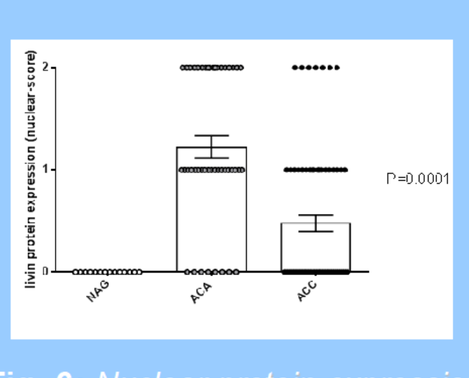

Fig. 6: *Nuclear protein expression levels of livin, evaluated as H-score. P<0.0001 per trend*

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

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