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Expression of inhibitor of apoptosis protein BIRC7/livin in adrenocortical tumors.

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INTRODUCTION: Adrenocortical tumors (ACT) consist of frequent benign adenomas (ACA) and rare highly malignant carcinomas (ACC). *BIRC7/livin,* a member of the inhibitors of apoptosis family, plays an important role in cancer development and progression in a variety of human malignancies. *BIRC7* encodes two splicing variants, livin α and livin β, which present anti–apoptotic property to different stimuli. Apoptosis initiated by etoposide, one of the first line chemotherapeutical drug used for ACC, is blocked only by the β isoform. Patients with livin β overexpression could be resistent to treatment with etoposide.
The aim of our study was to evaluate the expression of BIRC7 in normal adrenal glands and adrenocortical tumors.

METHODS: *BIRC7* mRNA expression was detected by quantitative real-time reverse transcription PCR (qRT-PCR) analysis in fresh-frozen tissue samples (27 ACC, 23 ACA, and 20 normal adrenal glands, NA) (Tab.1). The mean value of the threshold cycle of each sample was normalized with β-actin (ΔCt value). Among these, 15 were paired samples of tumor and corresponding normal adrenal tissue (12 ACA and 3 ACC). To evaluate the different expression of the two isoforms of BIRC7 in ACT and NA, we used specific primer to amplify 216-bp livinα and 162-bp livin β in all of 15 paired samples. Livin isoforms were then identified by 4x agarose gel.
The correlation between *BIRC7* levels and clinical and pathological parameters was also investigated.

RESULT: *BIRC7* mRNA levels were similar between NA and ACA, but significantly increased in ACC (*P<0.005* vs both ACA and NA) (Fig.1). *BIRC7* was more expressed in tumor than corresponding normal tissue in 12 out 15 paired samples (Fig. 2). No significant difference was found between cortisol- and non cortisol-secreting tumors.

	ACC	ACA
N° patients	27	23
Gender F:M	13:14	15:8
Age (years) mean±SD	51,8±14,4	47,3±12,0
Tumor size (cm) mean±SD	10,2±5,6	3,2±2,5*
Secreting (%): • cortisol • non-cortisol	12 (44,4%) 15 (55,6%)	11 (47,8%) 12 (52,2%)
ENSAT Tumor Stage • I-II • III • IV	13 (50%) 9 (34,6%) 4 (15,4%)	Not appl.
Ki67% mean±SD	17,5±10,6	Not appl.
Weiss score mean±SD	6,2±2,0	Not appl.

Tab.1: *Clinical characteristics of patients in ACC and ACA group. Not appl.: data not applicable. *P<0.05.*

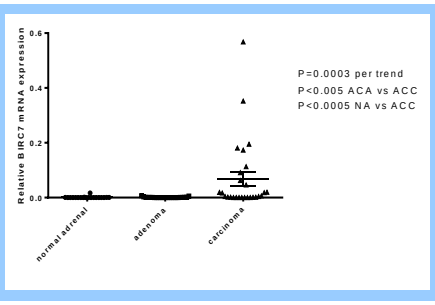

Fig. 1: *Relative BIRC7 mRNA expression levels in NA, ACA and ACC.*

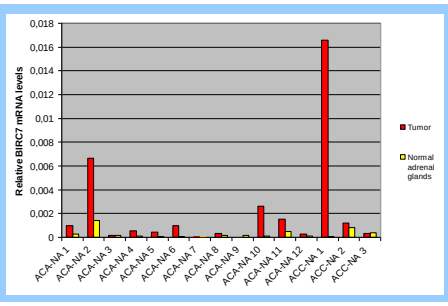

Fig. 2: *Relative BIRC7 mRNA expression levels in 14 paired samples of tumor (2 ACC and 12 ACA) and corresponding normal adrenal tissue.*

In the ACC group, we did not observe any significant correlation between *BIRC7* levels and clinical parameters, such as age, tumor size, Weiss score, ENSAT tumor stage, Ki67 index and number of metastasis at diagnosis. No significant differences were observed in terms of overall survival (OS) and time to progression/disease free survival (TTP/DFS) (Fig.3).
Both livin isoforms α and β were detected in all 15 paired samples (Fig.4), being livin β more expressed than α. The band of livin β was particularly strong in ACC in respect to ACA and NA. Both isoforms were higher in tumors then in NA..

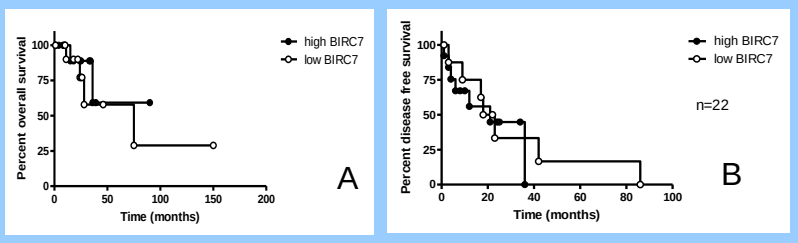

Fig. 3: *Impact of BIRC7 mRNA expression on overall survival (A) and TTP/DFS (B) in patient with ACC (n=27) by Kaplan-Meier curves (both P NS).*

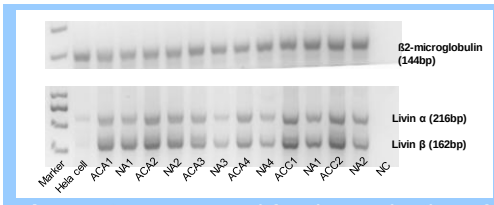

Fig. 4: *4x agarose gel for the evaluation of livin isoforms α and β. Example of six paired samples of tumor (ACA 1-4, ACA1-2) and adjacent normal adrenal glands (NA). β2-microglobulin was used as internal standard.* Hela cells was positive control.

CONCLUSION: To our knowledge, this is the first study that investigates the expression of BIRC7/livin in normal adrenal and adrenocortical tumors. We demonstrate that BIRC7 is specifically over-expressed in ACC. As previously reported for different human cancers, these findings open a new perspective for the use of livin as a potential therapeutic target in ACC.