Higher Expression of the Oncogene YAP1, a Wnt/ß-Catenin Target, is associated with Poor Outcome in Pediatric Patients with Adrenocortical Tumors

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Background

- Overexpression of the oncogene *Yes-Associated-Protein-1 (YAP1)*, a Hippo pathway target (Fig. 1), was recently associated with increased cell proliferation in some human cancers.
- Wnt/ß-catenin pathway abnormal activation plays an important role in adrenocortical tumors (ACTs).
- *YAP1* is a potential target of the Wnt/ß-catenin pathway (Fig. 1).
- To date, the role of YAP1 in adrenal tumorigenesis has not been evaluated.

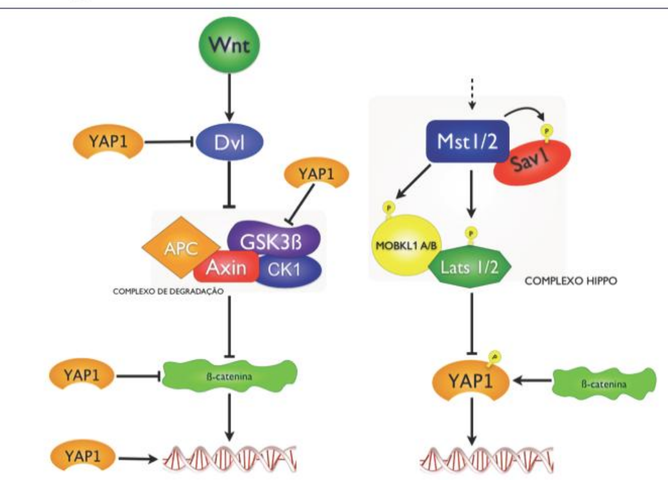

Figure 1. Hippo pathways is a negative regulator of YAP1: YAP1 nuclear translocation is prevented by phosphorylating YAP1. YAP1 is a target of the Wnt/beta-catenin pathway and its expression can increase cell proliferation in colon cancer (aapted from Konsavage et al., 2013).

Aim

•To evaluate the role of YAP1 in adrenal tumorigenesis and its potential interaction with the Wnt/ß-catenin pathway.

Material and Methods

- 42 pediatric patients with ACT
  81% females; median age: 31 months [5-186]
- 24 normal pediatric adrenal cortices (controls)
- 32 fetal adrenals
- 18 adult patients with ACT
  89% females; median age: 42.5 yearss [21-66]
- **qPCR:** *YAP1* mRNA expression (tumors and controls)
- *YAP1* mRNA expression *vs.* Clinical, biochemical and histopathological data
- *YAP1* mRNA expression and patient's outcome (survival analysis)
- ***In vitro* study:**

➢H295 cell line (harbors ß-catenin p.S45P mutation)

➢Treatment: TCF/ß-catenin complex antagonist (PNU-74654)

- **Western Blot:** YAP1 protein expression
- Statistics

➢mRNA expression: Mann-Whitney test

➢Survival analysis: Kaplan–Meier curves and log-rank test

➢*In vitro* study: ANOVA and Dunnett's

Results

Immunohistochemistry

- IHC showed high nuclear expression of YAP1 in fetal adrenals (Fig. 2) but not in postnatal normal adrenals (Fig. 3).
- YAP1 nuclear accumulation was observed in 97% of the pediatric ACTs (Fig. 4 and 5) and in 100% of the adult ACTs (Fig. 6).

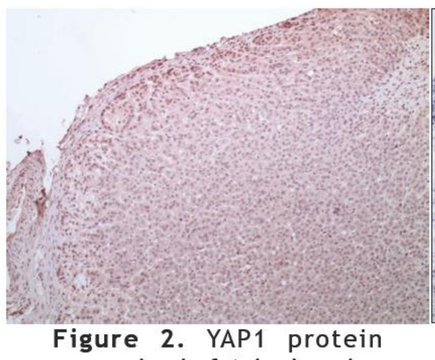

Figure 2. YAP1 protein expression in fetal adrenals.

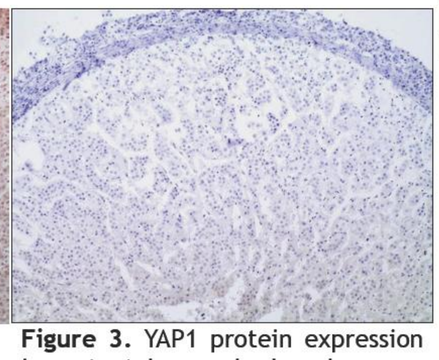

Figure 3. YAP1 protein expression in postnatal normal adrenals.

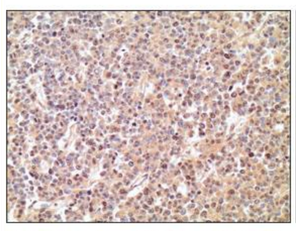

Figure 4. YAP1 protein expression in pediatric ACTs (nuclear accumulation)

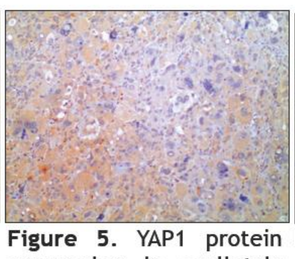

Figure 5. YAP1 protein expression in pediatric ACTs (cytoplasmic accumulation)

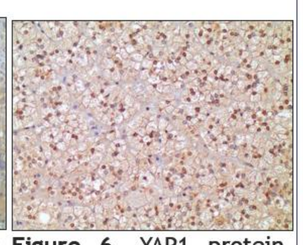

Figure 6. YAP1 protein expression in adult ACTs.

qPCR - Pediatric ACTs

- *YAP1* mRNA expression was detected both in normal adrenal as well as in pediatric adrenal tumors.
- Among the pediatric ACTs, *YAP1* mRNA expression was greatly variable.
- Expression of *YAP1* was not different between pediatric ACTs and normal pediatric adrenals (p=0.99; Fig. 7).

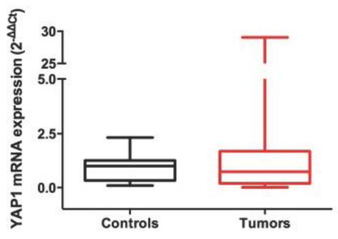

Figure 7. *YAP1* mRNA expression in normal adrenal and adrenal tumors. (n=41)

- Compared with survivors, *YAP1* mRNA expression was higher in the group of patients who died (p=0.02; Fig. 8).
- Compared with patients who had not reccurence and/or metastasis, *YAP1* mRNA expression was higher in the group of patients who had tumor recurrence or metastasis (p=0.002; Fig. 9).

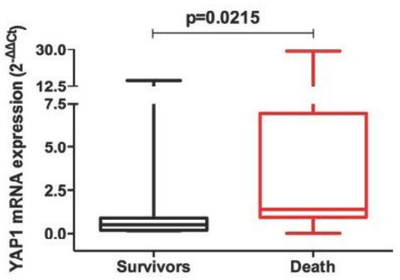

Figure 8. *YAP1* mRNA expression and patient's outcome. (n=28)

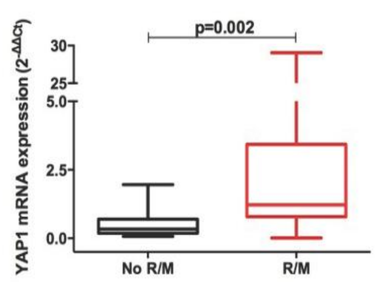

Figure 9. *YAP1* mRNA expression in patients with tumor recurrence or metastasis. (n=29)

Bayesian linear regression

- *YAP1* mRNA expression was higher in patients with recurrence/metastasis (5.02; 95% CrI: 2.09-7.94), death (5.09; 95% CrI: 2.09-8.16) and advanced tumor stage (4.63; 95% CrI: 0.61-8.64) (Table 1)

	Mean	CrI 95%
Pediatric ACTs	0,34	(-2.8 - 3.5)
Death	**5,09**	**(2.09 - 8.16)**
Recurrence/Metastasis	**5,02**	**(2.09 - 7.94)**
Histology	1,47	(-2.12 - 5.06)
Weiss score	-1,48	(-5.16 - 2.13)
Sandrini staging	**4,63**	**(0.61 - 8.64)**
TP53 mutation	2,76	(-4.18 - 6.83)

Table 1. Results of linear regression models: higher *YAP1* mRNA expression was associated with death, recurrence and/or metastasis and in advanced tumoral stage.

Results

Survival analysis

- *YAP1* mRNA overexpression was associated with lower survival (p=0.02; Fig. 10).

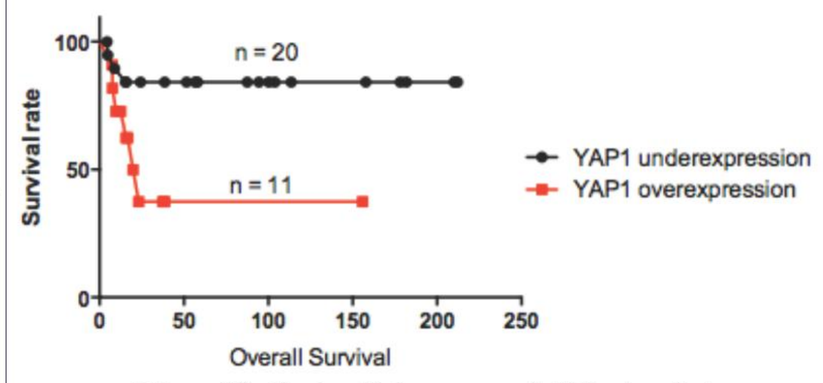

Figure 10. Kaplan-Meier curve of ACTs showing *YAP1* underexpression and overexpression.

qPCR - Adult ACTs

- In adult ACTs, lower YAP1 mRNA expression was associated with tumor reccurence and/or metastasis (p=0.04; Fig. 11).
- In adult ACTs, there was a significant positive association between lower YAP1 mRNA expression and advanced tumor stage (p=0.01; Fig. 12).
- *YAP1* mRNA expression was not associated with lower survival (p=0.94; Fig. 13).

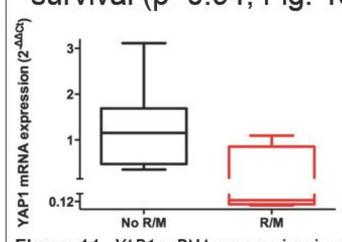

Figure 11. *YAP1* mRNA expression in patients with tumor recurrence or metastasis. (n=11).

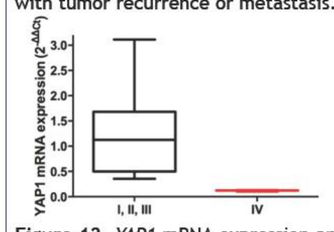

Figure 12. *YAP1* mRNA expression and tumor staging (MacFarlane-Sullivan). (n=11).

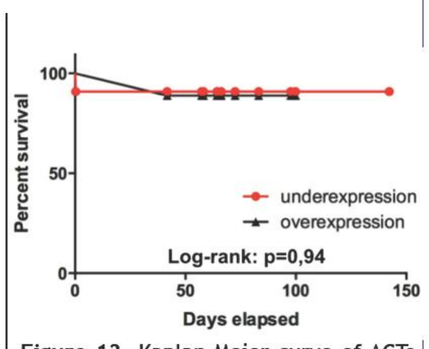

Figure 13. Kaplan-Meier curve of ACTs showing *YAP1* under-expression and overexpression.

In vitro study

- Compared with vehicle, TCF/ß-catenin complex inhibition decreased YAP1 protein expression to 56.2%, 42.1%, and 19.1% at 50µM, 100µM e 200µM PNU-74654, respectively, 48 hours after treatment (Fig. 14).

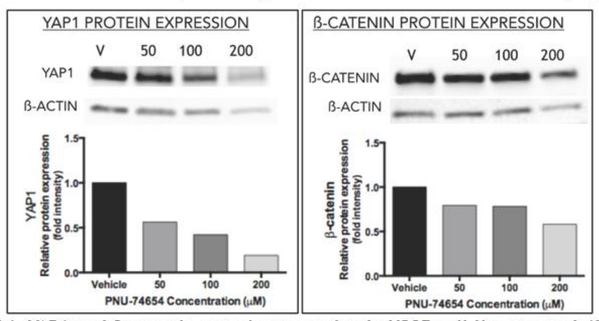

Figure 14. YAP1 and ß-catenin protein expression in H295 cell line treated 48h after treatment with PNU-74654. V=Vehicle, 50=50µM PNU-74654, 100=100µM PNU-74654, 200=200µM PNU-74654

Conclusion

- **Overexpression of the oncogene *YAP1* appears to be a marker of poor prognosis of pediatric patients with adrenocortical tumors.**
- **Higher expression of *YAP1* was associated with lower survival.**
- **In vitro, YAP1, a target of the Hippo pathway, is also a Wnt/ß-catenin target gene.**
- **Our results suggest that YAP1 may be an interesting target to treat invasive or recurrent adrenal tumors.**

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