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

Rafael H. Abdouch1, Ana C. Bueno1, Letícia F. Leal1, Marcelo M. Cavalcanti1, Silvia R. Brandalise2, Maria J. Masterllo3, José A. Yunes3, Carlos E. Martinelli Jr.3, Carlos A. Scrideli4, Luiz G. Tone1, Silvio Tucci1, Ayton C. Moreira1, Leandra Z. Ramalho1, Margaret de Castro2, Soni R. Antonini1

Department of Pediatrics1, Internal Medicine1, Surgery1 and Pathology2, Ribeirão Preto Medical School, University of Sao Paulo, Ribeirão Preto, Brazil; 3Boldrini Children’s Center, Campinas, Brazil

**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/β-catenin pathway abnormal activation plays an important role in adrenocortical tumors (ACTs).
- YAP1 is a potential target of the Wnt/β-catenin pathway (Fig. 1).
- To date, the role of YAP1 in adrenal tumorigenesis has not been evaluated.

**Aim**

To evaluate the role of YAP1 in adrenal tumorigenesis and its potential interaction with the Wnt/β-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 years [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 β-catenin p.S45P mutation)
> Treatment: TCF/β-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).

**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=0.09; Fig. 7).

**qPCR - Adult ACTs**

- In adult ACTs, lower YAP1 mRNA expression was associated with tumor recurrence and/or metastasis (p=0.04; Fig. 8).
- 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).

**in vitro study**

- 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 recurrence and metastasis, YAP1 mRNA expression was higher in the group of patients who had tumor recurrence or metastasis (p=0.002; Fig. 9).

**Bayesian linear regression**

- YAP1 mRNA expression was higher in patients with recurrence/metastasis (5.02; 95% CI: 2.09-7.94), death (5.09; 95% CI: 2.08-8.16) and advanced tumor stage (4.63; 95% CI: 0.61-8.64) (Table 1).

**Conclusion**

- Overexpression of the oncogene YAP1 appears to be a marker of poorer 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/β-catenin target gene.
- Our results suggest that YAP1 may be an interesting target to treat invasive or recurrent adrenal tumors.

**Acknowledgments**

Financial Support

**References**


---

**Figure Legends**

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

Figure 2. YAP1 protein expression in fetal adrenals.

Figure 3. YAP1 protein expression in postnatal normal adrenals.

Figure 4. YAP1 protein expression in pediatric adrenals.

Figure 5. YAP1 protein expression in pediatric adrenals.

Figure 6. YAP1 protein expression in pediatric ACTs (nucleosomal accumulation).

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

Figure 8. YAP1 mRNA expression and patient’s outcome. (n=38)

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

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

Figure 11. YAP1 mRNA expression in patients with tumor recurrence or metastasis (n=15).

Figure 12. YAP1 mRNA expression and tumor staging (n=60) (n=15).

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

Figure 14. YAP1 and β-catenin protein expression in H295 cell line treated 48h after treatment with PNU-74654. Vehiclae, 10μM PNU-74654, 50μM PNU-74654, 100μM PNU-74654, 200μM PNU-74654.