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EFFECT OF SILENCING ALDOSTERONE SYNTHASE ON CELL APOPTOSIS IN HAC15 HUMAN ADRENOCORTICAL CELLS

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INTRODUCTION

Aldosterone-producing adenomas (APA) are a surgically curable form of secondary hypertension caused by pathologic expression of aldosterone synthase, CYP11B2, in adrenal lesions. Overexpression of CYP11B2 leads to excessive synthesis of aldosterone, resulting in induction of hypertension. Somatic mutations in KCNJ5, ATP1A1, ATP2B3, CTNNB1 and CACNA1D have been identified in APA. Although the effect of the mutations on aldosterone production has been well-documented, the effect of modulating CYP11B2 on cell fate remains to be elucidated. We aimed to investigate the effect of silencing CYP11B2 on cell apoptosis in human adrenal cells.

METHODOLOGY

HAC15, a subclone of the H295R immortalized human adrenocortical cell line, was transfected with ONTARGET plus siRNA (Thermo Scientific) or relevant controls using the Neon™ Transfection System 100 μL Kit (MPK10096, Invitrogen) according to manufacturer's recommendations. Forty-eight hours after transfection, the apoptosis assay was performed using the Pacific Blue™ Annexin V/SYTOX™ AADvanced™ apoptosis kit (A35136, Invitrogen) on the BD FACSVerse™ system. The supernatants and cells were harvested for aldosterone (IS-3300, IDS-iSYS) and cortisol (06687733190, Roche Elecsys e100), and RNA isolation (12183018A, Invitrogen) for real-time PCR via Applied Biosystems ABI 7000. Experiments were repeated 3 times independently.

RESULTS

CYP11B2 silencing caused a reduction of aldosterone production (-69.4% ± 3.1) and CYP11B2 mRNA expression (-83.0% ± 12.7) compared to control ($p<0.05$), with no significant change in cortisol production. This suggested that silencing was specific to CYP11B2 despite the gene being highly homologous to CYP11B1. However, flow cytometric analysis of CYP11B2 silencing showed no significant difference on the percentage of apoptosis cells compared to control cells.

CONCLUSION

Our findings showed that silencing CYP11B2 decreases aldosterone synthesis but does not affect apoptosis rate in HAC15 cells. However, further investigation on cell proliferation is needed before ruling out that modulation of CYP11B2 can affect cell fate.