# Supplementary Material

# Human Antigen R (HuR) Facilitates miR-19 Synthesis and Affects Cellular Kinetics in Papillary Thyroid Cancer

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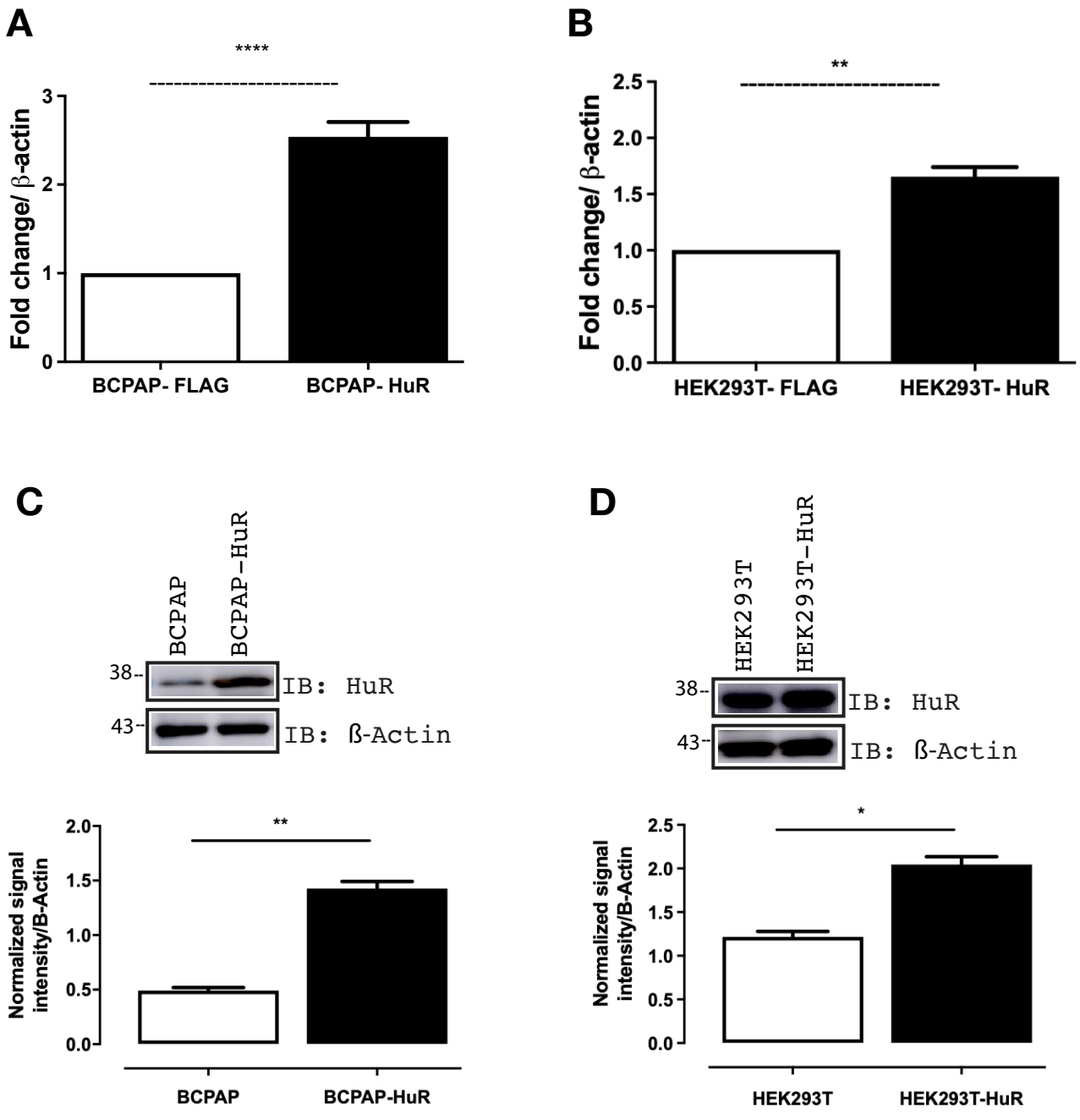
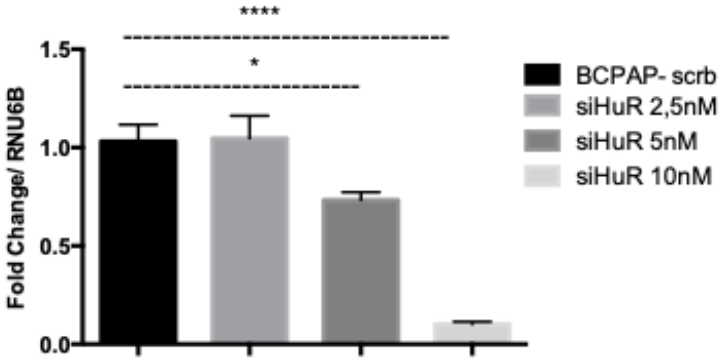
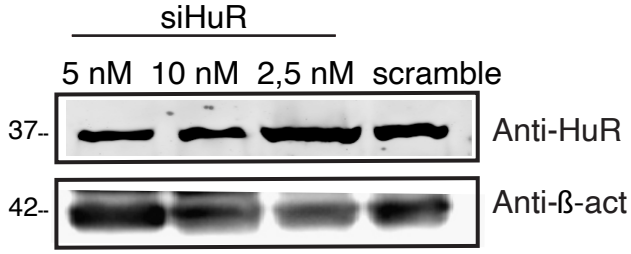

A
****
Fold change/ β-actin
3
2
1
0
BCPAP- FLAG
BCPAP- HuR
B
**
Fold change/ β-actin
2.5
2.0
1.5
1.0
0.5
0.0
HEK293T- FLAG
HEK293T- HuR
C
BCPAP
BCPAP-HuR
38-
IB: HuR
43-
IB: ß-Actin
**
Normalized signal intensity/B-Actin
2.0
1.5
1.0
0.5
0.0
BCPAP
BCPAP-HuR
D
HEK293T
HEK293T-HuR
38-
IB: HuR
43-
IB: ß-Actin
*
Normalized signal intensity/B-Actin
2.5
2.0
1.5
1.0
0.5
0.0
HEK293T
HEK293T-HuR

**Supplementary Figure 1.** Confirmation of FLAG-HuR over-expression in (A, C) BCPAP and (B, D) HEK-293T cells using qPCR and western blot. (A, B) Cell lines transfected with empty pFLAG were used as controls (white bars). The amplification of β-actin was used to normalize Ct values. The y-axis represents the fold change of expression calculated after normalization. Error bars represent standard deviations calculated from three independent measurements. (C, D) Western blot using anti-HuR and anti-β-actin were performed with the control and HuR over-expression cell lines, and optical densitometry was calculated using Image J software. The y-axis shows the signal intensity after β-actin normalization. *$P < 0.05$, **$P < 0.005$, ****$P<0.0005$.

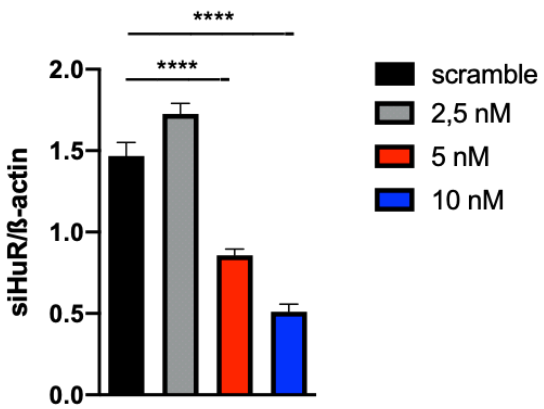
A


****
*
1.5
1.0
0.5
0.0
Fold Change/ RNU6B
BCPAP- scrb
siHuR 2,5nM
siHuR 5nM
siHuR 10nM

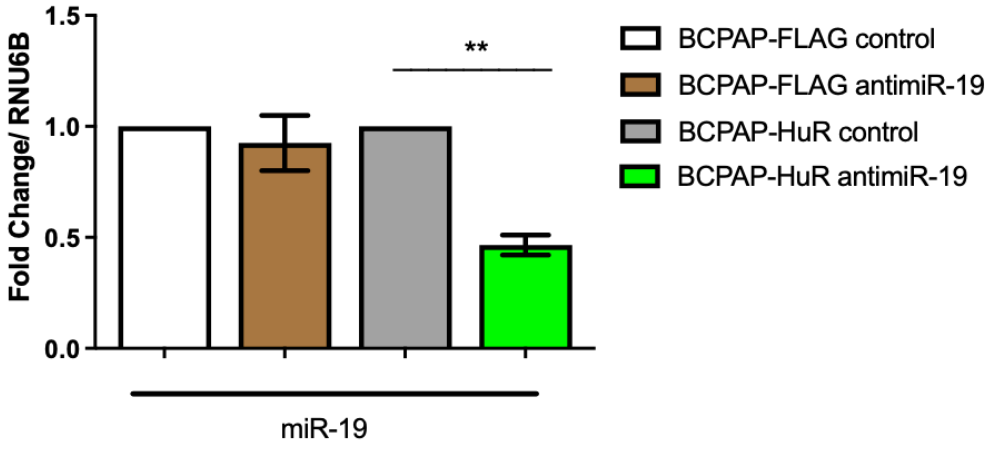
B


siHuR
5 nM
10 nM
2,5 nM
scramble
37-
Anti-HuR
42-
Anti-ß-act

C


****
****
2.0
1.5
1.0
0.5
0.0
siHuR/ß-actin
scramble
2,5 nM
5 nM
10 nM

**Supplementary Figure 2.** Confirmation of HuR knockdown. (A) qPCR analysis of HuR mRNA knockdown in BCPAP cells using siRNA-HuR (siHuR) at 2.5, 5, and 10 nM concentrations. HuR mRNA levels were assessed by qPCR with specific primers and normalization was performed with RNU6B amplification; BCPAP transfected with scrambled siRNA was used as a control (BCPAP-scrb). (B) HuR protein knockdown was analyzed after the use of 2.5, 5 and 10 nM siHuR by western blot using anti-HuR (30 kDa) and anti-β-actin as endogenous control (42 kDa). Cells transfected with scrambled siRNA were used as a control. (C) Optical densitometry was calculated using Image J software. The y-axis shows the signal intensity after β-actin normalization; ***** P < 0.0005*; **P < 0.05*.


1.5
1.0
0.5
0.0
Fold Change/ RNU6B
**
miR-19
BCPAP-FLAG control
BCPAP-FLAG antimiR-19
BCPAP-HuR control
BCPAP-HuR antimiR-19

**Supplementary Figure 3.** Confirmation of *miR-19a* inhibition in BCPAP-HuR cells. qRT-PCR analysis of *miR-19a* using BCPAP-FLAG (white), BCPAP-FLAG antimiR-19a (brown), BCPAP-HuR (grey), and BCPAP-HuR antimiR-19a (green). *miR-19a* levels were assessed using SYBR Green and specific primers. The y-axis represents the fold change of expression calculated after normalization with RNU6B primers. BCPAP-FLAG and BCPAP-HuR were used as controls to calculate *miR-19a* levels on the respective groups after antimiR-19a transfection. Error bars represent standard deviations calculated from three independent measurements. ***P < 0.005*.

**Supplementary Table S1.** Oligonucleotide sequences used in this work

| **Oligo** | **Sequence (5′-3′)** | **Source** |
|---|---|---|
| miR-17a - F | ATAGCCTCGAGGTCAGAATAATG | this work |
| miR-17a - R | ATGATAAGCTTGTCACCATAATG | this work |
| miR-18 - F | ATAGCCTCGAGTGTTCTAAGG | [1] |
| miR-18 - R | ATGATAAGCTTTGCCAGAAGG | [1] |
| miR-19a - F | ATAGCCTCGAGGCAGTCCTCTGTTAG | [1] |
| miR-19a - R | ATGATAAGCTTGCAGGCCACCATCAG | [1] |
| miR-92 - F | ATAGCCTCGAGCTTTCTACAC | this work |
| miR-92 - R | ATGATAAGCTTCCAAACTCAAC | this work |
| RNU6B - F | CTCGCTTCGGCAGCACATATAC | this work |
| RNU6B- R | GGAACGCTTCACGAATTTGCGTG | this work |
| HuR - F | GACTACAGGTTTGTCCAGAG | this work |
| HuR - R | GGGGGTTTATGACCATTGAA | this work |
| B-actin - F | ACCTTCTACAATGAGCTGCG | this work |
| B- actin - R | CCTGGATAGCAACGTACATGG | this work |
| hsa-miR19a | UGUGCAAAUCUAUGCAAAACUGA | Thermo |
| hsa-miR19b | UGUGCAAAUCCAUGCAAAACUGA | Thermo |
| hsa-miR18 | UAAGGUGCAUCUAGUGCAGAUAG | Thermo |
| hsa-miR423 | AGCUCGGUCUGAGGCCCCUCAGU | Thermo |
| anti-miR-19a | commercial | Ambion cat# 4464084 |
| anti-miR-negative control #1 | commercial | Ambion cat# 4464076 |
| siHuR | UGAACUACGUGACCGCGAATT | Thermo cat# 4390824 |
| siRNA-negative control | commercial | Thermo cat# 4390843 |

1. Paiva MM, Kimura ET,Coltri PP: miR18a and miR19a Recruit Specific Proteins for Splicing in Thyroid Cancer Cells.Cancer Genomics Proteomics 2017;14(5):373-381.