義大醫院 E-DA HOSPITAL I-SHOU UNIVERSITY

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基礎組-從業

探討位於14q32.31的MicroRNAs, miR-493, miR-494 和 miR-495對肝癌細胞株的生長、移行和侵襲之調控

MicroRNAs, miR-493, miR-494 and miR-495 mapped to the 14q32.31 locus, regulate proliferation, migration and invasion in hepatoma cell lines

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Background

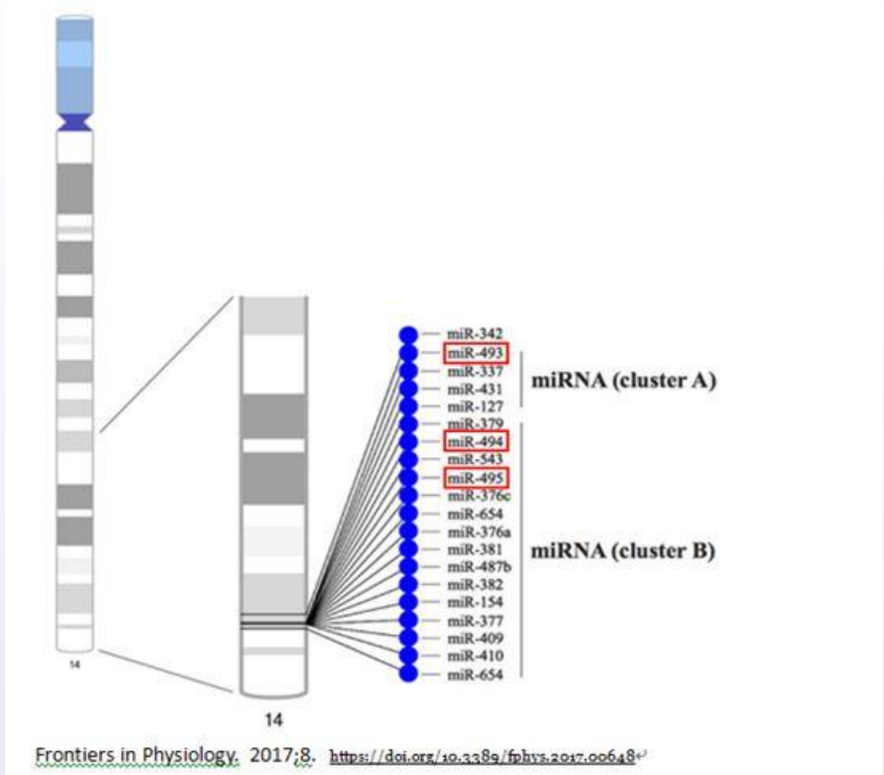
Hepatocellular carcinoma (HCC) is one of the most common tumors. MicroRNAs act as oncogenes or tumor suppressors in a wide variety of human cancers including HCC. A large cluster of microRNAs s located within the 14q32.2 DLK1-DIO3 region showed dysregulated in HCC. Our previous report demonstrated miR-493 decreased but with miR-495 overexpression in the HCC patients with early recurrence after hepatectomy. To ascertain the cellular function of those miRNAs, we transiently transfected miR-493, miR-494 and miR-495 in HuH7 cell lines and characterized their effect on cell behaviors including proliferation, migration and invasion.

Methods

Overexpression of miR-493, miR-494 and miR-495 were achieved by transfection of HuH7 cells with miRNA mimic separately or simultaneously. The wound healing and Trans-well migration were assessed.

Results

The chromosomal region 14q32.31 contains a large cluster of 20 intergenic miRNAs located within 10 kb of each other **(Fig 1)**. We analyzed the expression of miR493, miR494 and miR495 by RT-qPCR in HuH7 cell lines. As shown in **Fig 2**, miR493 and miR494 had markedly lower expression in Huh7 compared with L02. The only exception was the twice increased expression of miR-495.



Frontiers in Physiology. 2017;8. https://doi.org/10.3389/fphys.2017.00648

Fig 1. The clusters of miRNAs located in the 14q32.31 locus. The interested miR-493, miR-494 and miR-495 were highlighted.

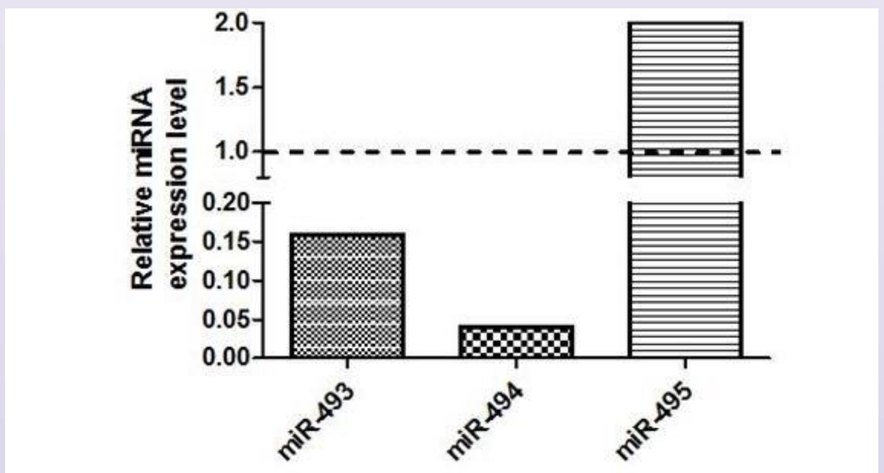


Fig 2. Basal expression level by quantified RT-PCR of miR-493, miR-494 and miR-495 in HuH7 cell lines relative to the normal hepatocyte cell line L02.

To determine the roles of miR-493, miR-494 and miR-495in HCC metastasis, we first performed a wound healing assay. As shown in **Fig 3**, wound healing assay suggested miR-493 overexpression attenuated HuH7 migration, whereas overexpression of miR-494 and miR-495 significantly enhanced the migration compared with the control cells. When combined effects of these miRNAs were analyzed, miR-493 significantly blocked the enhance effects of miR-494 and miR-495.

The transwell invasion assay was employed to measure the effects of miR-493, miR-494 and miR-495 on HCC invasion. The results presented in **Fig 4** demonstrated miR-493 suppressed HuH7 invasion, in the contrast, overexpression of miR-494 and miR-495 could boost the invasion. In addition, miR-493 significantly blocked the enhance effects of miR-495.

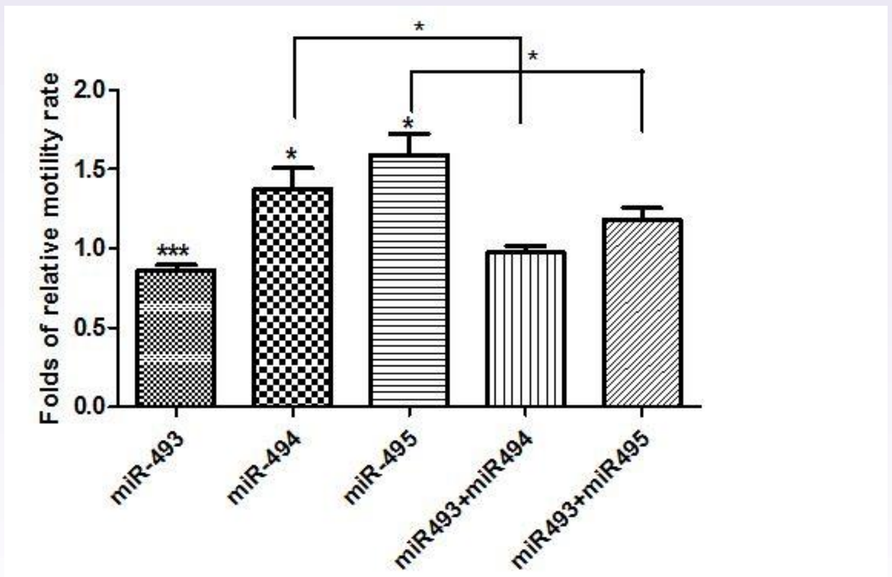


Fig 3. The effects of miR-493, miR-494 and miR-495 on HuH7 migration. HuH7 transfected with NC, miR-493, miR-494 and miR-495 mimics separately or simultaneously. Wound healing assay was performed with a 48-h recovery period. Quantification of the relative motility rate with NC transfected cells.

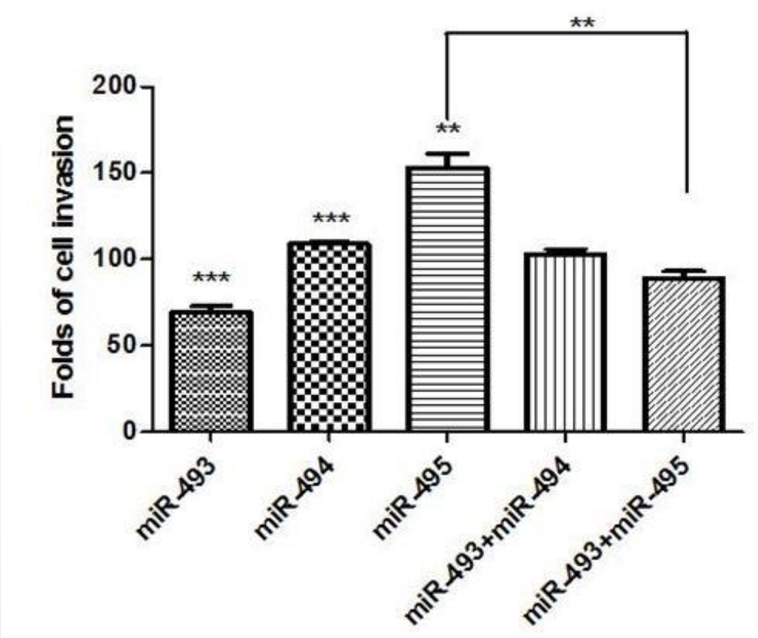


Fig 4. The effects of miR-493, miR-494 and miR-495 on HuH7 invasion. HuH7 was transfected with NC, miR-493, miR-494 and miR-495 mimics separately or simultaneously. Transwell invasion assay was analyzed post 24hr- transfection. Quantification of the relative folds of invasive cells compared with the cells transfected with NC.

Conclusion

There is aberrant expression of microRNAs of 14q32.31 cluster in HCC. The interaction of the miR-493, miR-494 and miR-495 on cell migration and invasion will affect HCC metastasis.

Reference

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2.MicroRNA 495 suppresses cell proliferation and invasion of hepatocellular carcinoma by directly targeting insulin like growth factor receptor 1. EXPERIMENTAL AND THERAPEUTIC MEDICINE 15: 1150-1158,2018.

3.MicroRNA-493 suppresses cell proliferation and invasion by targeting ZFX in human hepatocellular carcinoma.Cancer Biomark. 2018;22(3):427-434. doi: 10.3233/CBM-171036.