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INTRODUCTION

Background

Tumor cells adapt to the hypoxic microenvironment by forming vascular mimicry (VM) structures, allowing the tumor cells to be directly exposed to the blood, making the detached cells more prone to metastasis.

The mechanism by which CTHRC1, as a secreted protein, promotes tumor metastasis through the formation of VM has not been reported.

METHODS

Design:

- **Clinical sample analysis:** LUAD tumor tissues and serum were collected to assess the expression level of CTHRC1.
- **Construction of CTHRC1^{-/-} mice:** The CTHRC1 gene was modified using CRISPR/Cas9 technology, and CTHRC1^{-/-} mice and wild-type C57BL/6 mice were constructed.
- **Metastasis Functional Assays:** The effects of CTHRC1 expression on the metastasis of LUAD were evaluated through wound-healing, migration and invasion assays.
- **Mechanism study:** Utilizing multi-omics sequencing methods to elucidate the mechanism by which CTHRC1 promotes the formation of vascular patterning and facilitates metastasis.

DISCUSSION AND CONCLUSION

- This study comprehensively explores the correlation between VM and metastasis through clinical samples, CTHRC1^{-/-} mouse models, and molecular experiments.
- Our research indicates that under hypoxic conditions, the secretion of CTHRC1 increases through autophagy. It directly binds to integrin α V and activates the FAK signaling pathway.
- These findings not only establish the value of CTHRC1 as a prognostic biomarker, but also reveal that the CTHRC1-integrin α V-FAK-VM axis is a feasible therapeutic target.

REFERENCES & ACKNOWLEDGEMENTS

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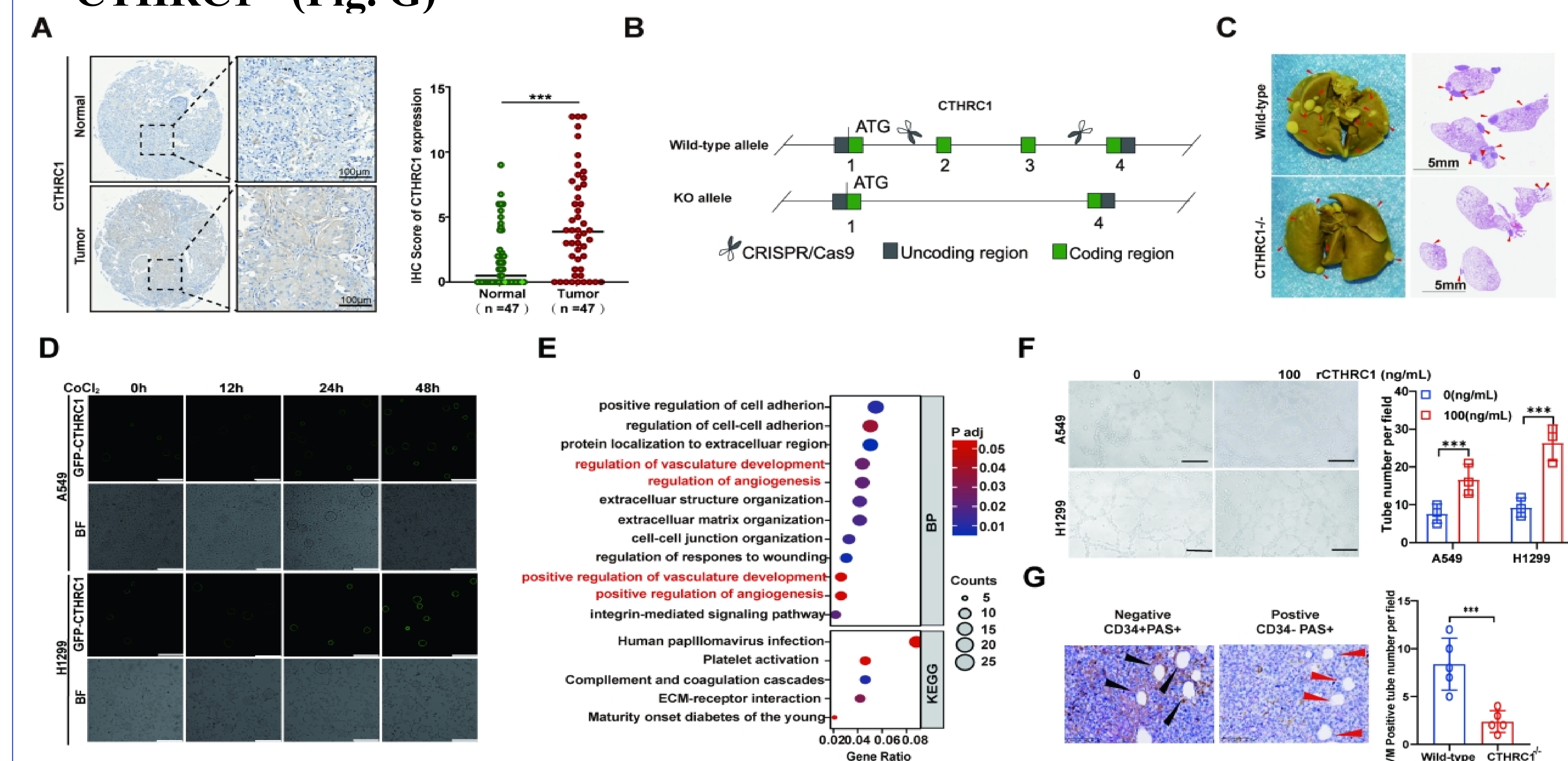
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RESULTS

- CTHRC1 is highly expressed from a clinical cohort study (n=47) (Fig A).
- CTHRC1 promotes vascular mimicry and metastasis in LUAD (Fig B-C).
- Hypoxic conditions enhance the autophagy-dependent secreted CTHRC1, which is released from the cell interior into the extracellular matrix,, a process visualized using a bead halo assay (Fig. D).
- CTHRC1 involved in vasculature development and angiogenesis (Fig. E).
- The rCTHRC1 treatment group exhibited significantly more tube-like structures than negative control group (Fig. F).
- CD34/PAS staining revealed a significant reduction in VM formation upon CTHRC1^{-/-} (Fig. G)



- (A) Immunohistochemical staining of CTHRC1 in LUAD patient tissues (n=47);
 (B) The construction strategy used for the CTHRC1^{-/-} mice;
 (C) Representative images of lung metastatic nodules and scanning photographs of H&E-stained lung micrometastatic foci.
 (D) CTHRC1 antibody-conjugated agarose beads were added to the cell culture supernatant, and the GFP-CTHRC1 signal captured by the beads was visualized.
 (E) KEGG enrichment analysis pathways;
 (F) The vascular mimicry formation ability after A549 and H1299 cells stimulation.
 (G) Representative images of CD34/PAS staining results.