Research on the Interaction Protein of Angiogenesis Factor AGGF1 and Long Non-coding RNA ANRIL

Jinyan Zhao, Qing K. Wang

Abstract

Angiogenic factor AGGF1 plays an important role in regulating the development of hematopoietic system and tumorigenesis. More evidence support that AGGF1 is involved in additional other cellular processes, such as autophagy, ER stress and glucose regulation. In order to understand the biological function of AGGF1 gene more comprehensively, we focus on looking for new proteins that directly interact with AGGF1 to discover new biological functions of AGGF1. Research results: two protein components, X and Y in HUVEC cell extracts were identified by HIS-PULL DOWN and MS, verified by CO-IP.

Genome wide association study showed that lncRNA ANRIL was a risk site for glioma, melanoma, breast cancer and nasopharyngeal carcinoma. In addition, ANRIL was found highly expressed in many cancer tissues and promoted tumor growth and migration. By expressing biotin-labeled ANRIL in vitro, we identified protein γ and protein δ from HUVEC cells as new RNA binding proteins that may interact with ANRIL through RNA pull down assay and mass spectrometry. It was found that overexpression of ANRIL slightly increased the expression of the above two proteins.

Result

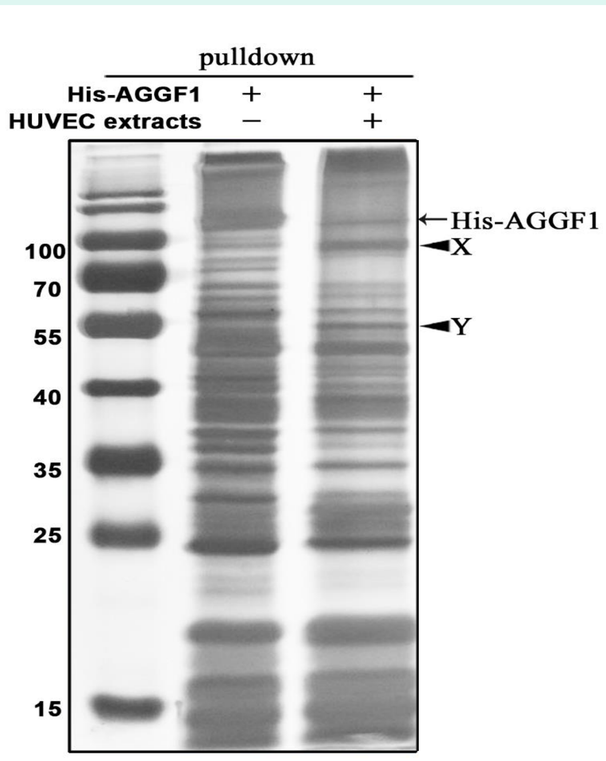

Figure 1 Silver staining polyacrylamine gel shows the result of pull-down in which His-tagged fusion proteins AGGF1 were used to incubated with or without HUVEC cell extracts. Short arrows show the position of X (91 kDa) and Y (54 kDa)

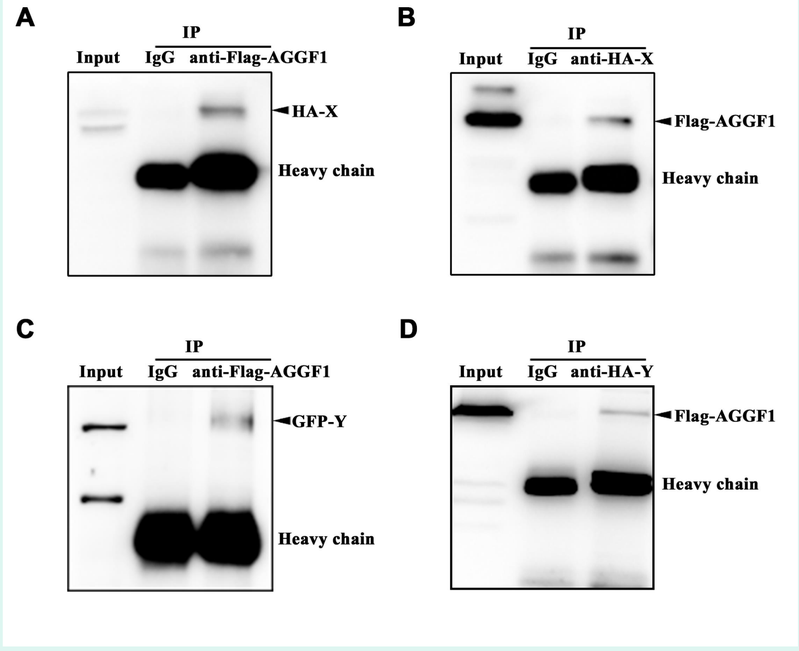

Figure 2 Western blot analysis of co-immunoprecipitation (co-IP) experiments demonstrating the interaction between AGGF1 and X (A-B) or Y (C-D) in Hela cells

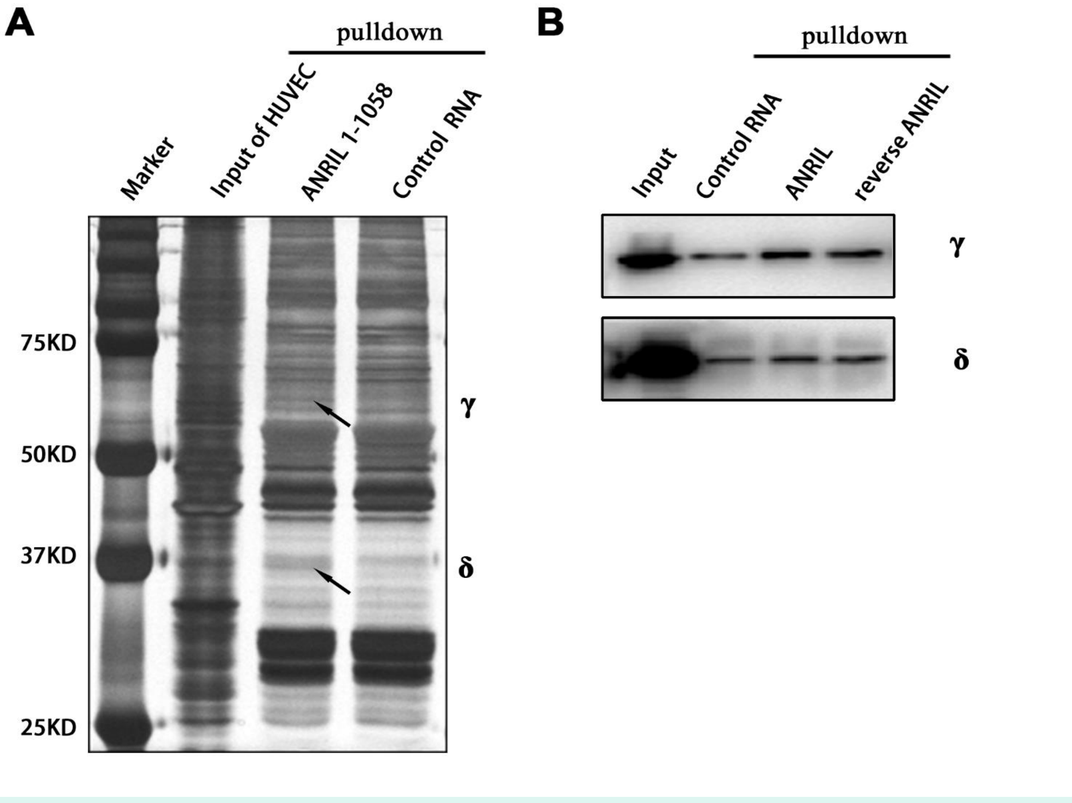

Figure 3 proteins γ, δ interact with ANRIL. (A) Silver staining polyacrylamine gel shows the result of pull-down in which truncated mutant RNA ANRIL 1-1058 or control RNA were used to incubated with HUVEC cell extracts. Arrows show the position of γ (60 kDa) and δ (37 kDa). (B) Western blot analysis of pull-down result between ANRIL, reverse ANRIL or control RNA and HUVEC cells with overexpression of γ or δ.

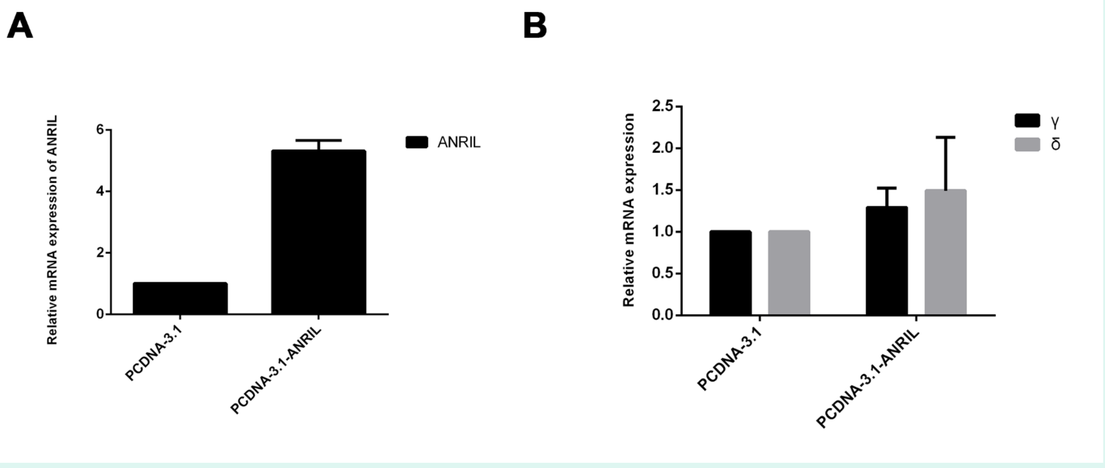

Figure 4 Overexpression of ANRIL slightly increased the expression of γ and δ. Relative mRNA expression was detected by qRT-PCR. Data are presented as the mean ± s.d. from at least three independent experiments.

Significance and Prospect

The discovery of new interacting proteins of cancer associated protein AGGF1 and lncRNA ANRIL will help to find new biological functions of them and better understanding their significance in biological regulation network. Subsequent functional studies showed that AGGF1, protein X and protein Y co-located in HeLa nucleus and collectively participated in a cellular process.

Note: as paper has not been published, only part of the results can be listed and the name of proteins was replaced by designation.

Supervisor: Jianfeng Liu
Key Laboratory of Molecular Biophysics of Ministry of Education,
School of Life Science and Technology,
Huazhong University of Science and Technology
Email: jfliu@mail.hust.edu.cn