FUNCTIONAL CHARACTERIZATION OF NOVEL CIRCULAR RNAS IN MOUSE GENOME

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Background

Noncoding RNAs (ncRNAs) often display tissue- and cell-type specific expression patterns, pointing to their involvement in biological processes such as cell fate specification and differentiation.

Circular RNAs (circRNAs) are single-stranded, covalently closed noncoding RNA molecules that are produced from pre-mRNAs through a process called backsplicing. However, the function and mechanism of circRNAs remains largely unknown.

Group 3 innate lymphoid cells (ILC3) are an important regulator for immunity, inflammation and tissue homeostasis in the intestine, but how ILC3 activation is regulated remains elusive.

Methods

Circular RNA knockout mice production and phenotyping pipeline

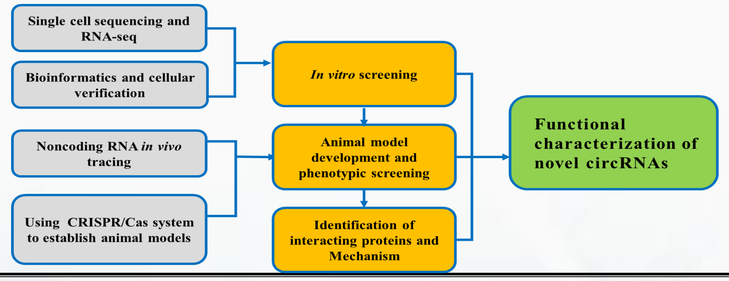


Fig. 1 CircIpo11 depletion impairs liver CSC self-renewal maintenance and inhibits HCC propagation. a-c) CircIPO11 is highly expressed in HCC. d) Construction of circIpo11-deficient mice. e) Macroscopic tumor images of circIpo11$^{+/+}$ and circIpo11$^{-/-}$ livers after treated by DEN for 8 months (8M) and 12 months (12M). CircIpo11$^{-/-}$ liver tumor foci were dramatically reduced in size compared with circIpo11$^{+/+}$ littermate control mice. CSC, Cancer Stem Cell; HCC, Hepatocellular Carcinoma.

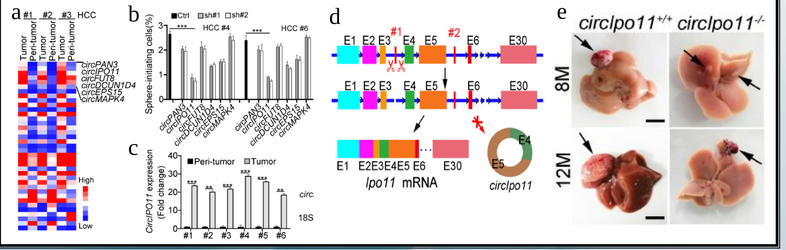


Fig.2 CircKcnt2 knockout induces ILC3 activation and causes spontaneous colitis. a) CircKcnt2 is highly induced in ILC3s during intestinal inflammation. b) Construction of circKcnt2-deficient mice. c) After DSS treatment, colon sections were analyzed by H&E staining. d) Construction of the host genes Kcnt2-deficient mice. e) Kcnt2 deficiency does not affect ILC3 maintenance.

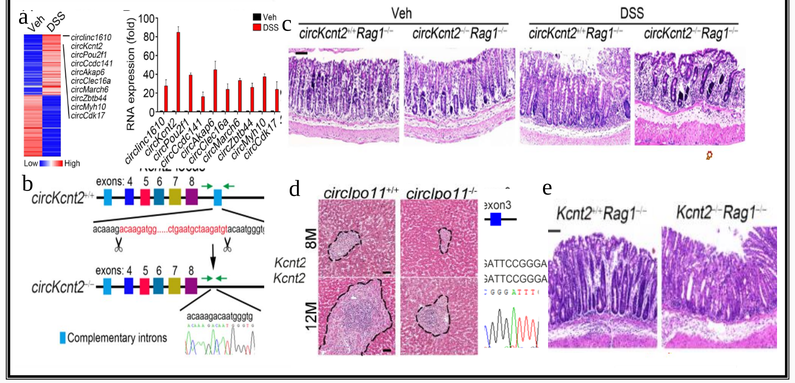
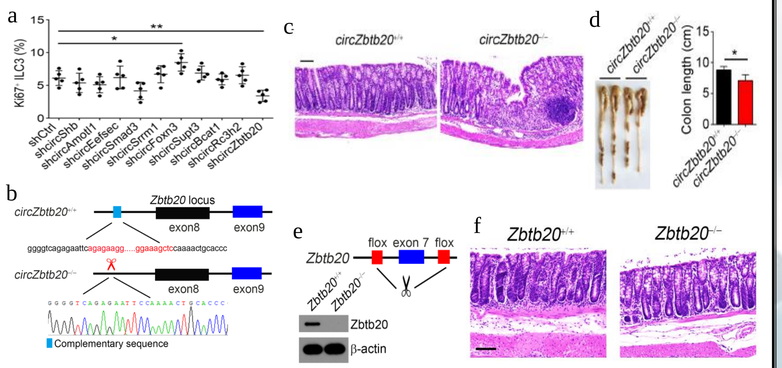


Fig. 3 CircZbtb20 knockout impairs ILC3 maintenance and function.

a) circZbtb20 depletion inhibited ILC3 proliferation. b) Construction of circZbtb20-deficient mice. c) Colon tissues from circZbtb20$^{+/+}$ and circZbtb20$^{-/-}$ mice were analyzed by H&E staining. d) Colon lengths were analyzed after infection with C. rodentium. e) Construction of the host gene Zbtb20-deficient mice. f) Zbtb20 deficiency does not affect ILC3 maintenance.

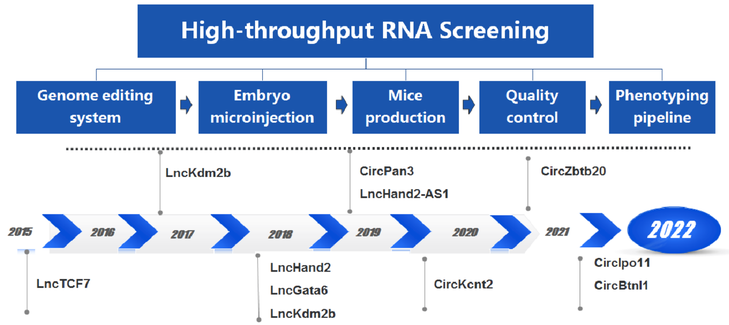


Results

Through systematic phenotyping and subsequent biochemical analysis, we showed that circular RNA circIpo11 drives self-renewal of liver cancer initiating cells. We also showed that circZbtb20 promotes ILC3 maintenance and function while circKcnt2 regulates ILC3 inactivation and resolution of innate colitis *in vivo*. Our findings reveal an important role of circular RNAs in the regulation of innate lymphoid cell homeostasis and stemness of CSCs.

Conclusions

We have developed a systematic pipeline to phenotype mouse mutants generated using CRISPR/Cas9 techniques to decipher the roles of circRNAs in organogenesis, embryogenesis, modulation of immune system and tumorigenesis. Our mouse screening study shows that ncRNAs and circRNAs are essentially invaluable targets for next-phase genome engineering.



References and Correspondence

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(2) Liu B#, Ye Bg#, **Zhu X#**, Yang L#, Li H Liu N, Zhu P, Lu T, He L, Tian Y*, Fan Z*. An inducible circular RNA circKcnt2 inhibits ILC3 activation to facilitate colitis resolution. ***Nature Communications.*** 2020;11(1):4076. (IF: 14.92)

(3) Liu B#, Liu N#, **Zhu X#**, Yang L, Ye B, Li H, Zhu P, Lu T, Tian Y*, Fan Z*. Circular RNA circZbtb20 maintains ILC3 homeostasis and function via Alkbh5-dependent m(6)A demethylation of Nr4a1 mRNA. ***Cellular & Molecular Immunology***, 2021: Jun;18(6):1412-1424. (IF: 11.53)

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