PNRC1 drives self-reinforcing activation of p53 in colorectal cancer

Chen Xiong[1,2,#], Qinghua Zhang[1,2,#], Tengfei Chao[3,#], Bangxiang Xie[4,#], Jing Tong[1,2], Yu Gan[5], Ruiwen Ruan[5], Jun Deng[5], Jianchun Chen[6], Tao Han[7], Xiang Zhou[1,2,8,9,*], Chunmeng Wang[2,10,*], and Qian Hao[1,2,8,9,*]

[1] Cancer Institute, Fudan University Shanghai Cancer Center, Shanghai 200032, China
[2] Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China
[3] Department of Oncology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei, China
[4] Beijing Institute of Hepatology, Beijing Youan Hospital, Capital Medical University, Beijing 100069 China
[5] The First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang 330006, Jiangxi, China
[6] Department of Neurosurgery, Changhai Hospital, Naval Medical University (Second Military Medical University), Shanghai 200433, China
[7] Xinxiang Key laboratory for Molecular Oncology, Institutes of Health Central Plains, Henan Medical University, Xinxiang 453003, China
[8] Shanghai Key Laboratory of Radiation Oncology, Department of Radiation Oncology, Fudan University Shanghai Cancer Center, Shanghai 200032, China
[9] Key Laboratory of Breast Cancer in Shanghai, Department of Breast Surgery, Fudan University Shanghai Cancer Center, Shanghai 200032, China
[10] Department of Musculoskeletal Oncology, Fudan University Shanghai Cancer Center, Shanghai 200032, China

[#] Equal contribution
[*] Correspondence:
Xiang Zhou, Email: xiangzhou@fudan.edu.cn
Chunmeng Wang, Email: cmwang1975@163.com
Qian Hao, Email: haoqian@fudan.edu.cn

Running title: Auto-activation of p53 by PNRC1

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Supplementary Figure S1.

(A, B) The volcano plot (A) and the differential expression ranking plot (B) of RNA sequencing show that PNRC1 is a p53-responsive gene in HCT116 $^{p53+/+}$ cells treated with 5-FU for 16 hours versus control.

(C-F) Nutlin-3, 5-FU, and Cisplatin do not affect the expression of PNRC1 mRNA (C, E) or protein (D, F) in HCT116 $^{p53-/-}$ and H1299 cells.

(G, H) Ectopic p53 induces PNRC1 mRNA (G) and protein (H) expression in H1299 cells.

(I) PNRC1 is abnormally expressed in multiple types of cancers (The Tumor Immune Estimation Resource (TIMER) database), ***$p < 0.001$, COAD, Colon Adenocarcinoma.

(J) PNRC1 is significantly decreased in COAD and other cancer types (The UALCAN database).

(K) Protein expression of PNRC1 is decreased in COAD samples (The Human Protein Atlas (HPA) database).

(L) Higher levels of PNRC1 are associated with better prognosis in patients with different cancer types (The Kaplan–Meier Plotter database).

Supplementary Figure S2.

(A, B) PNRC1 knockdown reduces apoptosis, whereas ectopic PNRC1 expression promotes apoptosis in colorectal cancer cells. HCT116 $^{p53+/+}$ and RKO cells were transfected with control or PNRC1 siRNAs for 48 h and harvested for the flow cytometric assay.

(C-E) p53 depletion attenuates the tumor-suppressive effects of PNRC1 in colorectal cancer cells. HCT116 $^{p53+/+}$ and RKO cells with PNRC1 overexpression were transfected with control siRNA or p53 siRNA, and cell proliferation (C), colony formation ability (D), and migration capacity (E) were evaluated by CCK-8 assay, colony formation assay, and transwell migration assay, respectively.

Supplementary Figure S3.

(A) IB analysis of p53 and p21 protein expression in mouse liver tissues.

(B) Schematic of the AOS/DSS treatment protocol.

Supplementary Tables

Table S1. Primers used for RT-qPCR

Primers	Sequences
p21-Forward	TGTCCGTCAGAACCCATGC
p21-Reverse	AAAGTCGAAGTTCCATCGCTC
PNRC1-Forward	ACTTGCCACTAACCAAGATCAC
PNRC1-Reverse	TTGGAAGAACACTAGGAGAAGGT
p53-Forward	CAGCACATGACGGAGGTTGT
p53-Reverse	TCATCCAAATACTCCACACGC
PUMA-Forward	GCCAGATTTGTGAGACAAGAGG
PUMA-Reverse	CAGGCACCTAATTGGGCTC
BAX-Forward	CCCGAGAGGTCTTTTTCCGAG
BAX-Reverse	CCAGCCCATGATGGTTCTGAT
BTG2-Forward	ACGGGAAGGGAACCGACAT
BTG2-Reverse	CAGTGGTGTTTGTAGTGCTCTG
MDM2-Forward	GAATCATCGGACTCAGGTACATC
MDM2-Reverse	TCTGTCTCACTAATTGCTCTCCT

Table S2. Sequences of PNRC1-siRNAs

siRNAs	Sequences
PNRC1-siRNA#1	GCATTTGAAGAAATCAGCATT
PNRC1-siRNA#2	GAACATTAAAAATTCGCATTT
PNRC1-siRNA#3	AAATTCGCATTTGAAGAAATT

Table S3. Sequences of PNRC1-sgRNAs

sgRNAs	Sequences
PNRC1-sgRNA1-F	caccgCAACCAAAACAGGGAGCTGA
PNRC1-sgRNA1-R	aaacTCAGCTCCCTGTTTTGGTTGc
PNRC1-sgRNA2-F	caccgTGAAAATTCCAACCAAAACA
PNRC1-sgRNA2-R	aaacTGTTTTGGTTGGAATTTTCAc
PNRC1-sgRNA3-F	caccgACCAAAACAGGGAGCTGATG
PNRC1-sgRNA3-R	aaacCATCAGCTCCCTGTTTTGGTc
PNRC1-sgRNA4-F	caccgAAGCCTCCTAGTCACTGGAT
PNRC1-sgRNA4-R	aaacATCCAGTGACTAGGAGGCTTc

Table S4. Primers used for ChIP-qPCR

Primers	Sequences
ChIP-qPCR Forward	GAAAAAATTTGCCCACCTTGTT
ChIP-qPCR Reverse	GTAACGCGGTAGTAAGCAAGAC