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Original Article

Overexpressed ACP5 has prognostic value in colorectal cancer and promotes cell proliferation and tumorigenesis via FAK/PI3K/AKT signaling pathway

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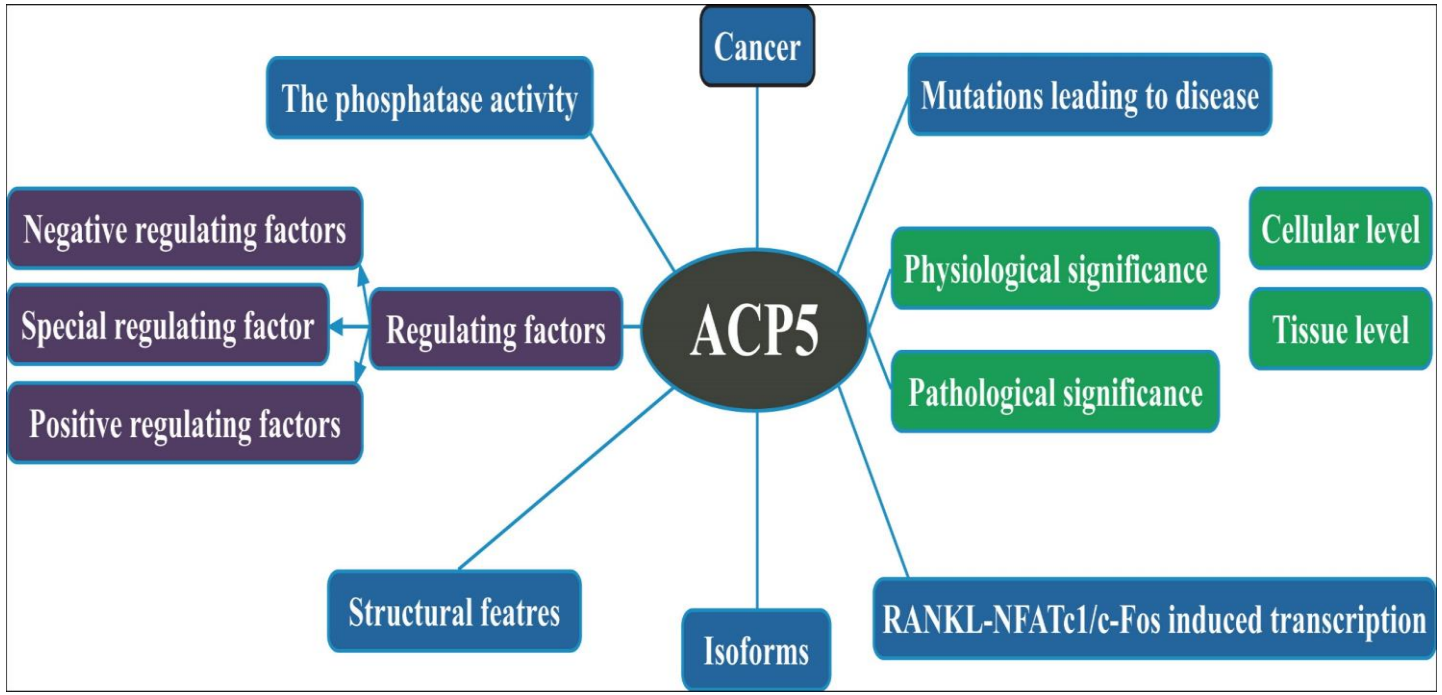
안 혜 빈

Introduction-ACP5 역할

- **Acid phosphatase 5, tartrate resistant (ACP5) or Tartrate-resistant acid phosphatase (TRAP)**



- Glycosylated monomeric metalloprotein enzyme expressed in mammals.
- Catalyze the conversion of ortho-phosphoric monoester to alcohol and ortho-phosphate.
- ACP5 broadly participates in various malignant process including uncontrolled proliferation, chemotherapy resistance, cellular invasion and metastasis.

Ren Xin , ACP5: Its Structure, Distribution, Regulation and Novel Functions, Anti-Cancer Agents in Medicinal Chemistry 2018; 18(8).

Introduction-선행연구

- ACP5 expression has been shown to be significantly up-regulated in hepatocellular carcinoma, breast cancer, and lung adenocarcinoma.

Immunology 2001 **102** 103–113

Mice lacking tartrate-resistant acid phosphatase (Acp 5) have disordered macrophage inflammatory responses and reduced clearance of the pathogen, *Staphylococcus aureus*

A. J. BUNE,* A. R. HAYMAN,* M. J. EVANS†‡ & T. M. COX* *Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge, †Wellcome CRC Institute of Cancer and Developmental Biology and Department of Genetics, University of Cambridge, Cambridge, UK

Wu *et al. BMC Cancer* 2010, **10**:158
http://www.biomedcentral.com/1471-2407/10/158

BMC Cancer

RESEARCH ARTICLE Open Access

Serum tartrate-resistant acid phosphatase 5b activity as a prognostic marker of survival in breast cancer with bone metastasis

Yi-Ying Wu[1], Anthony J Janckila[2], Chih-Hung Ku[3], Cheng-Ping Yu[1], Jyh-Cherng Yu[1], Su-Hui Lee[†1], Hsin-Yi Liu[†1], Lung T Yam[2] and Tsu-Yi Chao*[1,4]

Oncogene (2014) **33**, 1395–1406
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www.nature.com/onc

ORIGINAL ARTICLE

ACP5, a direct transcriptional target of FoxM1, promotes tumor metastasis and indicates poor prognosis in hepatocellular carcinoma

L Xia[1,4], W Huang[1,4], D Tian[2], Z Chen[1], L Zhang[1], Y Li[3], H Hu[1], J Liu[1], Z Chen[1], G Tang[1], J Dou[1], S Sha[1], B Xu[1], C Liu[1], J Ma[1], S Zhang[1], M Li[1], D Fan[1], Y Nie[1] and K Wu[1]

Molecular Therapy
Oncolytics
Original Article

AMERICAN SOCIETY of GENE & CELL THERAPY

Tartrate-Resistant Acid Phosphatase 5/ACP5 Interacts with p53 to Control the Expression of SMAD3 in Lung Adenocarcinoma

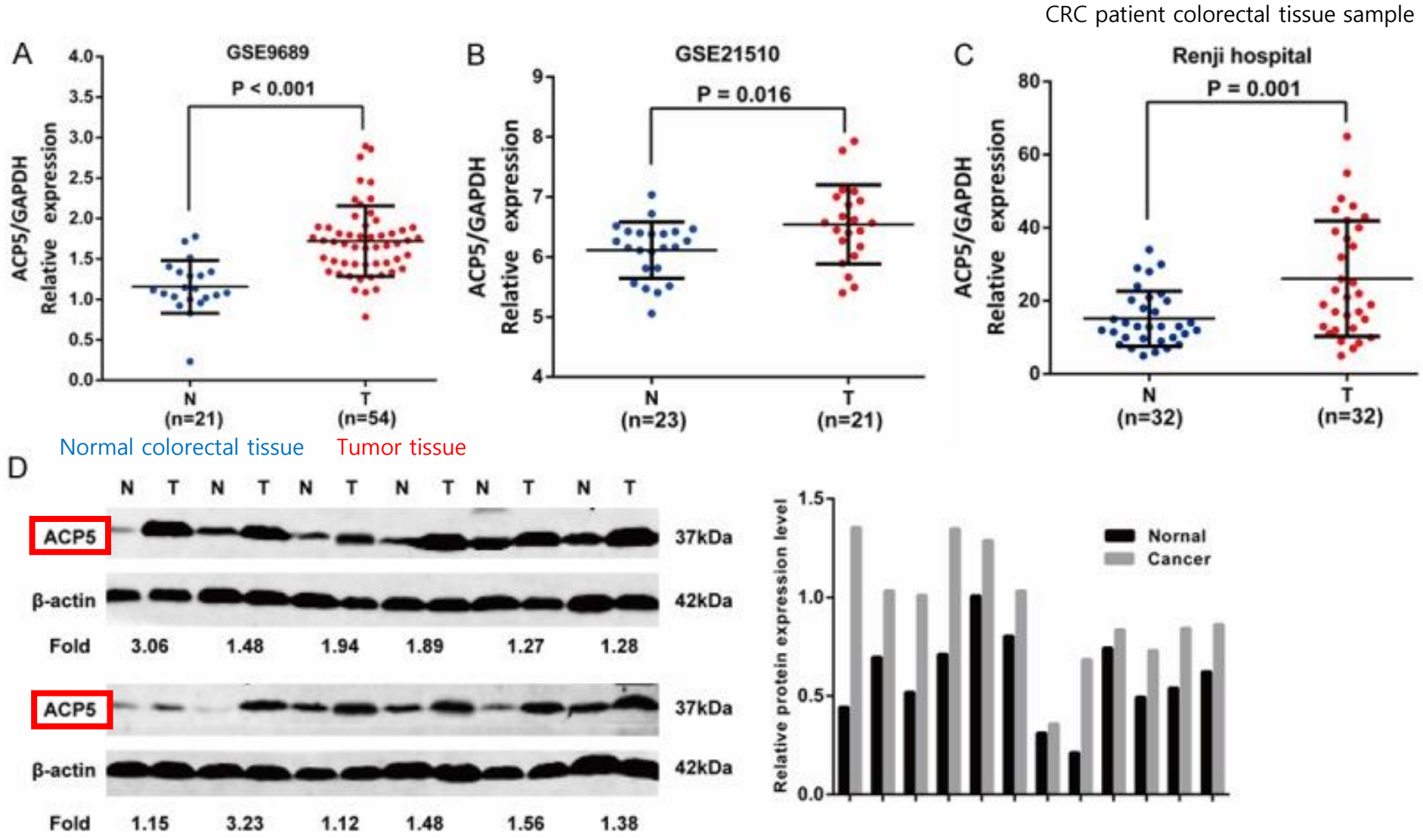
Yinan Hu,[1,5] Jun Yu,[2,5] Qi Wang,[1] Lei Zhang,[1] Xueying Chen,[1] Yong Cao,[1] Jianping Zhao,[1] Yongjian Xu,[1] Dingsheng Jiang,[3] Yi Wang,[1] and Weining Xiong[1,4]

- ✓ The functional role and underling molecular mechanism of ACP5 in CRC has not been investigated.
- ✓ These prompted to explore the role of ACP5 in human **colorectal cancer**.

Figure 1.

The expression of ACP5 in CRC tissues.

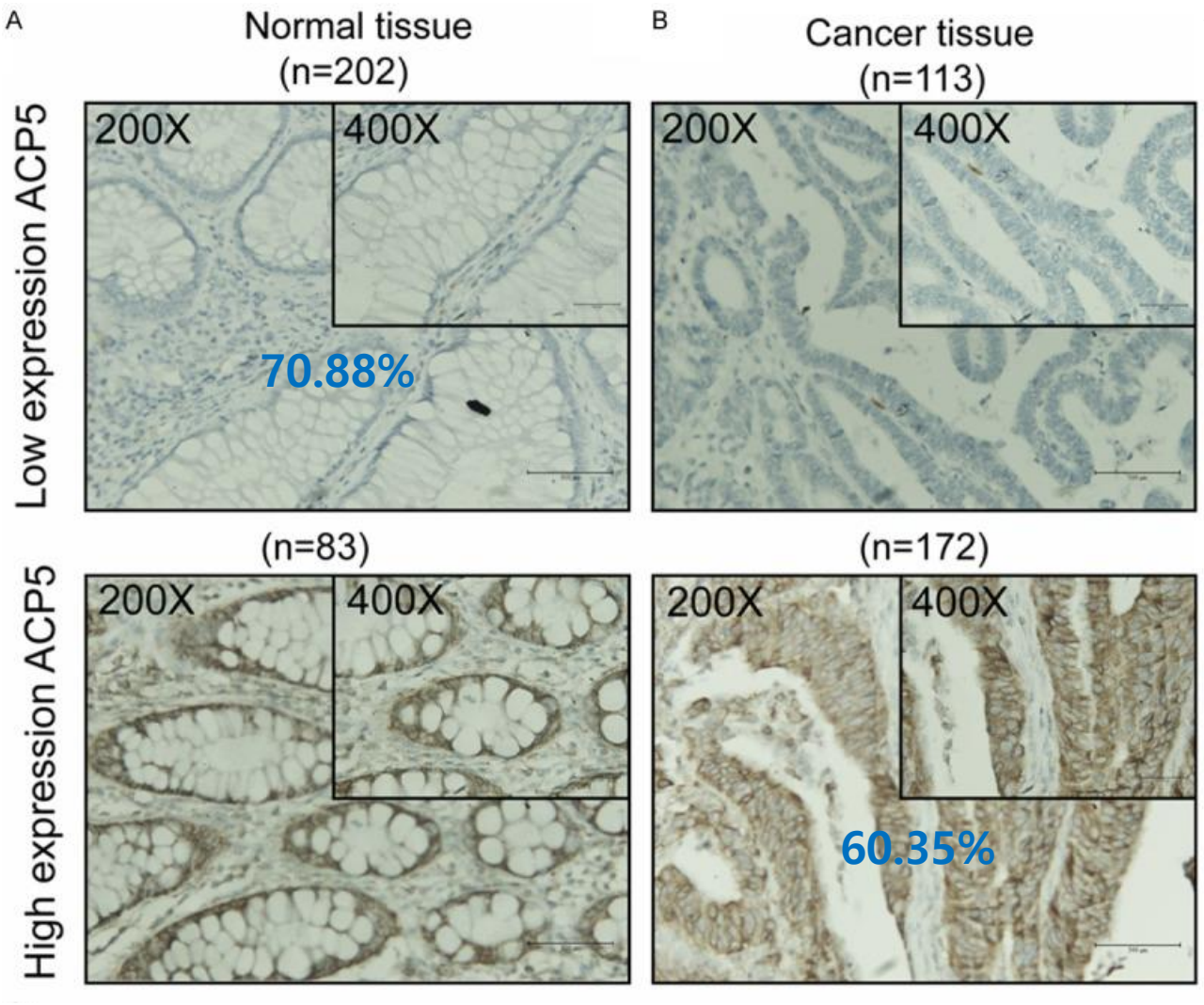
A) GSE9689
B) GSE21510
: microarray datasets from GEO(Gene Expression Omnibus) databases
C) RT-qPCR
D) Western Blot



✓ Both the mRNA and protein level of ACP5 were significantly up-regulated in CRC tissues.

Figure 2.

ACP5 expression in CRC tissues and matched adjacent non-tumor tissues.



✓ ACP5 protein level was measured by immunohistochemical analysis in normal tissues and CRC tissues.

C

	Tissue		*P* value (X^2)
	Normal (n=285)	Cancer (n=285)	
Immunohistochemical grade			
Low expression ACP5	202 (70.88%)	113 (39.65%)	<0.001
High expression ACP5	83 (29.12%)	172 (60.35%)	

Immunohistochemistry grading system

- Intensity of immunostaining score

: weak/moderate/strong immunostaining

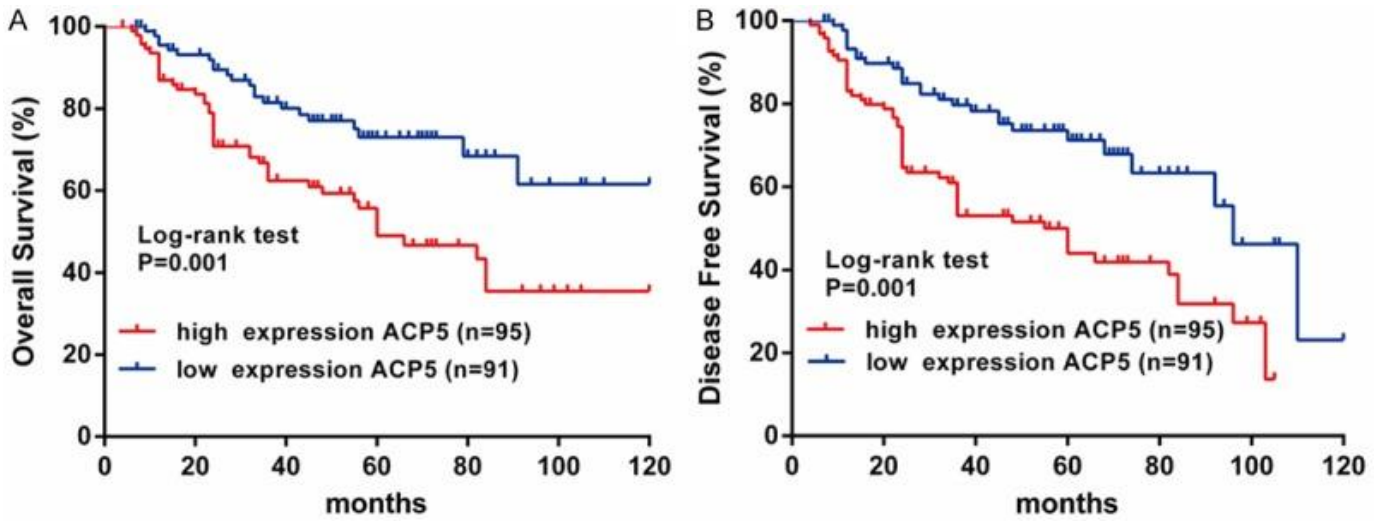
- Percentage of immunoreactive cell score

Grade	Descriptions
Negative	less than 1% positive cells
Grade 1	1–9% positive cells (light, moderate or heavy staining)
Grade 2	10–50% light cells with scattered moderate or heavy staining
Grade 3	mostly light, <50% moderate with scattered heavy staining
Grade 4	mostly moderate, <50% heavy staining
Grade 5	>50% heavy staining

Figure 3.

ACP5 expression invested associated the prognosis in CRC patients.

TNM stage: Tumor, Node, Metastasis/
classifying the extent of spread of cancer



- ✓ High ACP5 expression was remarkably associated with decreased OS and DFS.
- ✓ ACP5 expression was prominently correlated with tumor size, tumor classification, lymphatic metastasis, distant metastasis and TNM stage.
- ✓ ACP5 is correlated with clinicopathological features and prognosis in CRC patients.

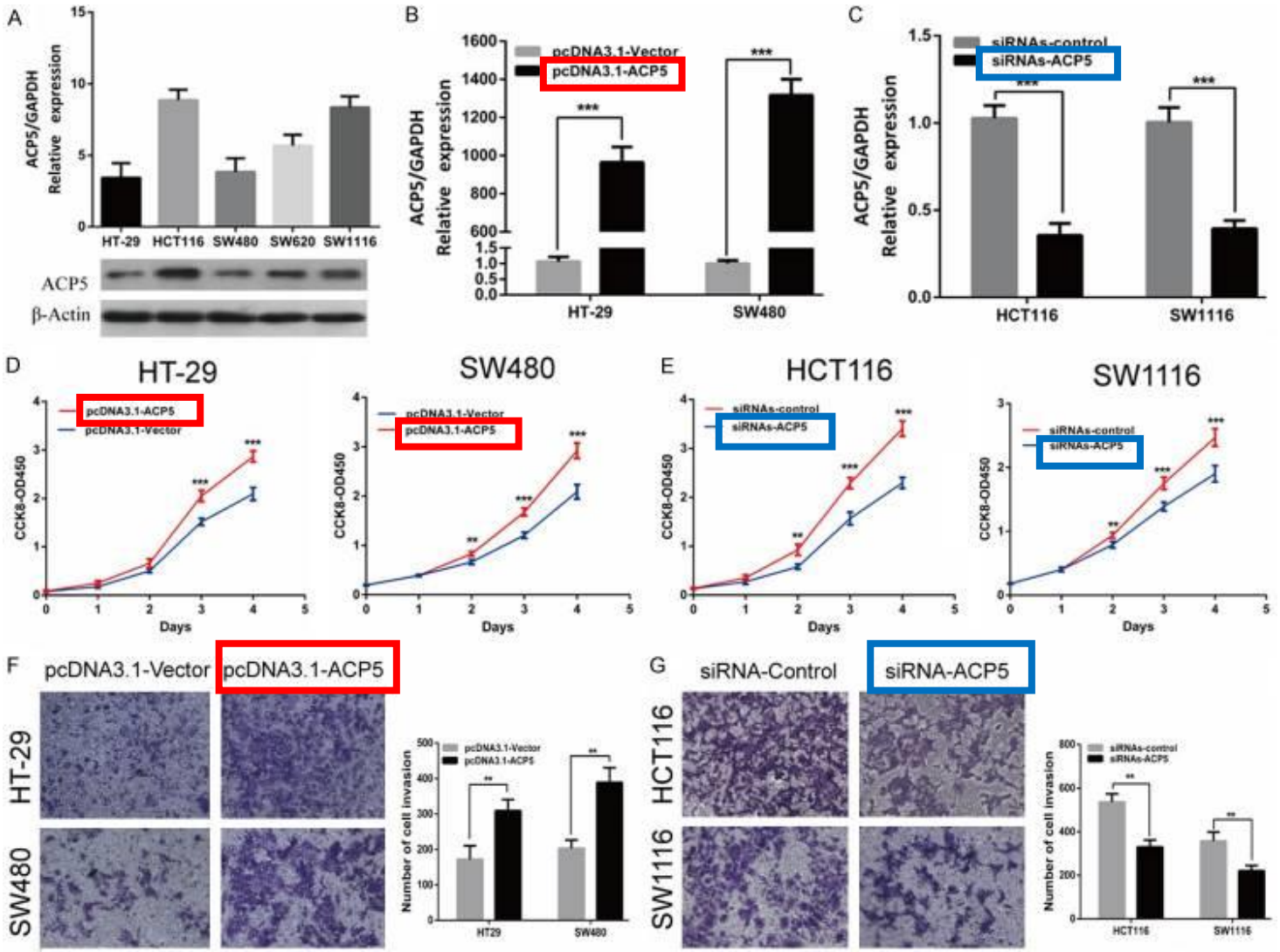
Correlations between ACP5 expression and clinicopathologic features in 285 CRC patients.

		ACP5 expression		
Clinicopathological feature	Total 285	Low (n=113, 39.65%)	High (n=172, 60.35%)	*P* value (χ^2 test)
Age (years)				
< 65	122	47 (41.59)	75 (43.60)	0.737
≥ 65	163	66 (58.41)	97 (56.40)	
Gender				
Male	170	69 (61.06)	101 (58.72)	0.694
Female	115	44 (38.94)	71 (41.28)	
Tumor location				
Rectum	176	73 (64.60)	103 (59.88)	0.423
Colon	109	40 (35.40)	69 (40.12)	
Tumor size				
≤ 5 cm	149	68 (60.18)	81 (47.09)	**0.031**
> 5 cm	136	45 (39.82)	91 (52.91)	
CEA level				
≤ 5 ng/ml	141	59 (52.21)	82 (47.67)	0.454
> 5 ng/ml	144	54 (47.79)	90 (52.33)	
Tumor classification				
T1-2	92	45 (39.82)	47 (27.33)	**0.027**
T3-4	193	68 (60.18)	125 (72.67)	
Lymph node metastasis				
Absent	144	71 (62.83)	73 (42.44)	**0.001**
Present	141	42 (37.17)	99 (57.56)	
Distant metastasis				
Absent	233	102 (90.27)	131 (76.16)	**0.003**
Present	52	11 (9.73)	41 (23.84)	
TNM stage(AJCC)				
Stage I	85	44 (38.94)	41 (23.84)	**0.005**
Stage II	59	24 (21.24)	35 (20.34)	
Stage III	89	34 (30.09)	55 (31.98)	
Stage IV	52	11 (9.73)	41 (23.84)	

Figure 4.

ACP5 promotes cell proliferation and invasion in CRC cells.

CRC cell line



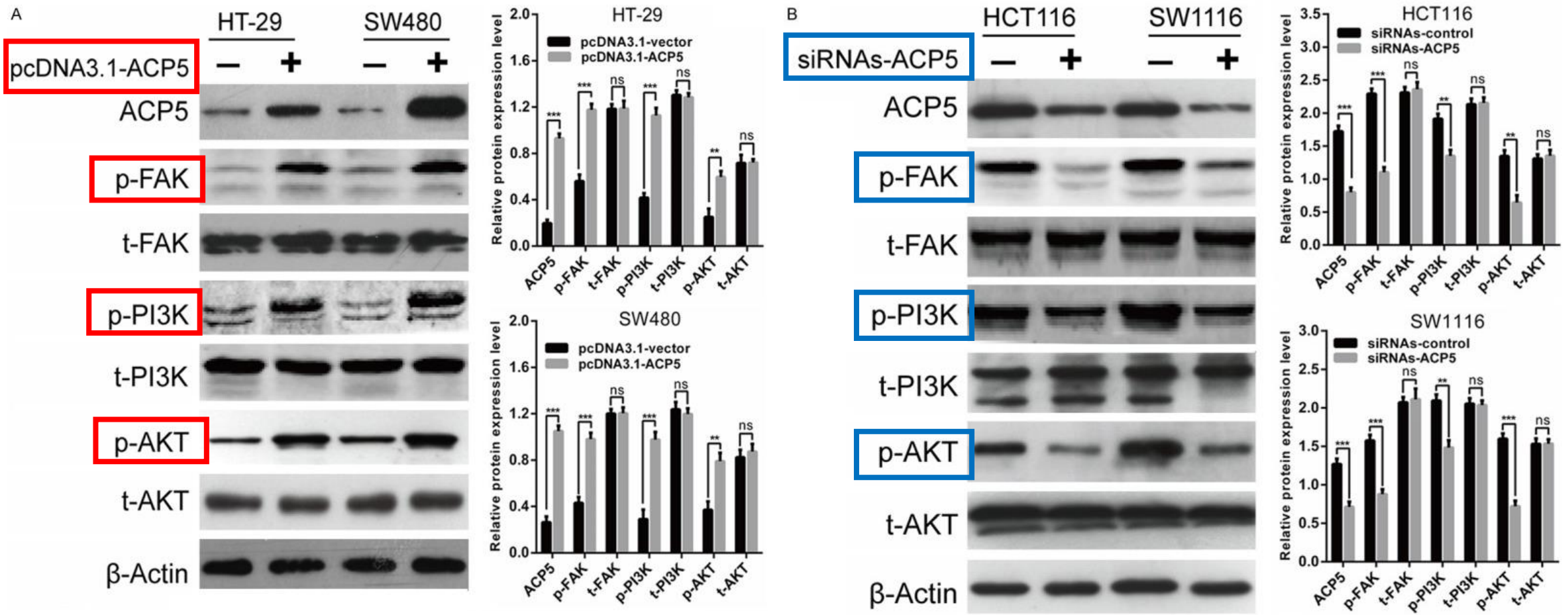
B,C) RT-qRCR
D) CCK-8 assay
F,G) Transwell assay

pcDNA3.1-ACP5
siRNAs-ACP5
: transfected into CRC cell lines.

- ✓ Growth rate of pcDNA3.1-ACP5 treated cells was promoted compared with pcDNA3.1-vector cells.
- ✓ pcDNA3.1- ACP5 significantly enhanced the invasive potential of HT-29 and SW480 cells .

Figure 5.

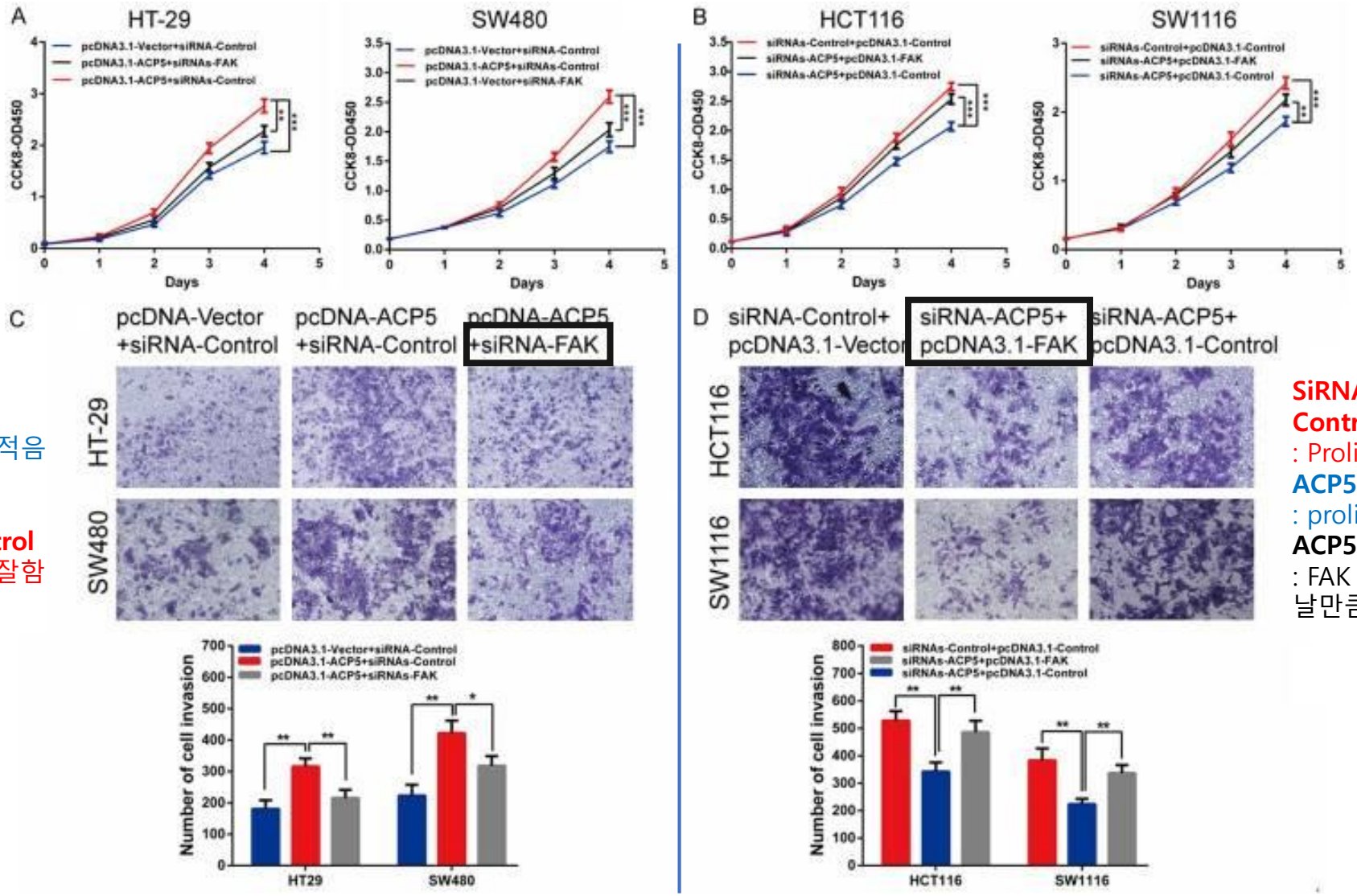
ACP5 up-regulates FAK expression and activates FAK/PI3K/AKT signaling pathway in CRC cells.



- ✓ Phospho-FAK, phospho-PI3K and phospho-Akt are marked increased in cells by pcDNA3.1-ACP5.
- ✓ Conversely, the phospho-FAK, phospho-PI3K and phospho-Akt protein are marked decreased in cells by siRNA-ACP5.

Figure 6.

Knock-down of FAK abolishes the oncogenic role of ACP5 in CRC cells.



C,D) Transwell assay

pcDNA3.1 Control
: proliferation 가장 적음
ACP5+siRNAs-FAK
: Proliferation 감소
ACP5+siRNAs-control
: Proliferation 가장 잘함

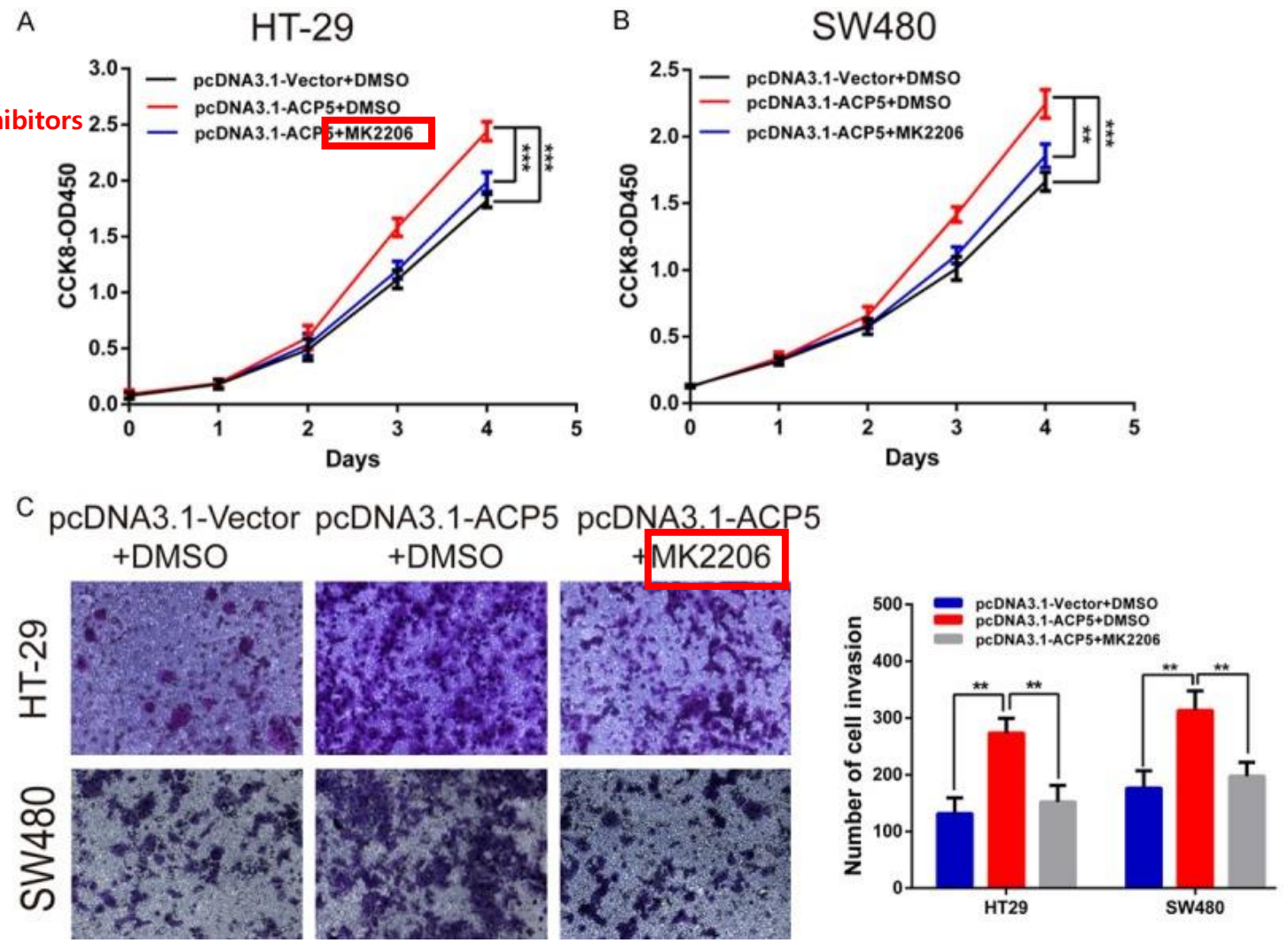
SiRNA Control
: Proliferation 가장 잘함
ACP5+pcDNA3.1-control
: proliferation 가장 적음
ACP5+pcDNA3.1-FAK
: FAK 과발현→ siRNA-ACP5 효과 안나타남만큼 proliferation, invasion 회복됨

✓ Overexpression of FAK completely recovered the proliferative and invasive potential of siRNAs-ACP5 cells.

Figure 7.

The Akt inhibitors could abolish the oncogenic role of ACP5 in CRC cells.

MK2206: Akt phosphorylation inhibitors

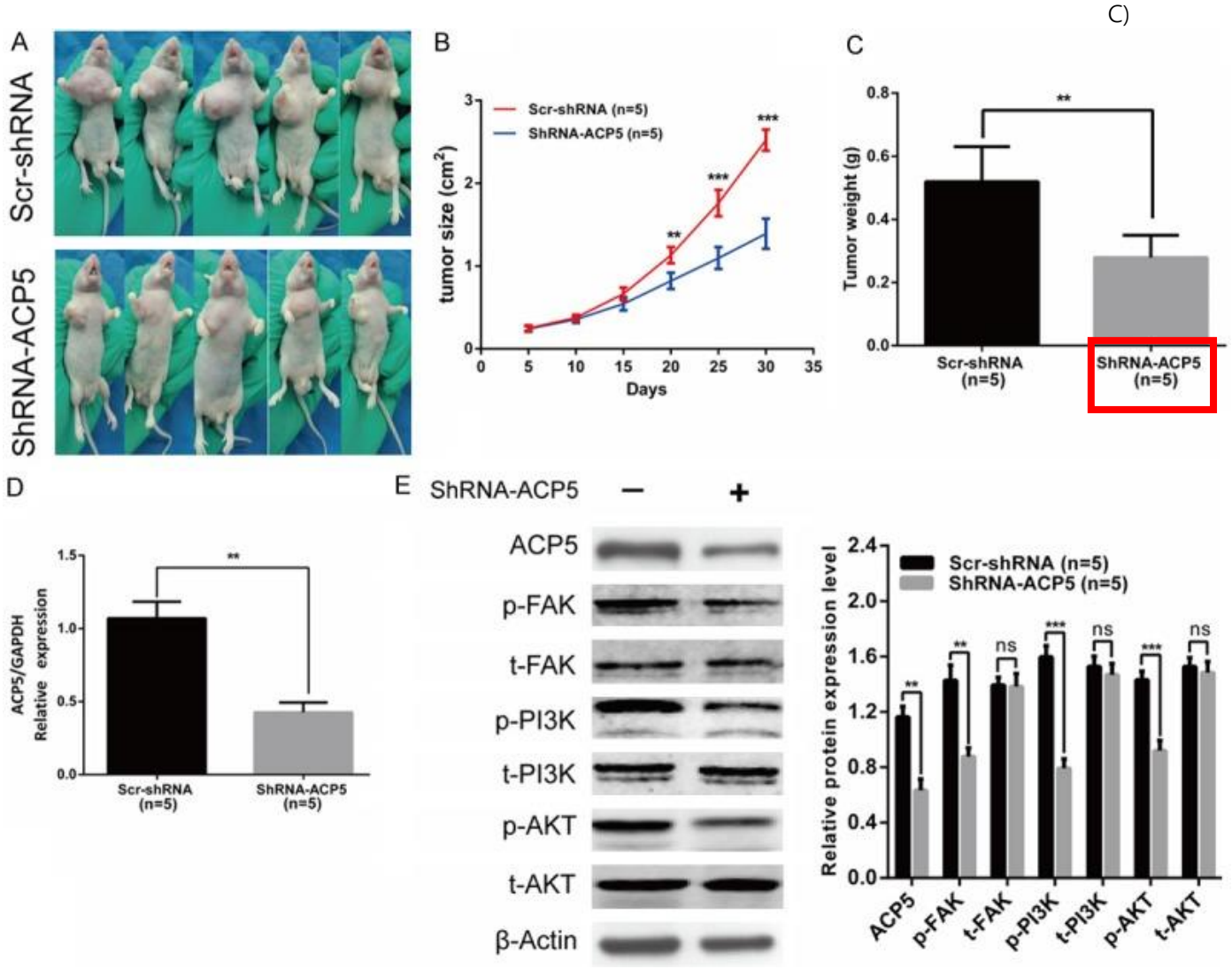


- ✓ The inhibition of activity by MK2206 could partially decrease the positive effects of ACP5 on CRC cell proliferation and invasion.
- ✓ ACP5 promoted CRC progression through the activation of the FAK/PI3K/AKT signaling pathway.

Figure 8.

shRNA-ACP5 inhibits tumor growth by FAK/PI3K/AKT signaling in vivo.

- **shRNA-ACP5-HCT116** cells were implanted subcutaneously into the flanks of BALB/C nude mice.
- Subcutaneous injection of 4x10^6 cells to BALB/C nude mice (4-6 weeks)



✓ ACP5 may activate FAK/PI3K/AKT signaling pathway to promote tumor progression.

Conclusion

- Overexpression of FAK completely recovered the proliferative and invasive potential of siRNAs-ACP5 cells.
- The inhibition of activity by Akt inhibitors could partially decrease the positive effects of ACP5 on CRC cell proliferation and invasion.
- ACP5 functions as an oncogene to facilitate the tumorigenesis and progression of colorectal cancer enhancing the FAK/PI3K/AKT signaling pathway.
- ACP5 could be a useful marker and potential therapeutic target in colorectal cancer.

- **ACP5의 중요성을 호흡기 질환 모델에서 확인**
- **ACP5 Knock out → DEP(Diesel Exhaust Particle) treatment → Apoptosis Assay → 호흡기 질환에 취약 → Mechanism Study**

- PI3K/AKT signaling pathway