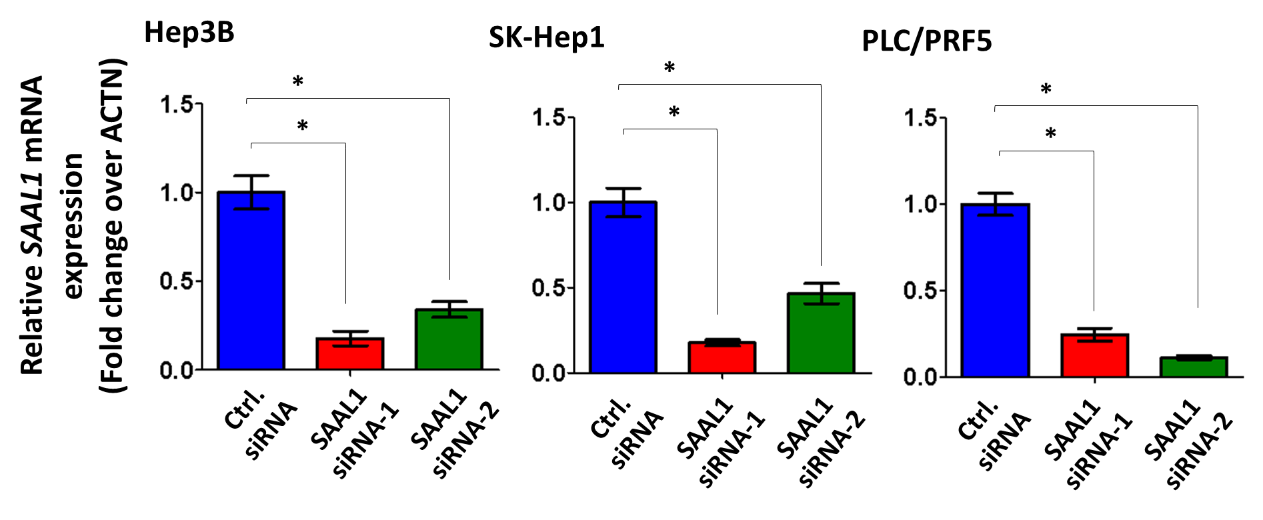
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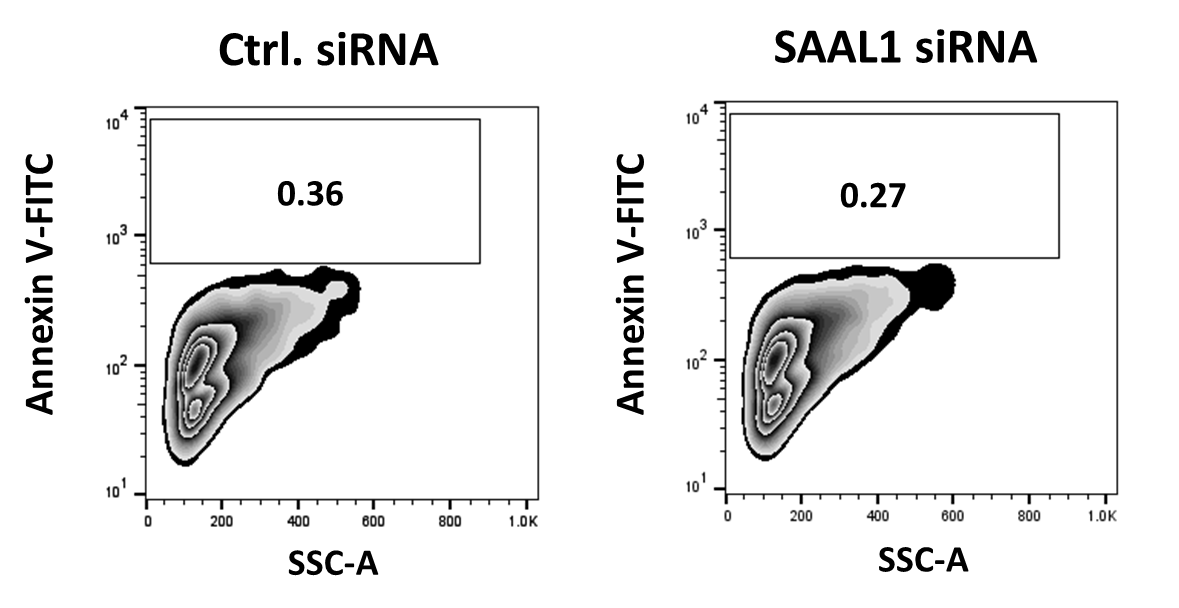
Identification of the Novel Oncogenic Role of SAAL1 and Its Therapeutic Potential in Hepatocellular Carcinoma

Pei-Yi Chu, Shiao-Lin Tung, Kuo-Wang Tsai, Fang-Ping Shen and Shih-Hsuan Chan

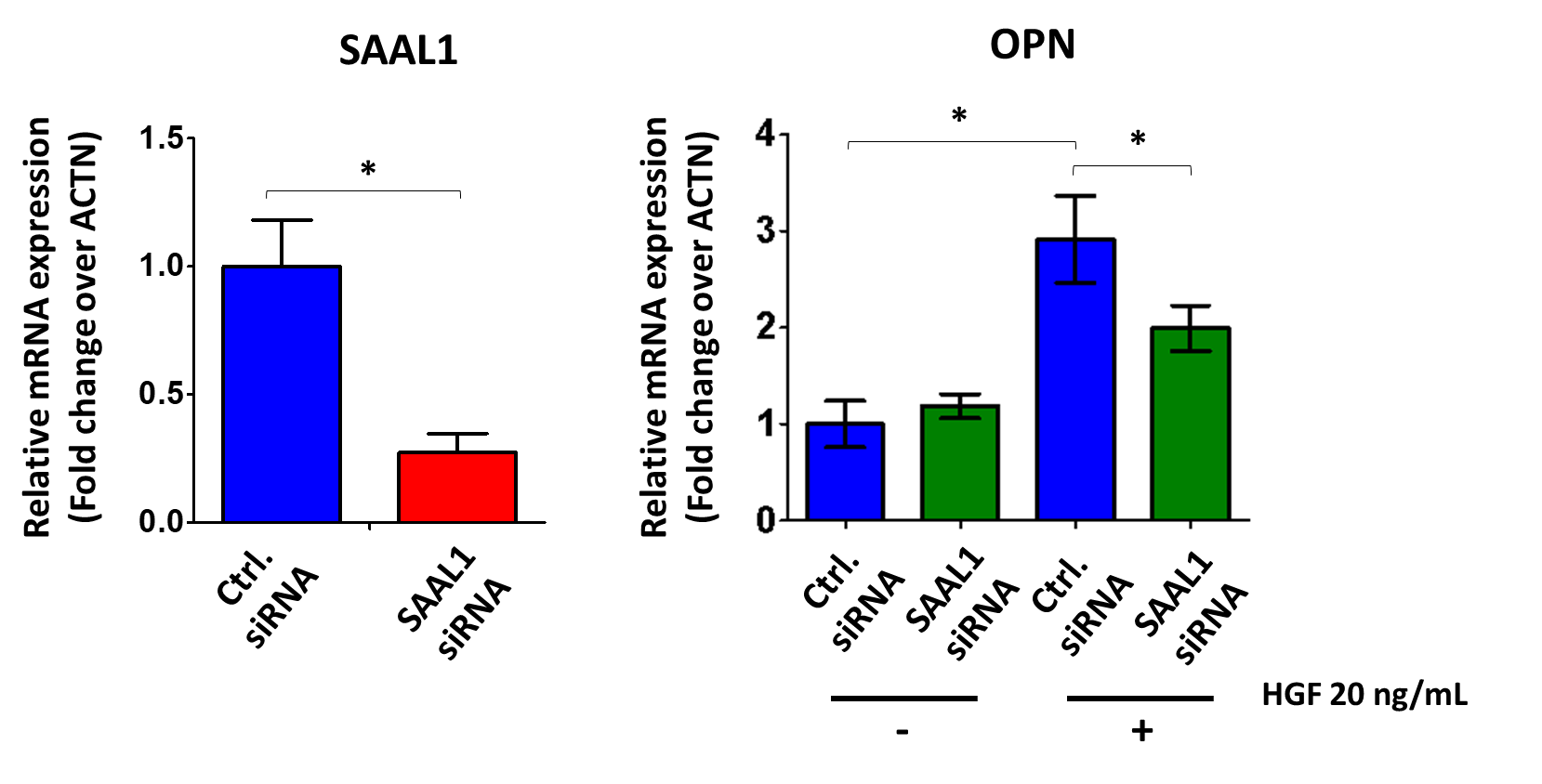
Supplementary Materials



**Supplementary Figure S1.** qRT-PCR analysis of SAAL1 siRNA Knockdown efficiency in three HCC cancer cell lines. SAAL1 mRNA expressions were normalized to Actin mRNA expressions. Data are means ± SEM (*n* = 3). \* *p* < 0.05.



**Supplementary Figure S2.** Flow cytometry analysis of annexin V expression in SAAL1-depleted SK-Hep1 cells. The SK-Hep1 cells were transfected with the control and SAAL1 siRNA respectively for 48 hours followed by annexin V-FITC staining. The representative figures were shown.



**Supplementary Figure S3.** SAAL1 depletion significantly abolishes HGF-induced Osteopontin (OPN) expression. Knockdown efficiency of SAAL1 siRNA and HGF-induced OPN expression were measured by qRT-PCR. Data are means ± SEM (*n* = 3). \* *p* < 0.05.

**Supplementary Table S1.** Correlation between expression levels of SAAL1 and clinicopathologic features of HCC patients.

|  |  |  |  |
| --- | --- | --- | --- |
| **Variables** | SAAL1 high (*n* = 151) | SAAL1 low (*n* = 195) | *p-*valuea |
| Number (%) | Number (%) |
| **AJCC Pathological stage** |  |  |  |
| I, II | 108 (71.5) | 148 (75.9) | 0.388 |
| III, IV | 43 (28.5) | 43 (24.1) |
| **pT stage** |  |  |  |
| T1, T2 | 111 (73.5) | 149 (76.4) | 0.616 |
| T3, T4 | 40 (26.5) | 46 (23.6) |
| **pN stage** |  |  |  |
| N0 | 148 (98.0) | 194 (99.5) | 0.321 |
| N1 | 3 (2.0) | 1 (0.5) |
| **pM stage** |  |  |  |
| M0 | 148 (98.0) | 194 (99.5) | 0.321 |
| M1 | 3 (2.0) | 1 (0.5) |

a *p-*value is estimated by Fisher’s exact test.

**Supplementary Table S2.** The detailed information of antibodies, reagents, chemicals and small interference RNA (siRNA) used in this study.

|  |  |  |
| --- | --- | --- |
| **Antibodies and Reagents** | **Vendor** | **Catalog Number** |
| Anti-Met antibody | Cell Signaling Technology, Danvers, MA, USA | 8198S |
| Anti-SAAL1 antibody | Bethyl Laboratory Inc., Montgomery, TX, USA | A304-966A |
| Anti-p-Met antibody | Cell Signaling Technology, Danvers, MA, USA | 3077S |
| Anti-Akt antibody | Cell Signaling Technology, Danvers, MA, USA | 4691S |
| Anti-p-Akt antibody | Cell Signaling Technology, Danvers, MA, USA | 9271S |
| Anti-mTOR antibody | Cell Signaling Technology, Danvers, MA, USA | #2983 |
| Anti-p-mTOR antibody | Cell Signaling Technology, Danvers, MA, USA | #2974 |
| Anti-β-actin antibody | Santa Cruz Biotechnology, Santa Cruz, CA, USA | sc-47778 |
| Anti-α-Tubulin antibody | Santa Cruz Biotechnology, Santa Cruz, CA, USA | sc-5286 |
| Anti-NA.K ATPase antibody | Cell Signaling Technology, Danvers, MA, USA | 23565 |
| Anti-Lamin B2 antibody | Abcam, San Francisco, CA, USA | Ab151735 |
| HGF recombinant protein | R＆D systems, MN, USA | 294-HG |
| *Trans*IT-X2® System | Mirus, Madison, WI, USA | MIR6000 |
| FITC annexin V | BD Biosciences | 51-65874X |
| propidium iodide | Sigma-Aldrich | P4170 |
| **siRNA** | **Oligonucleotide sequences used for siRNA**  **Transfection** | **Vendor** |
| Control siRNA | CCTAAGGTTAAGTCGCCCTCG | MDBIO, Taiwan |
| SAAL1 siRNA-1(448-467) | CCACCUACUCUGCUGGAAATT | MDBIO, Taiwan |
| SAAL1 siRNA-2(615-634) | GGUUGUGGACAAGCUCUUUTT | MDBIO, Taiwan |

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