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UPREGULATION OF ONCOGENE ACTIVIN A RECEPTOR TYPE I BY HELICOBACTER PYLORI INFECTION PROMOTES GASTRIC INTESTINAL METAPLASIA VIA REGULATING CDX2

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Background Activin A receptor type I (ACVR1) is involved in tumorigenesis. However, the underlying molecular mechanisms of ACVR1 in gastric cancer (GC) and its association with *Helicobacter pylori* (*H. pylori*) remained unclear.

Methods The Cancer Genome Atlas (TCGA) and Gene Expression Profiling Interactive Analysis (GEPIA) database were utilized to explore the ACVR1 expression in GC and normal control and its association with survival. The ACVR1 was knocked out using CRISPR/Cas-9, RNA sequencing analysis was performed in AGS cells with ACVR1 knockout and normal control. Functional experiments (CCK-8, colony-forming and transwell assays) were conducted to demonstrate the role of ACVR1 in cell proliferation, invasion and metastasis. ACVR1 and CDX2 were detected in AGS cells cocultured with wild type or *cagA*⁻ *H. pylori* strains (7.13 and PMSS1) at different MOIs or times. The CDX2 and key elements of BMP signaling pathway were detected in AGS cells with ACVR1 knockout and normal control. In addition, Immunohistochemistry was performed to detect the ACVR1 and CDX2 expression in gastric samples.

Results ACVR1 expression was higher in GC than normal control from TCGA, GEPIA and samples collected from our hospital ($P < 0.05$) (IDDF2021-ABS-0113 Figure 1. **ACVR1 is upregulated in GC and is associated with poor prognosis in GC patients.** (A) Normalized expression value of ACVR1 (log 2 transformation and Z correction) in 49 GC tissues and 35 adjacent normal tissues from the TCGA database. (B) The ACVR1 expression in GC and normal control from GEPIA database. (C) The ACVR1 expression in 6 pairs of GC and adjacent normal tissues, β -actin was used as an internal control. (D) The graph summarizes the WB results from 6 pairs of GC and adjacent normal tissues. (E) The survival plot shows the prognosis in 192 GC patients with high

ACVR1 expression and 192 GC patients with low ACVR1 expression. (F) The expression of ACVR1 in gastric mucosal epithelial cells and GC cells. (G) The graph summarizes the WB results from gastric mucosal epithelial cells and GC cells. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). Loss-of-function experiments showed that ACVR1 promoted cell proliferation, migration, and invasion in vitro (IDDF2021-ABS-0113 Figure 2. **ACVR1 promotes proliferation, invasion and metastasis of GC cells.** (A) The ACVR1 expression in AGS cells with ACVR1 knock-out and normal control. (B) The graph summarizes the WB results from AGS cells with ACVR1 knock-out and normal control. (C) CCK-8 assay is performed in AGS cells with ACVR1 knock-out and normal control, relative cell proliferation rate was calculated in each group. (D-E) Colony-forming assay is performed in AGS cells with ACVR1 knock-out and normal control, the number of colony formation was calculated in each group. (F-G) The invasion assay is performed in AGS cells with ACVR1 knock-out and normal control, stained invading cells were counted and shown for each group. Original magnification, $\times 100$. $**P < 0.01$, $***P < 0.001$). Both *cagA*⁺ and *cagA*⁻ *H. pylori* could upregulate the expression ACVR1 ($P < 0.05$) (IDDF2021-ABS-0113 Figure 3. ***H. pylori* upregulated ACVR1 expression independent of CagA** (A) Normalized expression value of ACVR1 (log 2 transformation and Z correction) in 16 *H. pylori*-positive GC patients and 33 *H. pylori*-negative GC patients. (B-C) WB analysis of ACVR1 in AGS-1 cells cocultured with *H. pylori* PMSS1 at different MOIs (0, 10, 50, 100 and 200). (D-E) WB analysis of ACVR1 in AGS-1 cells cocultured with *H. pylori* 7.13 at different MOIs (0, 10, 50, 100 and 200). (F-G) WB analysis of ACVR1 in AGS-1 cells cocultured with *cagA*⁺ and *cagA*⁻ *H. pylori* PMSS1. Hp: *H. pylori*. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). Downregulation of ACVR1 inhibited the *H. pylori*-induced cell proliferation, migration, and invasion ($P < 0.05$) (IDDF2021-ABS-0113 Figure 4. ***H. pylori* promotes cell proliferation, invasion and metastasis and ACVR1 downregulation inhibited this process.** (A-B) CCK-8 assay is performed in AGS cells with ACVR1 knock-out and normal control cocultured with *H. pylori* strains PMSS1, 7.13. (C-E) The invasion assay is performed in AGS cells with ACVR1 knock-out and normal control cocultured with *H. pylori* strains PMSS1, 7.13, stained invading cells were counted and shown for each

Abstract IDDF2021-ABS-0113 Figure 1

group. Original magnification, $\times 100$. Hp: *H. pylori*. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). The CDX2 and key elements of BMP signaling pathway were downregulated in AGS cells with ACVR1 knockout (IDDF2021-ABS-0113 Figure 5. **ACVR1 regulates the BMP signaling pathway and CDX2 expression.** (A) The ACVR1 and CDX2 expression in AGS cells with ACVR1 knock-out and normal control. (B) The graph summarizes the WB results of CDX2 expression in AGS cells with ACVR1 knock-out and normal control. (C)

The top 20 signaling pathway associated with ACVR1 from KEGG analysis. (D) The smad family expression in AGS cells with ACVR1 knock-out and normal control. (E) The CDX2 expression in AGS cells with ACVR1 knock-out and normal control from RNA sequencing data. (F-G) WB analysis of CDX2 in AGS-1 cells cocultured with *H. pylori* 7.13 at different MOIs (0, 10, 50, 100 and 200). (H-I) WB analysis of CDX2 in AGS-1 cells cocultured with *H. pylori* 7.13 from 0 to 24 h. Hp: *H. pylori*. * $P < 0.05$, *** $P < 0.001$). ACVR1

Abstract IDDF2021-ABS-0113 Figure 2

Abstract IDDF2021-ABS-0113 Figure 3

and CDX2 were upregulated in the stage of intestinal metaplasia (IM). Moreover, ACVR1 and CDX2 expression were higher in *H. pylori*-positive group than *H. pylori*-negative group ($P < 0.05$) (IDDF2021-ABS-0113 Figure 6. ACVR1 and CDX2 were significantly higher in *H. pylori*-positive subjects than *H. pylori*-negative subjects. (A) IHC staining is performed to detect the ACVR1 expression in chronic gastritis (CG), intestinal metaplasia (IM) and dysplasia (DYS) and GC

with or without *H. pylori* infection. (B-C) The graph summarizes the IHC staining results for ACVR1 in the gastric samples. (D) IHC staining is performed to detect the CDX2 expression in mild, moderate and severe IM with or without *H. pylori* infection. (E) The graph summarizes the IHC staining results for CDX2 in the gastric samples. Original magnification, $20 \times$, Scale bar = $50 \mu\text{m}$, Hp: *H. pylori*. * $P < 0.05$, *** $P < 0.001$).

Abstract IDDF2021-ABS-0113 Figure 4

Abstract IDDF2021-ABS-0113 Figure 5

Abstract IDDF2021-ABS-0113 Figure 6

Conclusions Our data indicate that *H. pylori* infection increases ACVR1 expression, promoting gastric IM via regulating CDX2, which is an essential step in *H. pylori* carcinogenesis.

IDDF2021-ABS-0123 STREPTOMYCES PLURIPOTENS MUSC 135T AS A TREASURE TROVE FOR ANTI-COLON CANCER AND ANTI-MRSA AGENTS

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Background The genus *Streptomyces* is well-known for the production of numerous bioactive compounds with interesting chemical scaffolds. *Streptomyces pluripotens* MUSC 135^T was isolated from a mangrove forest in Malaysia during a screening program for bioactive actinobacteria. The crude

fermentative extract of MUSC 135^T was shown to inhibit the growth of several pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA). Thus, this study aimed to explore the potentials of *Streptomyces pluripotens* in producing valuable bioactive compounds via fermentation experiments and genome mining to identify the presence of biosynthetic gene clusters (BGCs) for secondary metabolites.

Methods The genome mining analysis was performed by applying the anti-SMASH pipeline to annotate and analyze potential biosynthetic gene clusters of secondary metabolites in *S. pluripotens*. Gas chromatography-mass spectrometry (GC-MS) analysis was performed to detect the presence of bioactive compounds in the methanol extract of the fermentation product of *S. pluripotens*. Anti-colon cancer and anti-MRSA activities of identified bioactive compounds were evaluated.

Results GC-MS analysis detected several bioactive compounds that possess anti-colon cancer cells properties in the methanol extract of the fermentation product of *Streptomyces pluripotens*. For instance, compound (1) exhibits cytotoxic activity towards colorectal cancer HCT116 cell line (IDDF2021-ABS-0123 Figure 1. *Streptomyces pluripotens* MUSC 135^T, a treasure trove for anti-colon cancer and anti-MRSA agents). The result of BCGs prediction revealed 28 putative biosynthetic gene clusters, 8 of them showed high similarity to known gene clusters (>80%). These predicted gene clusters were reported to encode compounds with promising anti-colon