論文内容の要旨

論文題目　C12orf48, Termed PARP-1 Binding Protein (PARPBP), Enhances Poly(ADP-ribose) Polymerase-1 (PARP-1) Activity and Protects Pancreatic Cancer Cells from DNA Damage

膵がん標的分子、C12orf48 は PARP-1 の活性を増加させ DNA ダメージからがん細胞を保護する

氏名　Lianhua, Piao　(朴 蓮花)

Introduction

Pancreatic cancer is the fourth leading cause of cancer death in the western world and shows the worst mortality among common malignancies with a 5-year survival rate of lower than 5%. To identify novel therapeutic targets for aggressive and therapy-resistant pancreatic cancer, we here focus on the characterization of a novel gene C12orf48 (Chromosome 12 open reading frame 48) which was found to be trans-activated in pancreatic ductal adenocarcinoma (PDAC) cells according to our previous microarray expression profile. We demonstrate that C12orf48 protein can interact with PARP-1 directly and be involved in the repair of DNA breaks through enhancing PARP-1 activity. Thus, we termed this molecule PARP-1 binding protein (PARPBP).

Poly(ADP-ribose) polymerase-1 (PARP-1), a nuclear enzyme, catalyzes the transfer of the ADP-ribose unit from its substrate, NAD^+, to some protein acceptors such as histones, p53, and PARP-1 itself. PARP-1, a molecular nick-sensor of DNA breaks, is essential in the repair of both DNA single-strand breaks (SSB) as well as double-strand breaks (DSB). PARP-1 is involved in multiple cellular processes including DNA repair, transcriptional regulation, chromatin modification, cell cycle progression, or genomic stability. Inhibition of PARP-1 enhanced the cytotoxicity of DNA-damaging agents and seemed to overcome one of the causes of resistance in cancer cells to anticancer treatment.

Materials and Methods

Cell Lines In our studies, we mainly used KLM-1 and SUIT-2 PDAC cell lines, which highly expressed C12orf48 proteins.

Immunoprecipitation and Mass-Spectrometric Analysis Protein bands that specifically observed in the cell extracts transfected with pCAGGS Flag-C12orf48-HA were excised and analyzed by liquid chromatography-mass spectrometry (LC-MS/MS).

In-vitro PARP-1 Auto-Poly(ADP-ribosyl)ation Assays Briefly, purified C12orf48 recombinant protein and recombinant human PARP-1 were incubated in binding buffer (10mM Tris-HCl, pH 7.5, 1mM $MgCl_2$, 1mM DTT) plus sonicated DNA. The reactions were started by adding ^{32}P-labeled NAD^+, and terminated

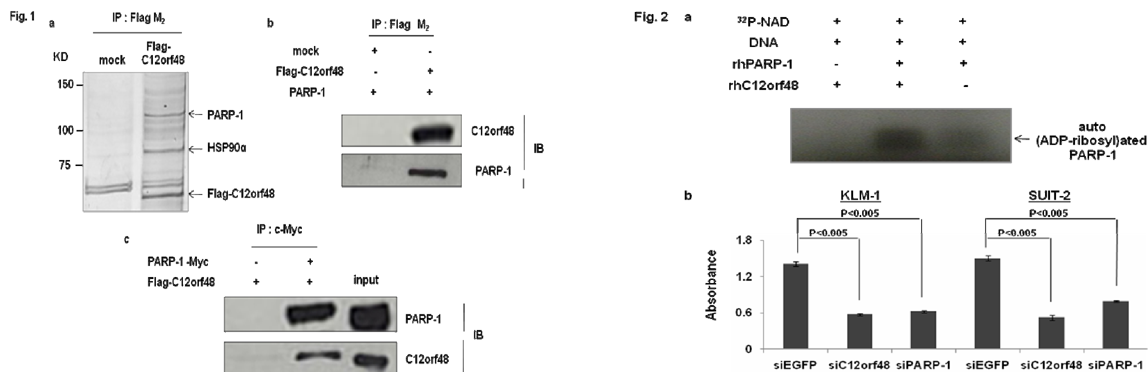
with SDS sample buffer. Incorporation of ^{32}P-labeled NAD^{+} to poly(ADP-ribosyl)ated proteins was visualized by autoradiography.

PARP-1 Activity in Cell Extracts PARP-1 activities in cell extracts were assayed using the universal colorimetric PARP assay kit based on the incorporation of biotinylated ADP-ribose onto histone H1 proteins. Briefly, cell extracts were loaded into a 96-well plate coated with histone H1, and incubated with biotinylated poly(ADP-ribose) and nicked DNA, size of which are 200-500 base pairs that are considered to be optimal for the PARP activation for 1 hour. Streptavidin-HRP (horseradish peroxidase) and TACS-Sapphire^{TM} was added subsequently to develop colors, and then the reaction was stopped by addition of 5% phosphoric acid. Finally, the absorbance was measured at 450nm in a spectrometrophotometer.

Sensitivity to DNA Damage Shcontrol and shC12orf48 KLM-1 cells were seeded into 6-well plates (5×10^5 cells/well), and incubated with indicated concentrations of adriamycin for 24 hours, H_2O_2 for 6 hours, or exposed to indicated intensity of UV radiation, and then incubated for 24 hours. Cell viability was measured using Cell-counting kit-8, and then the absorbance was measured at 490 nm, and at 630 nm with a Microplate Reader 550.

Results and Discussion

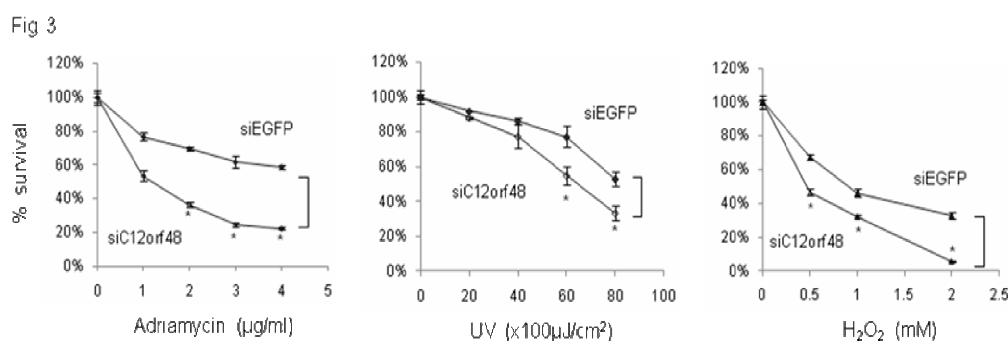
C12orf48, the gene trans-activated in pancreatic ductal adenocarcinoma (PDAC) cells through our genome-wide microarray analysis was confirmed to be overexpressed in five of the nine pancreatic cancer cases examined. Northern blot analysis using the C12orf48 cDNA fragment as a probe confirmed abundant expression of a 4-kb transcript in most of the eight PDAC cell lines we examined, but its expression was hardly detectable in any normal organs except the testis. Moreover, immunohistochemical analysis using anti-C12orf48 antibody showed positive signals in the nuclei of 21 of 31 PDAC tissues, whereas no staining was observed in any of normal pancreatic tissues. MTT assay and colony formation assay revealed that depletion of C12orf48 in KLM-1 and SUIT-2 cells caused dramatic reduction in the number of viable cells. Furthermore, we performed FACS analysis after depletion of C12orf48 by siRNA oligonucleotide in KLM-1 and SUIT-2 cells and found a drastic increase of cells at sub-G1 population. These findings implied its critical roles in pancreatic carcinogenesis.



Importantly, we demonstrated that C12orf48 protein could physically interact with PARP-1(Fig. 1) and positively regulate the enzymatic activity of PARP-1(Fig. 2). We observed that addition of C12orf48

protein significantly enhanced the incorporation of [^{32}P]NAD^{+} to recombinant PARP-1 protein in vitro when damaged DNA was co-incubated (Fig. 2a). PARP-1 activities to modify histone H1 were also significantly enhanced by overexpression of C12orf48 in HEK293 cells. Furthermore, Concordant with C12orf48 expression, the PARP-1 activities to modify histone H1 were decreased to 40.8% and 34.8% in C12orf48-depleted KLM-1 and SUIT-2 cells, respectively, compared with the control cells (Fig. 2b). The magnitude of this suppressive effect of C12orf48 on PARP-1 activity was almost same as the effect when PARP-1 itself was knocked down (Fig. 2b). It suggested that depletion of C12orf48 could decrease PARP-1 enzymatic activity both in vivo and in vitro. Together, these findings presumably explain that C12orf48 depletion lead to the reduction of pancreatic cancer cell viability, in part, through its direct interaction with PARP-1. However, it cannot be excluded that other C12orf48-specific and PARP1-independent effects can also affect cancer cell viability, and further study is required to clarify the roles of C12orf48 in cancer.

PARP-1 has an emerging and indispensable role in the repair of both DNA single-strand breaks (SSBs) and double-strand breaks (DSBs). In regard to DNA damage signaling, PARP-1 is promptly stimulated and recruits the enzymes required for DNA repair to the site of DNA damage. Hence, the activity of PARP-1 plays a key role in signaling and initiating these processes. It has been reported that inhibition of PARP-1 activity could increase the susceptibility of cells to DNA damaging agents. Given the findings that C12orf48 could regulate PARP-1 activity, we assessed that the C12orf48 depletion could sensitize cancer cells to various DNA damaging agents. As a result, C12orf48-depleted KLM-1 cells showed much higher sensitivities to adriamycin treatment, UV irradiation, and H_2O_2 treatment (Fig. 3). These findings indicated that C12orf48 might protect cancer cells from cell death following the DNA damage or cellular stresses in cancer cells through the regulation of poly(ADP-ribosyl)ation activity of PARP-1. It also suggested the possibilities that C12orf48, termed PARP-1 binding protein (PARPBP), might be involved in multiple cellular processes including DNA repair, chromatin modification, cell-cycle progression and genomic stability through the interaction and regulation of PARP-1.



In our studies, we also investigated that knockdown of C12orf48 as well as PARP-1 caused the failure of the G1/S cell-cycle checkpoint which would usually prevent the replication of cells having defects in DNA. Hence, this G1/S checkpoint failure induced by depletion of C12orf48 or PARP-1 in cancer cells could increase a possibility of accumulation of genetic mutations and/or genomic instability, resulting in growth retardation of cancer cells. Moreover, knockdown of C12orf48 in cancer cells enhanced G2/M arrest in

PDAC cells after gamma-irradiation, consistent with previous reports describing that PARP-1 inhibitors enhanced the G2 arrest after gamma-irradiation. However, since the underlying mechanism of PARP-1 enzymatic activity in G2-arrest regulation is unclear, additional studies will be required to clarify it.

Together, development of drugs inhibiting the interaction between C12orf48/PARPBP and PARP-1 should be a good therapeutic approach to achieve very specific cytotoxicy to some of pancreatic cancer cells with minimum risk of adverse effects to normal organs.

47-087342: Lianhua, Piao