

Full Length Research Paper

The role of GPR55 protein in proliferation, migration and invasion in human pancreatic neuroendocrine tumor cells

Ruihua Duan

Department of Hepatobiliary Surgery, People's Hospital of Huangpi District, Wuhan City
259 Baixiu St. Huangpi District, Wuhan, Hubei, China, 430300

*Corresponding author. Email: ruihuaduan@aol.com

Accepted 7 July, 2022

Objectives: GPR55 expression involves varieties of molecular pathways and is associated with carcinogenesis. Here human pancreatic neuroendocrine tumor cell lines and 79 human pancreatic neuroendocrine tumor tissues were used to investigate the roles of GPR55 in pancreatic neuroendocrine tumor. **Methods:** The impact of GPR55 on proliferation was determined by MTT assay. Transwell migration and matrigel invasion assays were performed to evaluate the effects of GPR55 on migration and invasion. Caspase 3/7 activities were measured to examine the impact of GPR55 on doxorubicin-induced apoptosis. Western blot and immunohistochemical studies were used to examine protein expression in human pancreatic neuroendocrine tumor cells and human pancreatic neuroendocrine tumor tissue, respectively. **Results:** GPR55 involved growth, migration and invasion and was associated with doxorubicin sensitivity. GPR55 decreased doxorubicin-induced apoptosis by regulating BAX and Bcl-2 expression. Pancreatic neuroendocrine tumor tissues showed higher expression of GPR55 than normal pancreatic tissue and the GPR55 level was associated with a higher stage. **Conclusions:** GPR55 gene displayed essential functions in pancreatic neuroendocrine tumor and might be used as genetic marker to predict doxorubicin treatment response and prognosis in human pancreatic neuroendocrine tumor.

Keywords: GPR55 gene, pancreatic neuroendocrine tumor, chemotherapy

INTRODUCTION

Pancreatic neuroendocrine tumors (PNETs) derive from neuroendocrine human cells in pancreas and account for approximately 7% of all pancreatic malignancies. In the past years, the incidence of PNETs is increased due to the advanced diagnostic techniques (Vagefi et al., 2007)..

The majority of PNETs are slow growing but can progress to metastatic disease with a poor prognosis. PNETs are classified as functioning and non-functioning tumors. Patients with functioning PNETs often have clinical syndromes due to the secretion of active peptides such as insulin, gastrin vasoactive intestinal peptide etc. Patients with non-functioning PNETs may be asymptomatic, which caused late diagnosis and poor

outcome (Cheslyn-Curtis et al., 1993; Eriksson and Oberg 2000). The therapeutic standardization and optimal management in PNETs are very difficult due to high heterogeneity. A better understanding the pathogenesis of PNETs will provide critical insight into its management and therapeutic strategies.

The orphan G protein-coupled receptor 55 (GPR55), a putative lysophosphatidylinositol receptor, plays a role in calcium release and ERK-MAP kinase phosphorylation (Nevalainen and Irving 2010; AISuleimani and Hiley 2015). Studies have demonstrated that GPR55 activation is associated with proliferation in variety of human cancers (Falasca and Ferro 2016; Andradas et al., 2011;

Piñeiro et al., 2011; Hu et al., 2011). Hasenoehrl et al. found that GRP55 promotes growth of colorectal cancer cells and acts as oncogene (Hasenoehrl et al., 2018). Ferro et al. showed that proliferation of pancreatic ductal adenocarcinoma can be decreased by inhibition of GRP55 level both in vitro and in vivo (Ferro et al., 2018). Andradas et al. reported that high expression of GPR55 is associated with metastasis in triple negative breast cancer (Andradas et al., 2016).

However, the function of GPR55 in the development of PNETs is unknown. Here, we examined the function of GPR55 in proliferation of PNETs cells and expression level in PNET human tissue.

MATERIALS AND METHODS

Cell lines

BON-1 and QGP-1 cells were cultured in growth medium (DMEM/F12, 10% fetal bovine serum and 1% penicillin/streptomycin).

Transfection

To make GPR55 stable expression cell lines, lentiviral-GPR55 particles were obtained from G&P Biosciences. BON-1-GPR55 and QGP-1 stable cells were made by incubating BON-1 and QGP-1 cells with virus-containing solutions. The stable cells were selected by ampicillin following the manufacturing protocol.

To make GPR55 low-expression cell lines, GPR55-specific siRNA was purchased from Santa Cruz and was transfected into BON-1 and QGP-1 cells using Lipofectamine RNAiMAX (Invitrogen). After 48 hour transfection, GPR55 expression was tested by western blot.

Cell proliferation assay

The impact of GPR55 on BON-1 and QGP-1 cells proliferation was examined using MTT assay. Briefly, transfected BON-1 and QGP-1 cells were grown in routine growth medium. After 24-hour culture, doxorubicin (0, 1, 2 and 4 μ M) were added to the growth medium and continue to culture the transfected cells. On different time points, MTT solution was added to the BON-1 and QGP-1 cells and continue to growth for 4 hours. The cell proliferation was determined by the OD value of each well.

Programmed cell death

Caspase 3/7 activities were measured to determine the impact of GPR55 on doxorubicin-induced programmed cell death. Briefly, BON-1 and QGP-1 cells were cultured with doxorubicin in the 96-well plates overnight. The OD value was measured to determine the caspase 3/7 activities after Caspase-Glo reagent (Promega, USA) was added into each well.

Transwell assay

The migration and invasion of BON-1 and QGP-1 cells were examined by transwell assays. Briefly, BON-1 and QGP-1 cells were seeded in upper chamber filled with growth medium without serum. The lower chambers were filled with growth medium with serum. After 48-hour culture, the membranes with invaded BON-1 and QGP-1 cells were stained. The invaded BON-1 and QGP-1 cells were counted under microscope.

Western blotting

Western blot assay was performed to examine the impact of GPR55 on protein expression on BON-1 and QGP-1 cells. Briefly, BON-1 and QGP-1 cells were mixed with RIPA buffer on ice. Sodium dodecyl sulfate polyacrylamide gel electrophoresis was carried out to separate different molecular weight proteins. Then all proteins were transferred to polyvinylidene difluoride (PVDF) membrane. Primary antibodies were incubated with the PVDF membrane overnight. After washing with cold PBS, the PVDF membrane was incubated with secondary antibodies for 1 hour at room temperature. The protein expression levels were analyzed.

GPR55 levels on human pancreatic neuroendocrine tumor

Pancreatic neuroendocrine tumor tissues from 79 patients from 2015 to 2020 were obtained from People's Hospital of Huangpi District. The study protocols were approved by the People's Hospital of Huangpi District ethical committee. Research consents were signed by all patients in this study. RNA was extracted from formalin-fixed paraffin-embedded (FFPE) pancreatic neuroendocrine tumor tissues.

Immunohistochemical analysis

FFPE pancreatic neuroendocrine tumor tissues were used to examine protein expression using immunohistochemical staining assay.

Statistical analysis

ANOVA and student's t test were used to analyze the statistical difference.

RESULTS

GPR55 protein promoted growth of pancreatic neuroendocrine tumor cells with or without doxorubicin

Altered expression of GPR55 in BON-1 cells and QGP-1 cells was confirmed by western blot (Figure 1 A-D).

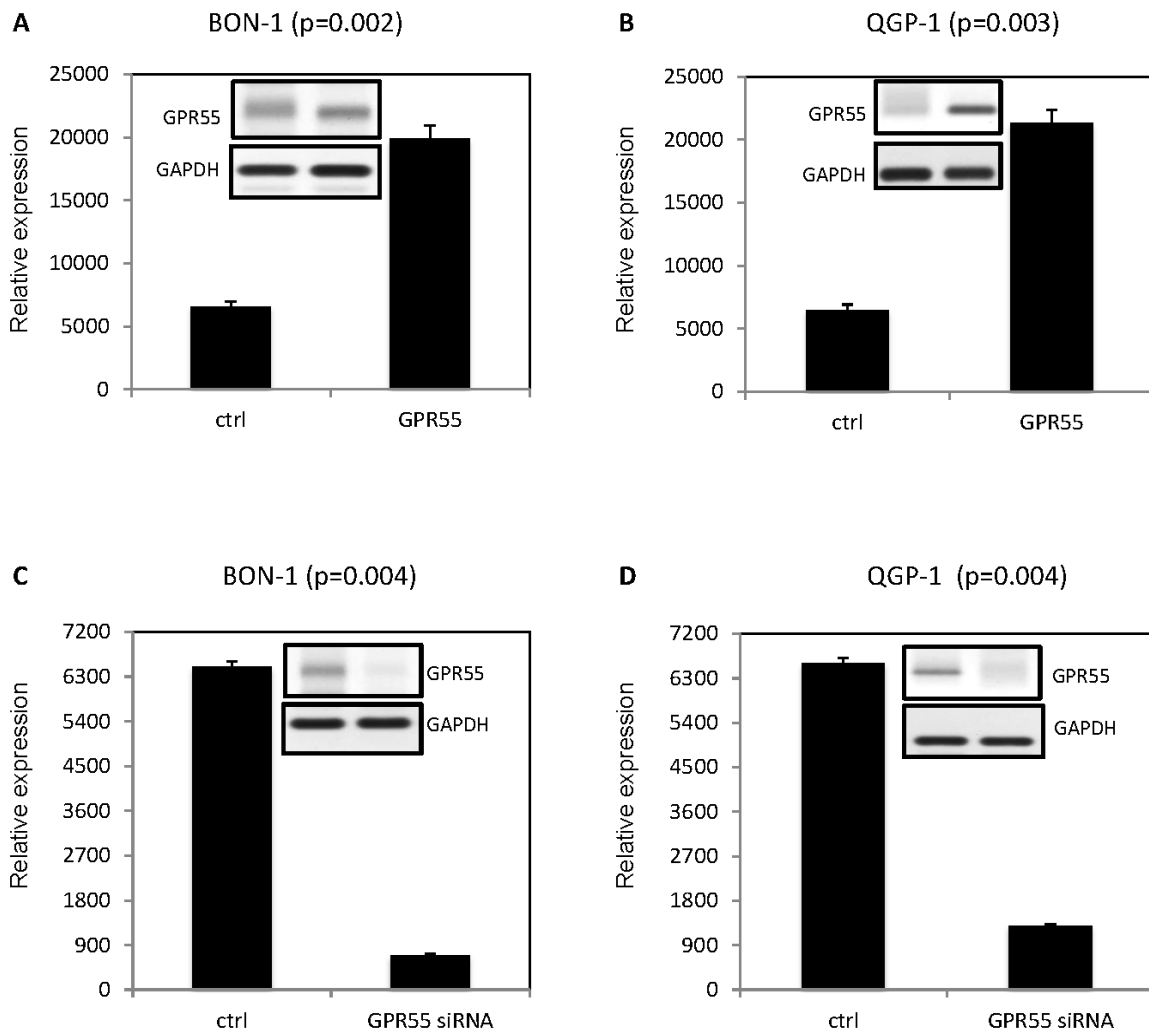


Figure 1. GPR55 expression level in BON-1 and QGP-1 cells after transfection

Overexpression of GPR55 increased growth of BON-1 cells (Figure 2A) and QGP-1 cells (Figure 2E) in absence of doxorubicin in a dose-dependent manner. Overexpression of GPR55 enhanced survival rate in BON-1 cells (Figure 2B-D) and QGP-1 cells (Fig. 2F-H) treated with doxorubicin. However, low-expression of GPR55 decreased growth of BON-1 cells (Figure 3A) and QGP-1 cells (Figure 3E) in absence of doxorubicin. Low-expression of GPR55 decreased survival rate in BON-1 cells (Figure 3B-D) and QGP-1 cells (Figure 3F-H) treated with doxorubicin.

GPR55 protein inhibited doxorubicin-induced apoptosis in pancreatic neuroendocrine tumor cells

Decreased caspase 3/7 activities were found in BON-1 cells (Figure 4A) and QGP-1 cells (Figure 4B) after overexpression of GPR55. Furthermore, GPR55

decreased BAX expression, but increased Bcl-2 expression in BON-1 cells (Figure 4 C-D) and QGP-1 cells (Figure 4 E-F).

GPR55 protein altered migration and invasion in pancreatic neuroendocrine tumor cells

High-level GPR55 increased migration and invasion in BON-1 cells (Figure 5 A-B) and QGP-1 cells (Figure 5 E-F). Low-level GPR55 decreased migration and invasion in BON-1 cells (Figure 5 C-D) and QGP-1 cells (Figure 5 G-H).

GPR55 protein expression on human pancreatic neuroendocrine tumor tissues

Higher level of GPR55 was detected in pancreatic neuroendocrine tumor than normal pancreatic tissue.

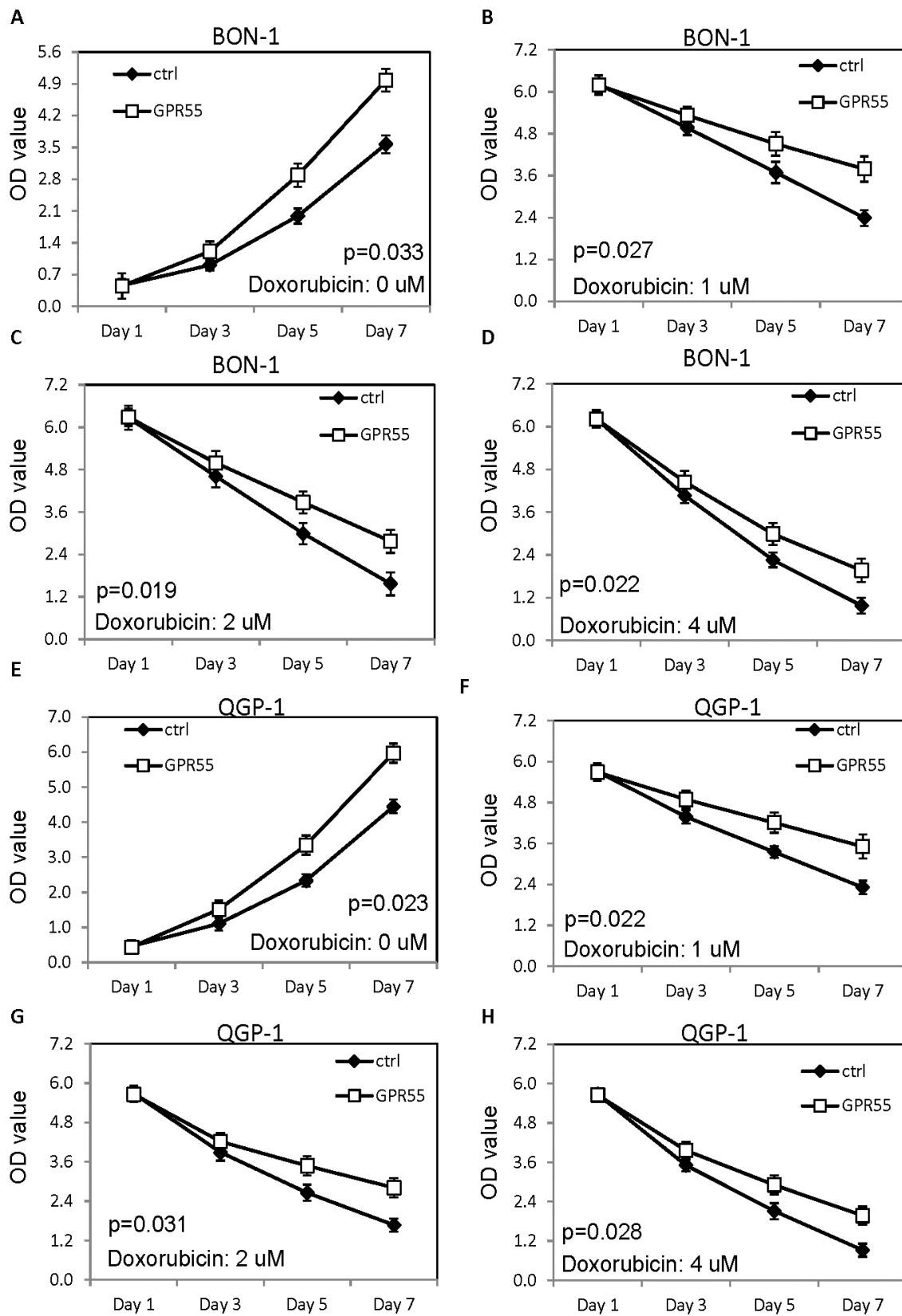


Figure 2. GPR55 increased growth of BON-1 and QGP-1 cells with or without doxorubicin treatment. (A-D) The growth of BON-1 cells with or without doxorubicin treatment after overexpression GPR55; (E-H) The growth of QGP-1 cells with or without doxorubicin treatment after overexpression GPR55.

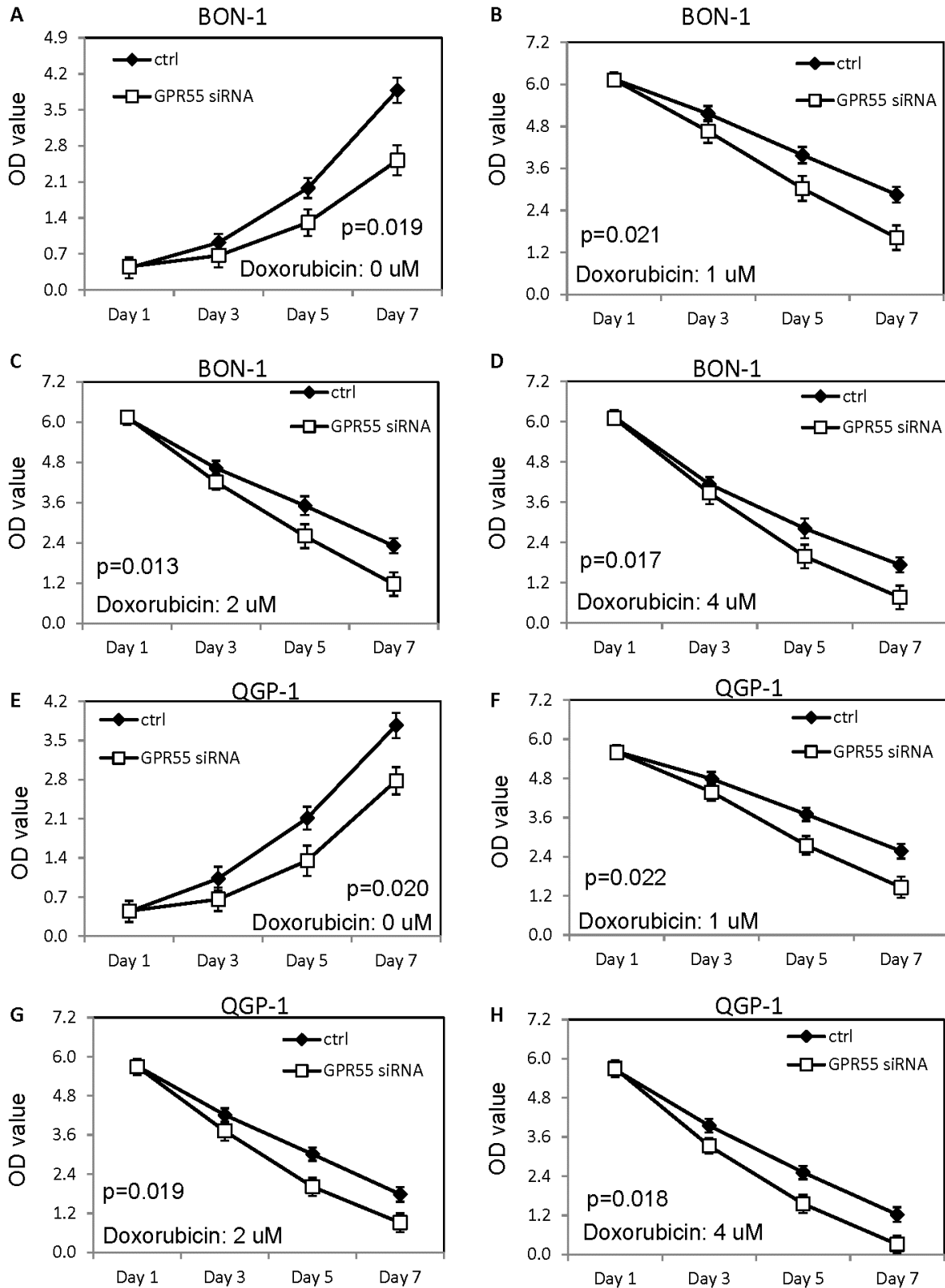


Figure 3. Knockdown GPR55 decreased growth of BON-1 and QGP-1 cells with or without doxorubicin treatment.

(A-D) The growth of BON-1 cells with or without doxorubicin treatment after knockdown GPR55; (E-H) The growth of QGP-1 cells with or without doxorubicin treatment after knockdown GPR55.

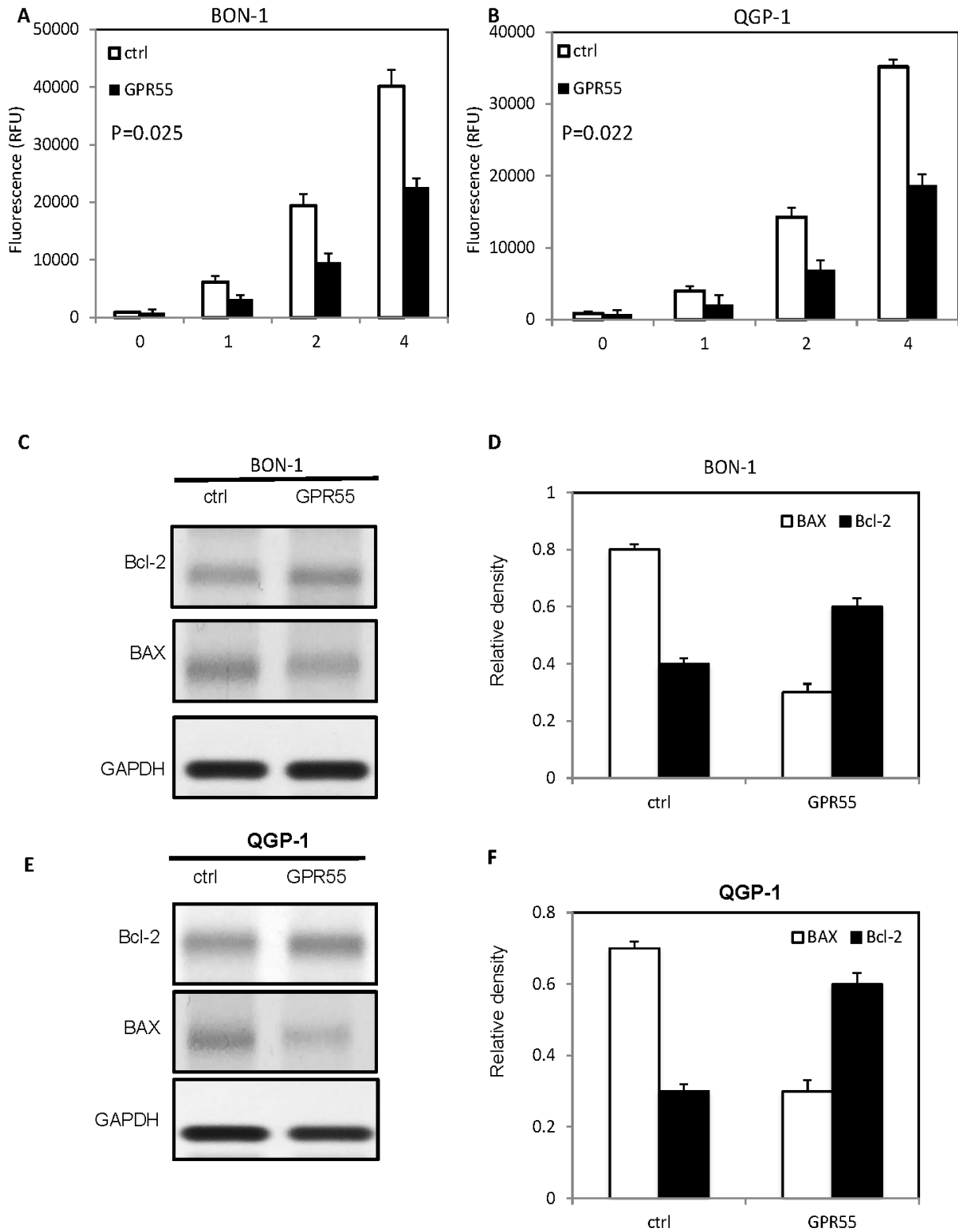


Figure 4. GPR55 decreased doxorubicin-induced apoptosis in pancreatic neuroendocrine tumor cells. (A) Caspase 3/7 activity in BON-1 cell with overexpressed GPR55; (B) Caspase 3/7 activity in QGP-1 cell with overexpressed GPR55; (C-F) Bcl-2 and BAX expression in BON-1 and QGP-1 cells.

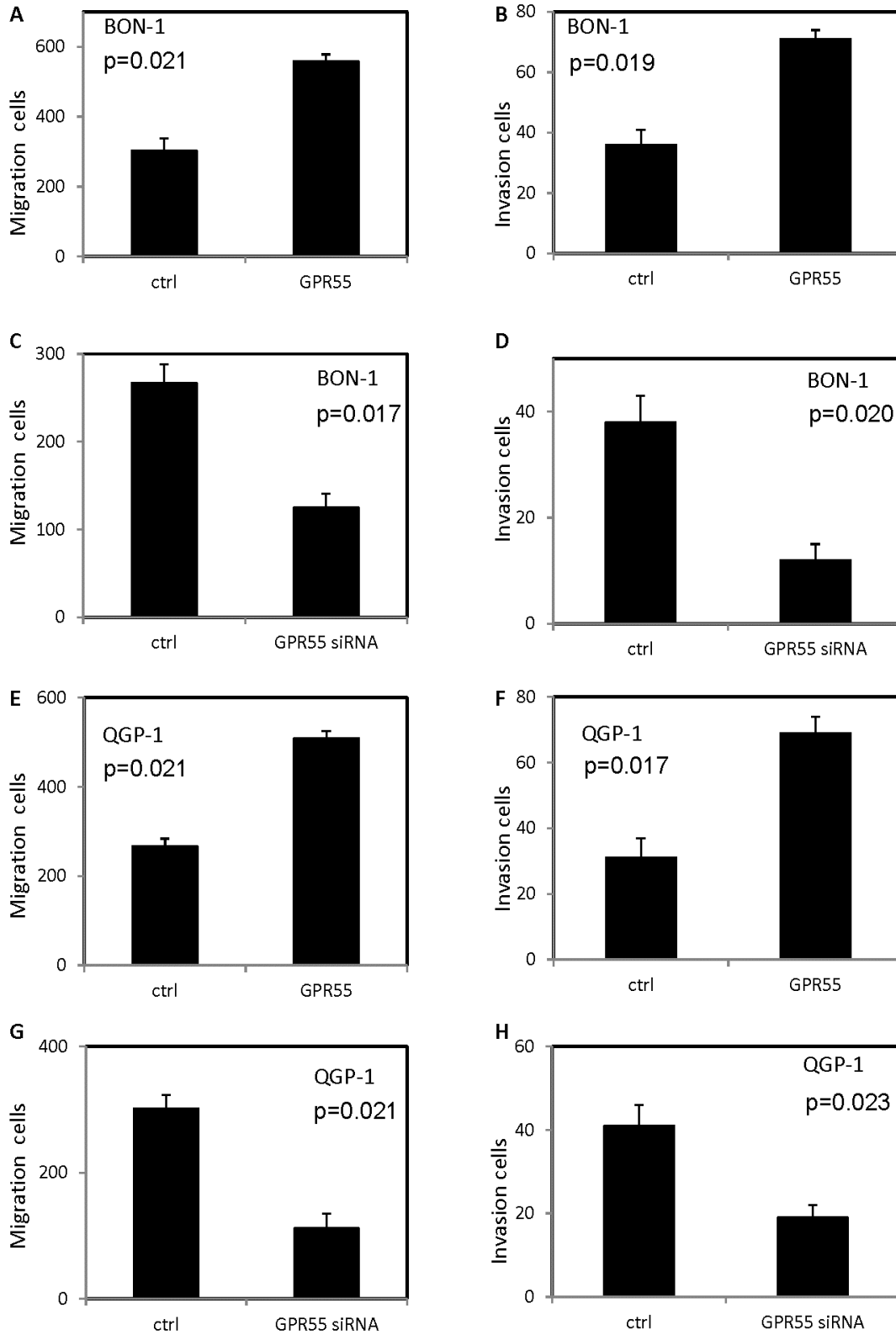


Figure 5. GPR55 altered invasion and migration of BON-1 and QGP-1 cells

(A-B) overexpression of GPR55 enhanced migration and invasion of BON-1 cells. (C-D) low-expression of GPR55 decreased migration and invasion of BON-1 cells. (E-F) overexpression of GPR55 enhanced migration and invasion of QGP-1 cells. (G-H) low-expression of GPR55 decreased migration and invasion of QGP-1 cells.

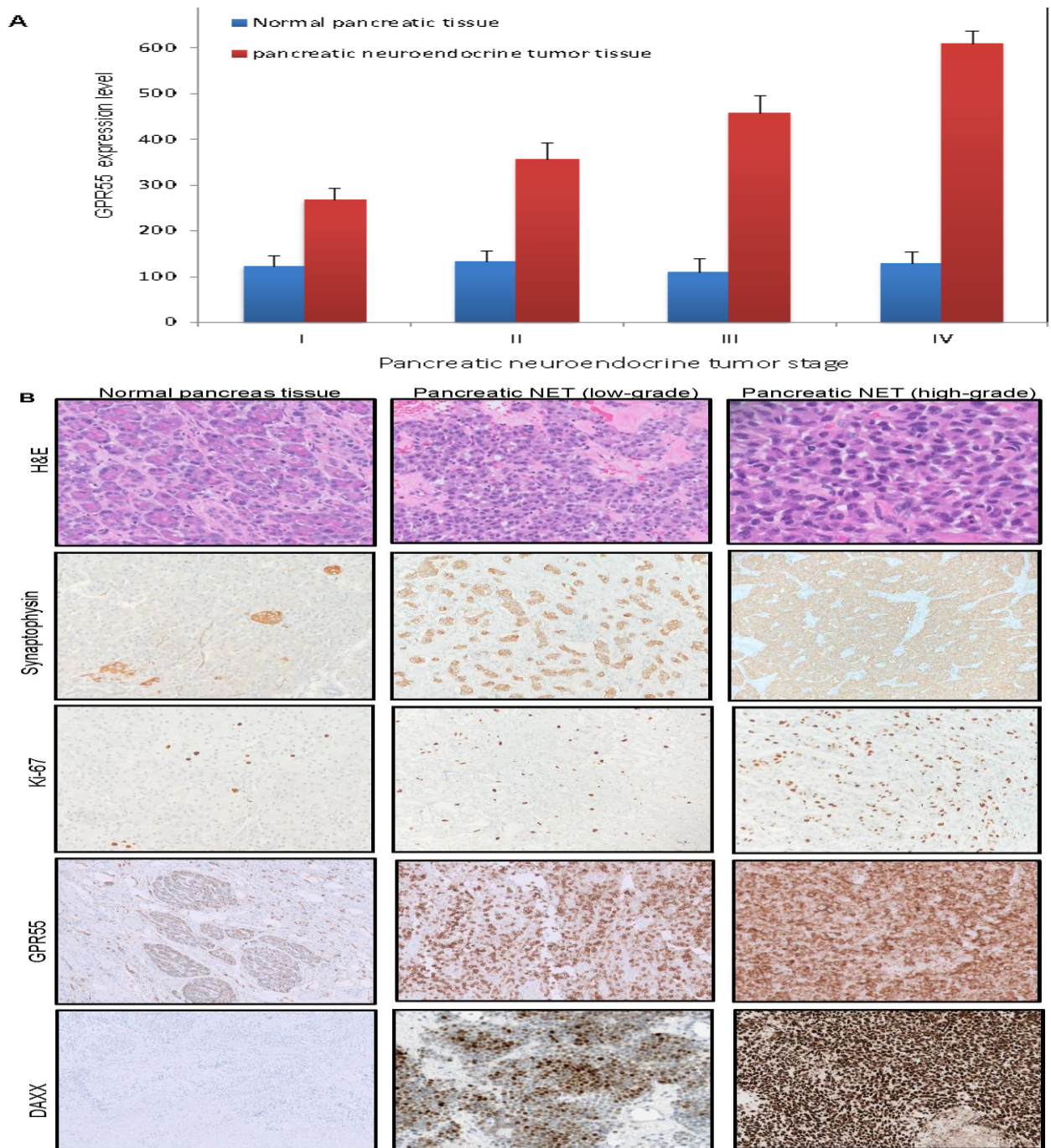


Figure 6. GPR55 expression in human pancreatic neuroendocrine tumor tissue (A) GPR55 expression levels in different stage of pancreatic neuroendocrine tumor tissue and normal pancreatic tissue. (B) Immunohistochemical studies in pancreatic neuroendocrine tumor tissues.

GPR55 expression correlates with pathologic stage of pancreatic neuroendocrine tumor (Figure 6A). Ki-67 immunostain demonstrated a higher proliferation index in pancreatic neuroendocrine tumor with high GPR55 expression. Meanwhile, pancreatic neuroendocrine tumor with high GPR55 expression showed higher DAXX

expression (Figure 6B).

DISCUSSION

Studies have shown evidence that GPR55 expression is associated with tumorigenesis in a variety of cancers in

different organs, such as and pancreas (Ferro et al., 2018), breast (Ford et al., 2010), ovary and prostate (Piñeiro et al., 2011). GPR55 is expressed in central nervous system and peripheral tissues by binding to Ga12/13 and Gq proteins (Ross 2009). Ferro et al. found GPR55 improved growth of pancreatic ductal adenocarcinoma and was regulated by p53 signaling pathway (Ferro et al., 2018). Hasenoehrl et al., reported that GPR55 promoted proliferation of colorectal cancer cells and showed an opposing role to the cannabinoid receptor 1 (CB1) Hasenoehrl et al., 2018. Andradas et al., showed that GPR55 increased cancer cell proliferation by activating extracellular signal-regulated kinase cascade Andradas et al., 2011. Interestingly, overexpression of GPR55 increased chemoresistance in pancreatic ductal adenocarcinoma cells (Singh et al., 2016). Our study showed overexpression of GPR55 increased proliferation of pancreatic neuroendocrine tumor cells, but low expression of GPR55 decreased proliferation of xxx cells. These results and prior studies indicate GPR55 expression is associated with tumorigenesis in pancreatic neuroendocrine tumor. Meanwhile, our study suggested GPR55 expression was closely related with the doxorubicin-induced apoptosis in pancreatic neuroendocrine tumor cells. These results indicated GPR55 might regulate chemotherapy resistance through apoptosis pathway.

It is very common for pancreatic neuroendocrine tumor to spread to other part of the body such as liver and lymph nodes (Trikalinos et al., 2020). Patients with metastatic pancreatic neuroendocrine tumor cancer have higher stage and often have poor outcome. Epithelial-to-mesenchymal transition (EMT) is a critical biological process of metastasis in cancer cells (Ribatti et al., 2020). Studies have demonstrated that GPR55 involve cancer metastasis and regulate a single step or multiple steps of EMT by targeting different signaling pathways (Paul et al., 2014; Kargl et al., 2016; Zhou et al., 2018). In our study, aberrant expression of GPR55 impacted migration and invasion in pancreatic neuroendocrine tumor cells. These results indicated GPR55 played a role in EMT in pancreatic neuroendocrine tumor cells. DAXX is a transcriptional repressor and mutation of DAXX is associated with higher grade and more aggressive behavior in pancreatic neuroendocrine tumor (Chan et al., 2018). We further examined impact of aberrant expression of GPR55 on protein expression in multiple signaling pathways. We found that overexpression of GPR55 enhanced DAXX expression in pancreatic neuroendocrine tumor tissues.

In summary, our study demonstrated GPR55 mediated proliferation and chemotherapy sensitivity in pancreatic neuroendocrine tumor. However, pancreatic neuroendocrine tumor is a heterogeneous disease and many factors contribute to chemotherapy resistance in pancreatic neuroendocrine tumor. Further studies are required to explore how GPR55 interacts with other

proteins in regulation pancreatic neuroendocrine tumor development and progression.

Declarations

Ethics approval and consent to participate: The study protocols were approved by the People's Hospital of Huangpi District ethical committee. Research consents were signed by all patients in this study.

Availability of data and materials: The datasets are available from the corresponding author on reasonable request.

Competing interests: No conflicts of interest

Authors' contributions:

Duan Ruihua: Design the project, performed experiments, write manuscript

REFERENCE

- AlSuleimani YM, Hiley CR (2015). The GPR55 agonist lysophosphatidylinositol relaxes rat mesenteric resistance artery and induces ca(2+) release in rat mesenteric artery endothelial cells. *Br J Pharmacol.* 172(12):3043-3057.
- Andradas C, Blasco-Benito S, Castillo-Lluva S, Dillenburger-Pilla P, Diez-Alarcia R, Juanes-García A, García-Taboada E, Hernando-Llorente R, Soriano J, Hamann S et al (2016). Activation of the orphan receptor GPR55 by lysophosphatidylinositol promotes metastasis in triple-negative breast cancer. *Oncotarget.* 7(30):47565-47575.
- Andradas C, Caffarel MM, Pérez-Gómez E, Salazar M, Lorente M, Velasco G, Guzmán M, Sánchez C (2011). The orphan g protein-coupled receptor GPR55 promotes cancer cell proliferation via erk. *Oncogene.* 30(2):245-252.
- Chan CS, Laddha SV, Lewis PW, Koletsky MS, Robzyk K, Da Silva E, Torres PJ, Untch BR, Li J, Bose P et al (2018). ATRX, DAXX or men1 mutant pancreatic neuroendocrine tumors are a distinct alpha-cell signature subgroup. *Nat Commun.* 9(1):4158
- Cheslyn-Curtis S, Sitaram V, Williamson RC (1993). Management of non-functioning neuroendocrine tumours of the pancreas. *Br J Surg.* 80(5):625-627.
- Eriksson B, Oberg K (2000). Neuroendocrine tumours of the pancreas. *Br J Surg.* 87(2):129-131.
- Falasca M, Ferro R (2016). Role of the lysophosphatidylinositol/ GPR55 axis in cancer. *Adv Biol Regul.* 60:88-93.
- Ferro R, Adamska A, Lattanzio R, Mavrommati I, Edling CE, Arifin SA, Fyffe CA, Sala G, Sacchetto L, Chiorino G et al (2018). GPR55 signalling promotes proliferation of pancreatic cancer cells and tumour growth in mice,

- and its inhibition increases effects of gemcitabine. *Oncogene*. 37(49):6368-6382.
- Ferro R, Adamska A, Lattanzio R, Mavrommati I, Edling CE, Arifin SA, Fyffe CA, Sala G, Sacchetto L, Chiorino G et al (2018). GPR55 signalling promotes proliferation of pancreatic cancer cells and tumour growth in mice, and its inhibition increases effects of gemcitabine. *Oncogene*. 37(49):6368-6382.
- Ford LA, Roelofs AJ, Anavi-Goffer S, Mowat L, Simpson DG, Irving AJ, Rogers MJ, Rajnicek AM, Ross RA (2010). A role for l-alpha-lysophosphatidylinositol and GPR55 in the modulation of migration, orientation and polarization of human breast cancer cells. *Br J Pharmacol*. 160(3):762-771.
- Hasenoehrl C, Feuersinger D, Sturm EM, Bärnthaler T, Heitzer E, Graf R, Grill M, Pichler M, Beck S, Butcher L et al (2018). G protein-coupled receptor GPR55 promotes colorectal cancer and has opposing effects to cannabinoid receptor 1. *Int J Cancer*. 142(1):121-132.
- Kargl J, Andersen L, Hasenöhr C, Feuersinger D, Stančić A, Fauland A, Magnes C, El-Heliebi A, Lax S, Uranitsch S et al (2016). GPR55 promotes migration and adhesion of colon cancer cells indicating a role in metastasis. *Br J Pharmacol*. 173(1):142-154.
- Nevalainen T, Irving AJ (2010). GPR55, a lysophosphatidylinositol receptor with cannabinoid sensitivity? *Curr Top Med Chem*. 10(8):799-813.
- Paul RK, Wnorowski A, Gonzalez-Mariscal I, Nayak SK, Pajak K, Moaddel R, Indig FE, Bernier M, Wainer IW (2014). (r,r')-4'-methoxy-1-naphthylfenoterol targets GPR55-mediated ligand internalization and impairs cancer cell motility. *Biochem Pharmacol*. 87(4):547-561.
- Piñeiro R, Maffucci T, Falasca M (2011). The putative cannabinoid receptor GPR55 defines a novel autocrine loop in cancer cell proliferation. *Oncogene*. 30(2):142-152.
- Piñeiro R, Maffucci T, Falasca M (2011). The putative cannabinoid receptor GPR55 defines a novel autocrine loop in cancer cell proliferation. *Oncogene*. 30(2):142-152.
- Ribatti D, Tamma R, Annese T (2020). Epithelial-mesenchymal transition in cancer: A historical overview. *Transl Oncol*. 13(6):100773.
- Ross RA (2009). The enigmatic pharmacology of GPR55. *Trends Pharmacol Sci*. 30(3):156-163.
- Singh NS, Bernier M, Wainer IW (2016). Selective GPR55 antagonism reduces chemoresistance in cancer cells. *Pharmacol Res*. 111:757-766.
- Trikalinos NA, Tan BR, Amin M, Liu J, Govindan R, Morgensztern D (2020). Effect of metastatic site on survival in patients with neuroendocrine neoplasms (nens). An analysis of seer data from 2010 to 2014. *BMC Endocr Disord*. 20(1):44.
- Vagefi PA, Razo O, Deshpande V, McGrath DJ, Lauwers GY, Thayer SP, Warshaw AL, Fernández-Del Castillo C (2007). Evolving patterns in the detection and outcomes of pancreatic neuroendocrine neoplasms: The massachusetts general hospital experience from 1977 to 2005. *Arch Surg*. 142(4):347-354.
- Zhou XL, Guo X, Song YP, Zhu CY, Zou W (2018). The LPI/ GPR55 axis enhances human breast cancer cell migration via HBXIP and P-MLC signaling. *Acta Pharmacol Sin*. 39(3):459-471.