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Evaluation of APEX1 and Jagged-1 as chemoresistance factors in advanced gastric cancer

Graduate School of Chosun University

Department of Medicine

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ABSTRACT

Evaluation of APEX1 and Jagged-1 as chemoresistance factors in Advanced gastric cancer

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배경

위암은 전세계적으로 매우 흔한 암 종중의 하나로 빈도는 한국을 포함한 동아시아에서 특히 높다. 최근 위암은 조기진단의 비율이 올라가고 위암절제술의 수술적 절제율이 늘어나면서 사망률은 급격히 감소중이다.

그러나 수술이 불가능한 진행성 전이성 위암으로 진단되는 빈도는 여전히 높고 이러한 경우 매우 치명적으로 완치가 불가능하다.

진행성, 전이성 위암환자는 항암화학요법이 유일한 치료대안이지만 항암제 내성이 가장 큰 문제이다.

본 연구는 진행성 위암 환자에서 APEX1 과 Jagged-1 이 항암제 내성인자로서 어느 정도의 역할을 하는지 알아보고자 한다.

방법

우리는 6 종류의 인간 위암세포주를 이용하여 *W*estern blot 을 이용하여 APEX1 및 Jagged-1의 발현빈도를 살펴본 뒤 APEX1 과 Jagged-1 이 동시에 발현되는 세포주와 APEX1 만 단독 발현되는 세포주를 선택하였다. 선택된 2가지 세포주를 이용하여 위암에서 현재 표준적 항암치료제인 5-Fluorouracil (5-FU) 와 Cisplatin 의 항암 감수성을 *3-94, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium*

bromide (MTT) *assay*를 이용하여 검사 하였다.

결과

APEX1 과 Jagged-1 이 동시에 발현되는 세포주에서 IC_{50}은 APEX1 만 단독 발현되는 세포주보다 Cisplatin의 1.5배, 5-FU의 3배로 APEX1 과 Jagged-1 이 동시에 발현된 경우 항암제(Cisplatin, 5-FU)에 더욱 내성을 가지고 있다.

반면 APEX1 과 Jagged-1 이 동시에 발현되는 세포주에 APEX1의 발현을 억제시키면 Jagged-1이 같이 억제되고 APEX1을 억제 시킨 뒤 MTT 검사를 시행해보면 APEX1 과 Jagged-1 이 동시에 발현되는 세포주와 APEX1 단독 발현된 세포주의 IC_{50}이 거의 비슷해진다.

결론

APEX1에 의해 Jagged-1이 활성화된 위암은 표준항암요법제인 5FU 와 Cisplatin에 내성을 가지게 된다. APEX1에 의한 Jagged-1의 과발현은 항암치료의 반응을 예측하는 인자중 하나가 될수 있고 더 나아가 진행성 위암의 항암치료에 치료표적으로 연구될 수 있겠다.

Key words : 위암, APEX1, Jagged-1 , 항암내성

I. Introduction

Gastric cancer is common cancer worldwide, especially in eastern Asia, including Korea. The incidence and mortality rate of gastric cancer has declined dramatically owing to the early detection and increased surgical resection of gastric cancer in Korea. However, gastric cancer remains a highly lethal malignancy, and locally advanced unresectable and metastatic gastroesophageal cancers are not curable diseases [1].

Cytotoxic chemotherapy in advanced gastric cancer is only palliative therapy. 5-Fluorouracil (5-FU) and platinum, including cisplatin or oxaliplatin, combination chemotherapy is considered the standard first-line treatment for advanced gastriccancer; however, the survival time of advanced gastric cancer (AGC) is only approximately 10 months [2-4]. The major clinical problem of cytotoxic chemotherapy is chemoresistance and many theories regarding chemoresistance mechanisms have been proposed [5].

Among them, enhanced DNA repair is one such proposed theory and the DNA base excision repair (BER) pathway is well known to enhance the DNA repair pathway[6-8].

Apurinic/apyrimidinic endodeoxyribonuclease1 (APEX1) is one of the enzymes involved in the base excision repair pathway; some studies have shown that APEX1 expression levels were correlated with chemoresistance in various cancer cells and an elevated APEX1 protein level has also been associated with poor outcome in various cancers. Jagged-1 is one of expressed Notch receptor ligands and Jagged-1-induced Notch activation plays a role in various aspects of tumor biology [9,10].

Many studies of the molecular mechanisms related to the progression of gastric cancer have been performed, as there are few known as therapeutic targets, such as HER2 [11]. However, the incidence of HER2-positive

advanced gastric cancer is only approximately 10% and there are no definitive biomarkers related to chemoresistance for AGC.

In this study, we evaluated APEX1 and Jagged-1 as potential chemoresistance factors in advanced gastric cancer.

II. Materials and Methods

1. Cell cultures

Six human gastric tract cancer cell lines (SNU-1, SNU-5, SNU-16, NCI-N87, KATO III, and AGS) were procured from the Korea Cell Line Bank (Seoul, Korea). The cell lines were cultured in RPMI1640 medium supplemented, 10% fetal bovine serum and 1% penicillin/streptomycin. This cells were maintained in a humidified atmosphere with 5% CO^2 at 37°C.

2. Preparation of drug solution for vitro assays

Aqueous solutions of all drugs were prepared in distilled water and were stored in a deep freezer (CLN-51U). Cisplatin was obtained from JW Pharmaceutical Corp. (Seoul, Korea) in aqueous form as 10 mg in 20 mL. 5-FU was obtained from JW Pharmaceutical Corp. in aqueous form as 250 mg in 5 mL.

3. 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay

The cell viability was determined by using an MTT (3-4, 5-dimethylthiazole -2-yl)-2, 5-diphenyl tetrazolium bromide) assay. The MTT assay was performed in accordance with a standard protocol. After treatment, 10 μL MTT (1 mg/mL) in PBS was incubated with cells in a 96-well plate for 4 h at 37 °C. Subsequently, the medium containing MTT was removed, and 100 μL dimethyl sulfoxide (DMSO) was added. The cells were incubated for an

additional 10 min at 37 °C with gentle shaking. The absorbance was read on an ELISA plate reader with a 540-nm filter.

4. Small interfering RNA (siRNA)-based experiments

The cells were transfected with siRNA by using RNAiMAX (Invitrogen, Carlsbad, CA, USA). The sequence used to target APEX1 was 5′-AAGTCTGGTACGACTGGAGTA-3′; for control shRNA, a nontargeting scrambled sequence was cloned into psilencer2.1-U6. The gastric cancer cells were transfected with APEX1 siRNA or scrambled control siRNA by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) and cultured in a selection medium containing 400 μg/mL hygromycin for 4-5 weeks.

III. Results

1.Western blot analysis of APEX-1 expression in gastric cancer cell lines

First, we checked APEX1 and Jagged-1 protein expression in gastric cancer cell lines (SNU-1, SNU-5, SNU-16, NCI-N87, KATO III, and AGS). APEX1 and Jagged-1 expression were detected by western blotting with α-tubulin used as a loading control.

The analysis of the western blots revealed that all gastric cancer cell lines expressed a high level of APEX1, but only two gastric cancer cell lines (NCI-N87, KATO-III) simultaneous expressed APEX1 and Jagged-1; in particular, only KATO-III strongly expressed APEX1 and Jagged-1 simultaneously (Fig. 1).

Therefore, we selected two cell lines (KATO III and AGS): KATO III strongly expressed both APEX1 and Jagged-1, whereas AGS strongly expressed only APEX1.

2.MTT assay for chemotherapeutic drugs (5 FU, cisplatin)

An MTT assay was performed to assess the sensitivity of two gastric cancer cell lines (KATO III vs AGS) to well-known effective chemotherapeutic agents: 5-FU and cisplatin (Fig. 2). The cell line (KATO-III) was more resistant to all chemotherapeutic agents (5-FU and cisplatin) than the others (AGS).

Additionally, the IC_{50} values in the resistant cell line (KATO-III) were higher than those in the sensitive cell line(AGS). The IC_{50} values in KATO-III were 1.5-fold and 3-fold higher for cisplatin and 5FU, respectively). These results showed that the simultaneous expression of APEX1 and Jagged-1 might be

related to the resistance factor to the chemotherapeutic agents (cisplatin and 5FU) (Table 1).

3.Western blot analysis of Jagged-1 expression in gastric cancer cell lines after APEX-1 knockdown

We evaluated the change in APEX1 and Jagged-1 expression levels in the KATO III and AGS cell lines after APEX1 knockdown.

We performed a control experiment for the APEX1 knockdown, in which APEX1-specific siRNA was transfected into KATO III and AGS. The western blots showed that when APEX1 siRNA was transfected into gastric cancer cells, the endogenous APEX1 protein level was knocked down by 80% compared with the level in control siRNA-transfected cells.

Jagged-1 protein expression in KATO III cells was clearly decreased, which suggested that Jagged-1 was activated by APEX1 in KATO III cells (Fig. 3).

4.MTT assay after APEX-1 knockdown

Finally, we assessed the change in drug sensitivity of two gastric cell lines (KATO III and AGS) after APEX1 knockdown.

First, APEX1 is believed to be a chemoresistance factor to cisplatin and 5-FU by enhancing DNA repair. However, after APEX1 knockdown, the MTT assay in the chemosensitive cell line (AGS) showed only a minimal decrease in IC_{50} (approximately 7% and 15%) for the chemotherapeutic agents (cisplatin and 5-FU ,respectively); however, the chemoresistant cell line (KATO-III) displayed a definite decrease in IC_{50} (approximately 21% and 67% for cisplatin and 5-FU, respectively).

These results suggested that APEX1, by itself, is minimally related or almost

unrelated to chemoresistance in gastric cancer. However, gastric cancer cells simultaneously expressing APEX1 and Jagged-1 were chemoresistant to cisplatin and 5-FU. Furthermore, Jagged-1 was activated by APEX1 (Fig 4).

The final result suggested that Jagged-1 activation by APEX1 was a chemoresistance factor in gastric cancer for cisplatin and, in particular, 5-FU.

IV. Discussion

1. Incidence

Gastric cancer is one of the most common cancers worldwide. It was described in 3000 BC in hieroglyphic inscriptions and in the first major analysis of cancer incidence and mortality performed in Verona, Italy, between 1760 and 1839. The incidence of gastric cancer varies according to geographic regions; higher prevalence is found in eastern Asia, Eastern Europe, and South America, whereas the lowest rates occur in North America and parts of Africa [1].

Some studies have been conducted on the gastric cancer risk in Japanese migrants to the United States. The results indicated that early exposure to environmental factors, rather than genetic factors, had a greater influence on mortality and incidence rates [12,13]. Such findings strongly suggest that environmental factors play most important role in the etiology of gastric cancer and that exposure to risk factors occurs early in life.

2. Risk factor of gastric cancer

Gastric cancer has significant geographical, ethnic, and socioeconomic differences in distribution.

The environmental risk factors of gastric cancer can be divided into factors such as diet, obesity, smoking, and Helicobacter pylori. Among these factors, a diet of salt-preserved foods and nitroso compounds, and H. pyloriare the most well-known risk factors for gastric cancer; however, no definitive

molecular pathway has been identified for gastric cancer [14-17].

3. Prognosis and treatment of Gastric cancer

The treatment outcomes of gastric cancer are determined by the stage at presentation. However, surgery is the only potential curative treatment. The five-year overall survival rate after surgery varies from 70%-95% in patients with stage I-III gastric cancer. Moreover, the incidence and mortality rates of gastric cancer in Korea has declined dramatically owing to early detection and improvements in the surgical resection technique. However, patients with unresectable advanced gastric cancer have extremely poor survival rates of less than 10 months after diagnosis. Cytotoxic chemotherapy is only the palliative treatment. 5-Fluoropyrimidines and platinum, including cisplatin or oxaliplatin, combination chemotherapy is considered the standard first-line treatment for advanced gastriccancer[2-4,18-22].

4. History of chemotherapy for the advanced gastric cancer

Most patients with advanced gastric cancer are treated with palliative chemotherapy. Since 1995, chemotherapy has provided data about palliation of symptoms, improved survival, and quality of life compared with the best supportive care in advanced and metastatic settings. Since the 2000s, various fluorouracil combination regimens, such epirubicin, cisplatin, and fluorouracil (ECF), FAMTX (fluorouracil, doxorubicin, and methotrexate), or MCF (mitomycin, cisplatin, and fluorouracil) have been studied and proven to improve survival and quality of life [18,19]. Several phase III trials by the German Study Group, including the REAL 2 and ML 17032 trials, established 5-FU, including oral 5-FU agents such as capecitabine, and platinum agents,

including cisplatin and oxaliplatin combination chemotherapy, as the standard first-line treatment in advanced gastric cancer [20-22].

In summary, 5-FU-based platinum chemotherapy has had a major impact on gastric cancer; however, cytotoxic chemotherapy even if any combination revealed a less than 1 years.

5. Target treatment for the gastric cancer

Chemotherapeutic approaches have changed drastically in the last decade owing to the development of targeted therapies. Therapies targeting receptors or signaling cascades related to tumor growth or suppression have improved responses and overall survival in cancer patients. Targeted chemotherapy was developed through an understanding of the genetic profile and the pathogenesis of specific cancers, such as non-small cell lung cancer, colon cancer, and breast cancer.

In gastric cancer, only trastuzumab has been approved as a target-based therapy, and it is only viable for approximately 10% of HER2-positive gastric cancers [17]. The other targets investigated for gastric cancer were epidermal growth factor receptor (EGFR), MET, and immune checkpoint proteins, such as programmed cell death 1 (PD-1) [23-25]. However, no definite positive results have yet emerged from various ongoing clinical trials.

6. Chemoresistance mechanism

The chemoresistance mechanism is diverse and is not yet fully understood. However, various theories have been suggested, such as membrane transporters, drug inactivation, DNA repair for chemotherapy induced DNA damage, dysregulation of cell survival, and a combination of mechanisms

[5,26-32]. There is little information available about targeted therapies for gastric cancer [5]; therefore, our study aimed to investigate molecular targets (APEX1 and Jagged-1) or cascades (Jagged-1 activation by APEX1) as chemoresistance mechanisms, especially to cisplatin and 5-FU, in gastric cancer cells.

Currently, 5-fluorouracil and platinum, including cisplatin or oxaliplatin, are the most effective chemotherapy regimens for gastric cancer[2-4,18-22].

Cisplatin is known as a highly potent DNA-damaging chemotherapeutic agent, which interferes with DNA replication and kills the fastest proliferating cells [33].

The mechanism of 5-FU cytotoxicity has been recognized to the misincorporation of fluoronucleotides into RNA and DNA through inhibition of the nucleotide synthetic enzyme such as thymidylate synthase [34].

This effect enhances the severe disruption of DNA synthesis and repair, which results in lethal DNA damage. Chemotherapeutics, such as cisplatin and 5-FU, usually enhances DNA damage to cancer cells; however, the upregulation of DNA repair enzymes induces chemoresistance through the repair of chemotherapy-induced DNA damage [5,32]. It is still unclear whether the chemoresistance of cisplatin and 5-FU is related to the DNA repair mechanism.

7. Relationship between APEX-1 and chemoresistance

Our target DNA repair enzyme was APEX1, which is a multifunctional protein that is essential for base excision repair. Although there are some reports on the relationship between APEX1 and chemoresistance, no such studies have been conducted in gastric cancer cells [6-8].

Our research suggested that APEX1 by itself is minimally related or almost

unrelated to chemoresistance in gastric cancer.

8. Relationship between Jagged-1 and chemoresistance

Notch signaling has emerged as a focus for research in various solid tumors; in particularly, the Notch signaling pathway has been determined as driver of gastric epithelial cell proliferation. Jagged-1 is one of five Notch receptor ligands expressed by mammalian cells. Jagged-1-induced Notch activation participates in various aspects of tumor growth through the maintenance of cancer stem cells, promotion of cancer cell survival, inhibition of tumor apoptosis, and driving malignancy proliferation and metastasis. Furthermore, it affects not only cancer cells, but also the microenvironmental components of tumors. Usually, a high expression of Jagged-1 is a poor prognostic factor in breast cancer, bladder cancer, leukemia, prostate cancer, biliary cancer, and gastric cancer [35-39].

In the case of gastric cancer, the activation of Jagged-1 was related to a poor prognosis and Jagged-1 was also a potential prognostic biomarker of overall survival of postoperative clinical outcome. Moreover, the involvement of Jagged-1-induced Notch pathway activity in chemoresistance has been reported [40-43].

However, the role of the Jagged-1-induced Notch pathway in chemoresistance in gastric cancer has been not investigated.

9. Role of APEX1-mediated upregulation of Jagged-1

Two clinical roles of Jagged-1 activation by APEX1 has been determined. First, APEX1 was shown to act as a positive regulator of Jagged-1/Notch activity in colon cancer; therefore, colon cancer progression was driven by

APEX1-mediated upregulation of Jagged-1. Second, Jagged-1 activation by APEX1 was a chemoresistance factor in advanced biliary cancer and the simultaneous high expression of APEX1 and Jagged-1 was associated with chemoresistance in biliary cancer [9-10].

Our results showed that APEX1 expression in the absence of Jagged-1 elevation is almost completely unrelated to chemoresistance in gastric cancer. However, the simultaneous expression both of APEX1 and Jagged-1 resulted in the chemoresistance of gastric cancer cells to cisplatin and 5-FU. Furthermore, APEX1 acted as a positive regulator of Jagged-1/Notch activity in gastric cancer.

V. Conclution

We have suggested that Jagged-1 activation by APEX1 is a chemoresistance factor for 5-FU and cisplatin in gastric cancer, especially 5-FU. Our results suggested that patients with advanced gastric cancer who simultaneously express APEX1 and Jagged-1 may have a potentially poor prognosis owing to chemoresistance; therefore, this may be a potential biomarker of a poor response of chemotherapy and a therapeutic target in chemoresistant advanced gastric cancers that exhibit high levels of Jagged-1/Notch signaling.

Although we demonstrated that Jagged-1 was activated by APEX1, and that Jagged-1 activation by APEX1 was a chemoresistance factor in gastric cancer, further research is still required.

Direct proof and identification of the exact mechanism by which Jagged-1 activation was driven by APEX1 should be a topic of future studies; in addition, the main pathway involving Notch, Hedgehog, Wnt, and Jagged-1 for chemoresistance remains to be elucidated.

The main Notch pathway among the various notch signaling pathways (1,2,3,4) responsible for such chemoresistance should also be investigated further. The molecular factor that stimulates APEX1 and the molecular factors that are stimulated by the signaling pathway of Jagged-1 activation by APEX1 should also be identified.

Finally, clinical studies in patient tissues and other clinical information should be evaluated.

However, our results showed that the simultaneous expression of Jagged-1 and APEX1 is generally associated with chemoresistance in gastric cancer, which suggests that APEX1 and Jagged-1 are predictors of the

chemoresponse and may provide an important tool for the selection of the appropriate therapeutic strategy for neoadjuvant chemotherapy for patients with borderline resectable gastric cancer.

Furthermore, they may also represent a potential therapeutic target to overcome chemoresistance in advanced gastric cancer.

References

1. Van Cutsem E, Haller D, Ohtsu A. The role of chemotherapy in the current treatment of gastric cancer. Gastric Cancer 2002; 5 Suppl 1: 17-22.

2.Wöhrer SS, Raderer M, Hejna M. Palliative chemotherapy for advanced gastric cancer. Ann Oncol2004; 15: 1585-1595.

3. Casaretto L, Sousa PL, Mari JJ. Chemotherapy versus support cancer treatment in advanced gastric cancer: a meta-analysis. Braz J Med Biol Res 2006 ; 39: 431-440.

4. Wagner AD, Grothe W, Haerting J, Kleber G, Grothey A, Fleig WE. Chemotherapy in advanced gastric cancer: a systematic review and meta-analysis based on aggregate data. J Clin Oncol. 2006; 24: 2903-2909.

5. Shi WJ, Gao JB. Molecular mechanisms of chemoresistance in gastric cancer. World J Gastrointest Oncol. 2016; 15; 8: 673-81.

6. Zhang Y, Wang J, Xiang D, Wang D, Xin X. Alterations in the expression of the apurinic/apyrimidinic endonuclease-1/redox factor-1 (APE1/Ref-1) in human ovarian cancer and indentification of the therapeutic potential of APE1/Ref-1 inhibitor. Int J Oncol. 2009; 35: 1069-79.

7. Madhusudan S, Smart F, Shrimpton P, Parsons JL, Gardiner L, Houlbrook S, Talbot DC, Hammonds T, Freemont PA, Sternberg MJ, Dianov GL, Hickson ID. Isolation of a small molecule inhibitor of DNA base excision repair. Nucleic Acids Res 2005; 33: 4711-24.

8. Fishel ML, Kelley MR. The DNA base excision repair protein Ape1/Ref-1 as a therapeutic and chemopreventive target. Mol Aspects Med. 2007; 28:

375-95.

9. Kim MH, Kim HB, Yoon SP, Lim SC, Cha MJ, Jeon YJ, Park SG, Chang IY, You HJ. Colon cancer progression is driven by APEX1-mediated upregulation of Jagged. J Clin Invest. 2013 Jul 1. pii: 65521. doi: 10.1172/JCI65521.

10. Kim HB, Cho WJ, Choi NG, Kim SS, Park JH, Lee HJ, Park SG. Clinical implications of APEX1 and Jagged1 as chemoresistance factors in biliary tract cancer.Ann Surg Treat Res. 2017; 92:15-22.

11.Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK; ToGA Trial Investigators. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 2010 ;376: 687-97.

12. Haenszel W, Kurihara M. Studies of Japanese migrants. I. Mortality from cancer and other diseases among Japanese in the United States. J Natl Cancer Inst 1968; 40:43-68.

13. Haenszel W, Kurihara M, Segi M, Lee RK. Stomach cancer among Japanese in Hawaii. J Natl Cancer Inst 1972; 49: 969-88.

14. Shikata K, Kiyohara Y, Kubo M, Yonemoto K, Ninomiya T, Shirota T, Tanizaki Y, Doi Y, Tanaka K, Oishi Y, Matsumoto T, Iida M. A prospective study of dietary salt intake and gastric cancer incidence in a defined Japanese population: the Hisayama study. Int J Cancer 2006; 119: 196-201.

15. Tricker AR. N-nitroso compounds and man: sources of exposure, endogenous formation and occurrence in body fluids. Eur J Cancer Prev 1997; 6: 226-68.

16. Tsugane S, Tei Y, Takahashi T, Watanabe S, Sugano K. Salty food intake and risk of Helicobacter pylori infection. Jpn J Cancer Res 1994; 85: 474-8.

17. Fox JG, Dangler CA, Taylor NS, King A, Koh TJ, Wang TC.High-salt diet induces gastric epithelial hyperplasia and parietal cell loss, and enhances Helicobacter pylori colonization in C57BL/6 mice. Cancer Res. 1999; 59: 4823-8.

18. Glimelius B, Hoffman K, Haglund U, Nyrén O, Sjödén PO. Initial or delayed chemotherapy with best supportive care in advanced gastric cancer. Ann Oncol 1994; 5: 189-90.

19. Pyrhonen S, Kuitunen T, Nyandoto P, Kouri M. Randomised comparison of fluorouracil, epidoxorubicin and methotrexate (FEMTX) plus supportive care with supportive care alone in patients with non-resectable gastric cancer. Br J Cancer 1995; 71: 587-91.

20. Cunningham D, Starling N, Rao S, Iveson T, Nicolson M, Coxon F, Middleton G, Daniel F, Oates J, Norman AR; Upper Gastrointestinal Clinical Studies Group of the National Cancer Research Institute of the United Kingdom. Capecitabine and oxaliplatin for advanced esophagogastric cancer. N Engl J Med 2008; 358: 36-46.

21. Al-Batran SE, Hartmann JT, Probst S, Schmalenberg H, Hollerbach S, Hofheinz R, Rethwisch V, Seipelt G, Homann N, Wilhelm G, Schuch G, Stoehlmacher J, Derigs HG, Hegewisch-Becker S, Grossmann J, Pauligk C,

Atmaca A, Bokemeyer C, Knuth A, Jäger E; Arbeitsgemeinschaft Internistische Onkologie. Phase III trial in metastatic gastroesophageal adenocarcinoma with fluorouracil, leucovorin plus either oxaliplatin or cisplatin: a study of the Arbeitsgemeinschaft Internistische Onkologie. J Clin Oncol 2008; 26: 1435-42.

22. Kang YK, Kang WK, Shin DB, Chen J, Xiong J, Wang J, Lichinitser M, Guan Z, Khasanov R, Zheng L, Philco-Salas M, Suarez T, Santamaria J, Forster G, McCloud PI. Capecitabine/cisplatin versus 5-fluorouracil/cisplatin as first-line therapy in patients with advanced gastric cancer: a randomised phase III noninferiority trial. Ann Oncol 2009; 20: 666-73.

23. Wilke H, Muro K, Van Cutsem E, Oh SC, Bodoky G, Shimada Y, Hironaka S, Sugimoto N, Lipatov O, Kim TY, Cunningham D, Rougier P, Komatsu Y, Ajani J, Emig M, Carlesi R, Ferry D, Chandrawansa K, Schwartz JD, Ohtsu A; RAINBOW Study Group. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised phase 3 trial. Lancet Oncol. 2014; 15: 1224-35.

24. Iveson T, Donehower RC, Davidenko I, Tjulandin S, Deptala A, Harrison M, Nirni S, Lakshmaiah K, Thomas A, Jiang Y, Zhu M, Tang R, Anderson A, Dubey S, Oliner KS, Loh E. Rilotumumab in combination with epirubicin, cisplatin, and capecitabine as first-line treatment for gastric or oesophagogastric junction adenocarcinoma: an open-label, dose de-escalation phase 1b study and a double-blind, randomised phase 2 study. The Lancet Oncology 2014; 15: 1007-18.

25. Satoh T, Lee KH, Rha SY, Sasaki Y, Park SH, Komatsu Y, Yasui H, Kim TY, Yamaguchi K, Fuse N, Yamada Y, Ura T, Kim SY, Munakata M, Saitoh S, Nishio K, Morita S, Yamamoto E, Zhang Q, Kim JM, Kim YH, Sakata Y.

Randomized phase II trial of nimotuzumab plus irinotecan versus irinotecan alone as second-line therapy for patients with advanced gastric cancer. Gastric Cancer 2015; 18: 824-32.

26. Sugimoto Y, Asami N, Tsuruo T. Expression of P-glycoprotein mRNA in human gastric tumors. Jpn J Cancer Res1989; 80: 993-99

27. Wallner J, Depisch D, Gsur A, Götzl M, Haider K, Pirker R. MDR1 gene expression and its clinical relevance in primary gastric carcinomas. Cancer 1993; 71: 667-71.

28. Gürel S, Yerci O, Filiz G, Dolar E, Yilmazlar T, Nak SG, Gülten M, Zorluoğlu A, Memik F. High expression of multidrug resistance-1 (MDR-1) and its relationship with multiple prognostic factors in gastric carcinomas in patients in Turkey. J Int Med Res. 1999; 27: 79-84.

29.Kang MH, Reynolds CP. Bcl-2 inhibitors: targeting mitochondrial apoptotic pathways in cancer therapy. Clin Cancer Res2009; 15: 1126-32

30. Bhola PD, Letai A. Mitochondria-Judges and Executioners of Cell Death Sentences. Mol Cell2016; 61: 695-704

31. Geng M, Wang L, Li P. Correlation between chemosensitivity to anticancer drugs and Bcl-2 expression in gastric cancer. Int J Clin Exp Pathol 2013; 6: 2554-9

32. Ronchetti L, Melucci E, De Nicola F, Goeman F, Casini B, Sperati F, Pallocca M, Terrenato I, Pizzuti L, Vici P, Sergi D, Di Lauro L, Amoreo CA, Gallo E, Diodoro MG, Pescarmona E, Vitale I, Barba M, Buglioni S, Mottolese M, Fanciulli M, De Maria R, Maugeri-Saccà M. DNA damage repair and survival outcomes in advanced gastric cancer patients treated

with first-line chemotherapy. Int J Cancer. 2017; 140: 2587-95.
33. Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol 2014; 740: 364-78.

34.Longley DB, Harkin DP, Johnston PG.5 -fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer. 2003; 3: 330-8.

35. Penton AL, Leonard LD, Spinner NB. Notch signaling in human development and disease. Semin Cell Dev Biol 2012;23: 450-7.

36. Steg AD, Katre AA, Goodman B, Han HD, Nick AM, Stone RL, et al. Targeting the notch ligand JAGGED1 in both tumor cells and stroma in ovarian cancer. Clin Cancer Res 2011;17:5674-85.

37. Yoon HA, Noh MH, Kim BG, Han JS, Jang JS, Choi SR, Jeong JS, Chun JH. Clinicopathological significance of altered Notch signaling in extrahepatic cholangiocarcinoma and gallbladder carcinoma. World J Gastroenterol 2011; 17: 4023-30.

38. Li D, Masiero M, Banham AH, Harris AL. The notch ligand JAGGED1 as a target for anti-tumor therapy. Front Oncol 2014;4: 254.

39. Wang Z, Li Y, Ahmad A, Azmi AS, Banerjee S, Kong D, et al. Targeting Notch signaling pathway to overcome drug resistance for cancer therapy. Biochim Biophys Acta 2010; 1806: 258-67.

40. Du X, Cheng Z, Wang YH, Guo ZH, Zhang SQ, Hu JK, Zhou ZG. Role of Notch signaling pathway in gastric cancer: a meta-analysis of the literature.World J Gastroenterol. 2014; 20: 9191-9.

41. Demitrack ES, Samuelson LC.Notch as a Driver of Gastric Epithelial

Cell Proliferation.Cell Mol Gastroenterol Hepatol. 2017 ;3: 323-30.

42. Liu H, Zhang H, Shen Z, Wang X, Wang Z, Xu J, Sun Y. Expression of Jagged1 predicts postoperative clinical outcome of patients with gastric cancer.Int J Clin Exp Med. 2015; 8: 14782-92.

43. Yao Y, Ni Y, Zhang J, Wang H, Shao S.The role of Notch signaling in gastric carcinoma: molecular pathogenesis and novel therapeutic targets.Oncotarget. 2017; 8: 53839-53.

	KATO III	AGS
CISPLATIN(IC50) ug/ml	4.924	3.292
5-FU (IC50) ug/ml	21.381	7.254
	KATO III /siAPEX1	AGS /siAPEX1
CISPLATIN(IC50) ug/ml	3.875	2.913
5-FU (IC50) ug/ml	8.279	6.384

Table 1. MTT assay for chemotherapeutic drugs in gastric cancer cells

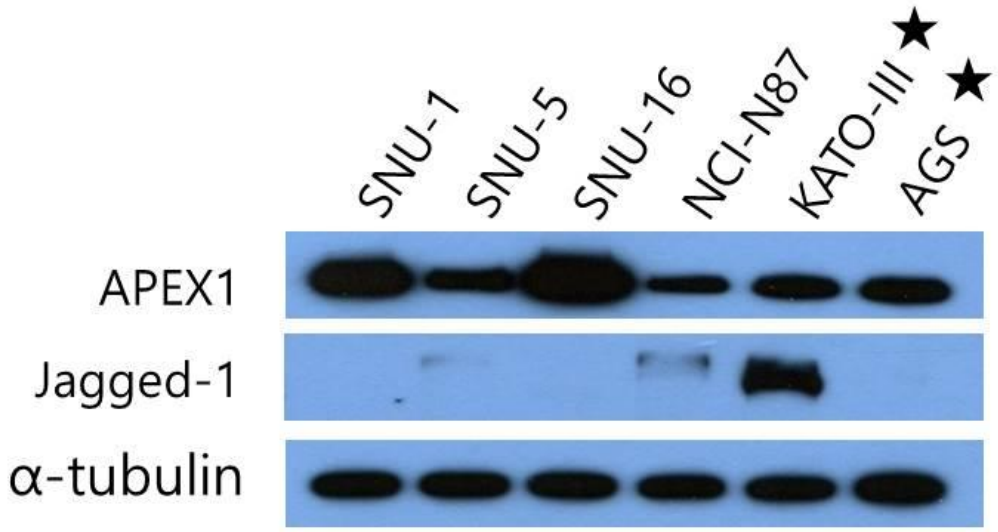

Figure 1. APEX1 expression was found in all gastric cancer cell lines, as shown by western blot. However, KATO-III and NCI-N87 also expressed Jagged-1. We selected two cell lines for further experiments: KATO-III (strong expression of both proteins) and AGS (strong expression of APEX1 but no Jagged-1 expression).

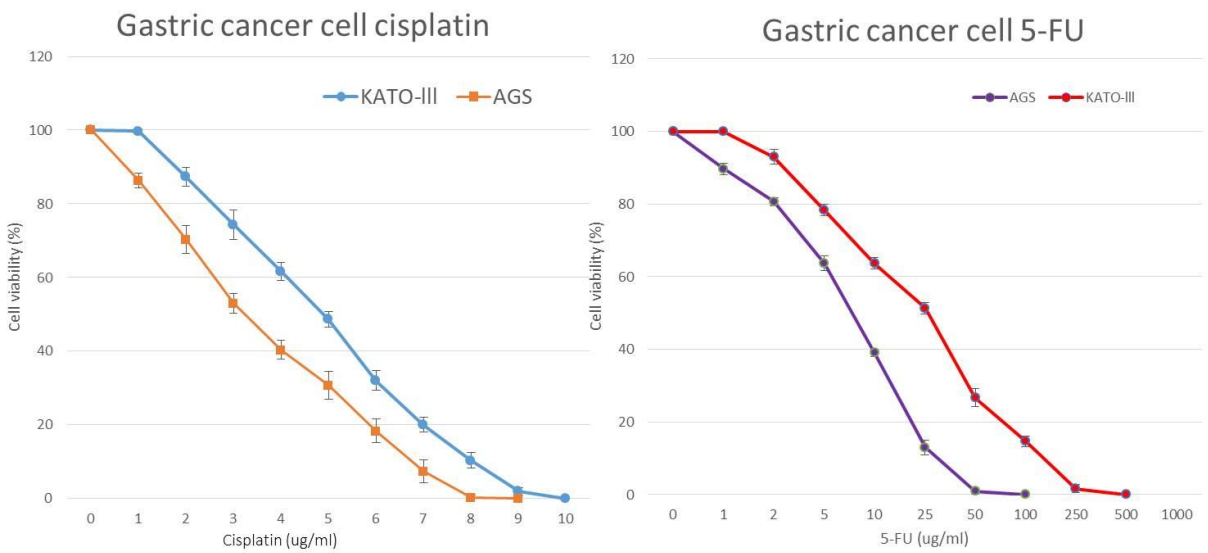

Figure 2. MTT assay of the chemotherapeutic drugs in gastric cancer cells. (A: 5-FU, B: cisplatin). The cells were plated in 96-well plates and treated with cisplatin or 5-FU. The IC_{50} values of KATO-III cells were higher than those of AGS cells; the values were1.5-fold and 3-fold higher for cisplatin and 5-FU, respectively).

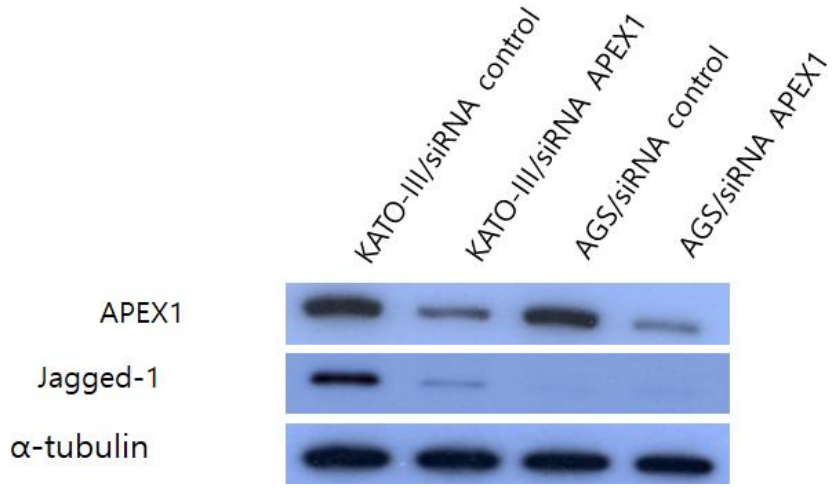

Figure 3. Western blot of APEX1 and Jagged-1 expression in gastric cancer cell lines (KATO-III and AGS) after APEX1 knockdown. Clear decreases in expression were found in KATO III cells.

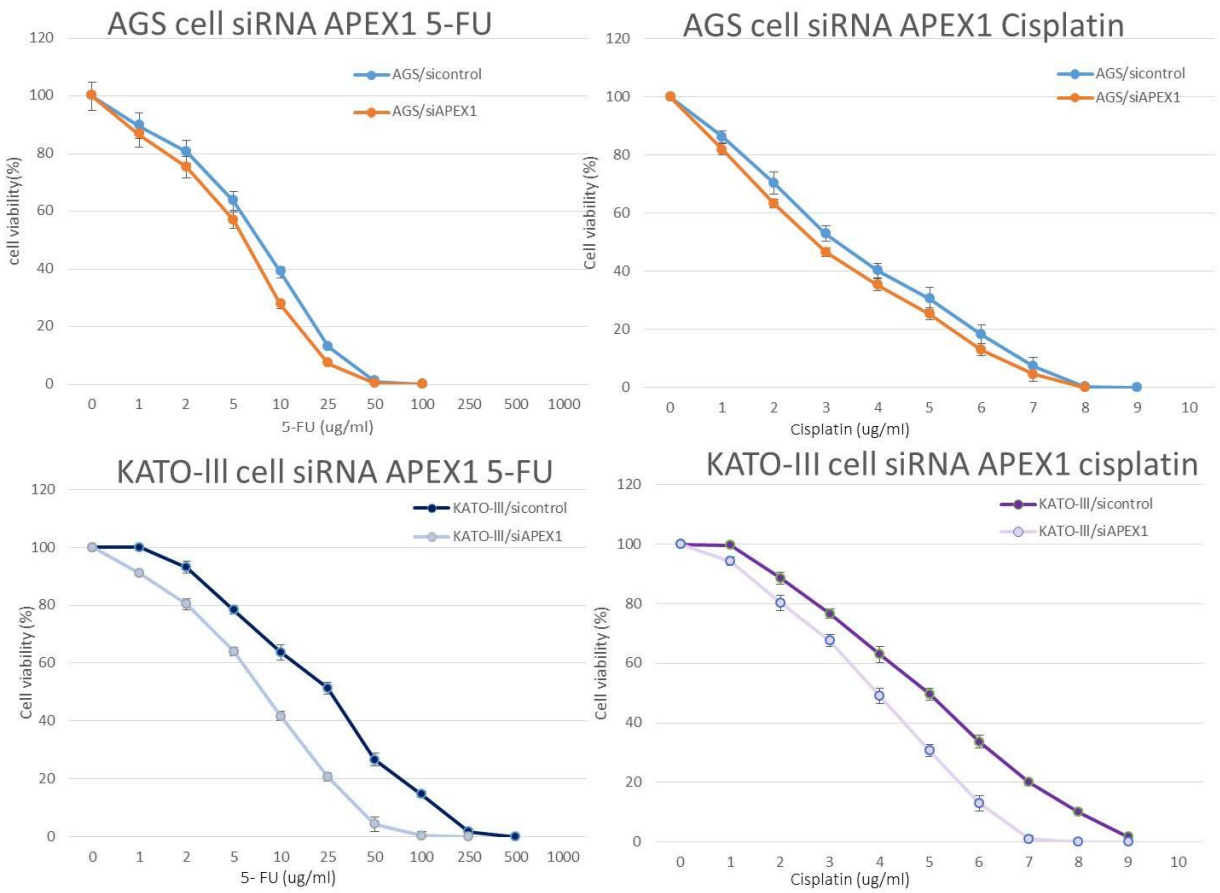

Figure 4. MTT assay of chemotherapeutic drugs in gastric cancer cells. Cells were plated in 96-well plates and treated with cisplatin or 5-FU. After APEX1 knockdown, the MTT assay in the chemosensitive cell lines (AGS) showed only a minimal decrease in IC_{50} (approximately 7% and 15%) for each chemotherapeutic agent (cisplatin and 5FU, respectively) (A,B). The chemoresistant cell line (KATO-III) displayed a clear decrease in IC_{50} (approximately 21% and 67%) for each chemotherapeutic agent (cisplatin and 5-FU, respectively)(C,D).

저작물 이용 허락서

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주 소	광주광역시				
논문제목	영어 : Evaluation of APEX1 and Jagged-1 as chemoresistance factors in advanced gastric cancer				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제,
   기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만,
   저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한
   권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의
   전송·출력을 허락함.

동의여부 : 동의(O) 반대( )

2018년 2월 23일

저작자: 김기범 (서명 또는 인)

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