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Ph.D. Dissertation

A Study on Jagged-1 Activated by APEX1 acts as a Chemoresistance Factor in Colorectal Cancer

Graduate School of Chosun University

Department of Medicine

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ABSTRACT

A Study on Jagged-1 Activated by APEX1 acts as a Chemoresistance Factor in Colorectal Cancer

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배경

대장암은 전 세계적으로 매우 흔한 암중의 하나이고 우리나라에서도 발병율이 급격히 상승중이다. 대장암은 수술적인 절제가 완치의 유일한 방법이다. 그러나 수술 후 재발하거나 수술이 불가능한 진행성 전이성 대장암은 표적 치료제와 함께 투여하는 복합항암화학요법이 유일한 치료대안이다. 현재 진행성 재발성 대장암의 기대여명은 3년 이내로 가장 큰 문제는 항암제 내성이다.

대장암에서 APEX1에 의하여 활성화되는 Jagged-1가 Norch 신호전달체계를 자극하여 대장암을 진행시킨다는 사실은 알려져 있지만 항암제 내성인자로서 역할은 알려지지 않았다.

본 연구는 APEX1 과 Jagged-1의 항암제 내성인자로서 임상적 역할에 대하여 알아보고자 한다.

방법

우리는 7종류의 인간 대장암 세포주를 Western blot 을 이용하여 APEX1 및

Jagged-1의 발현빈도를 살펴보았다. 이중 APEX1 과 Jagged-1 이 동시에 강하게 발현되는 세포주와 APEX1만 강하게 발현되는 세포주를 선택하였다. 선택된 세포주를 이용하여 대장암에서 현재 표준적 항암치료제인 5-Fluorouracil (5-FU) 및 Oxaliplatin의 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 세포주의 IC_{50}은 APEX1만 강하게 발현되는 세포주보다 현저히 높은 항암제내성을 가지고 있었다. 반면 APEX1 과 Jagged-1 이 동시에 발현되는 세포주에 APEX1을 억제 시키면 Jagged-1이 같이 억제되었고 이세포주에 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 대장암은 표준항암요법제인 5-FU 와 Oxaliplatin에 내성을 가지게 된다. APEX1에 의한 Jagged-1의 과발현은 항암치료의 반응을 예측하는 인자중 하나가 될 수 있고 더 나아가 진행성 대장암의 항암치료에 치료표적으로 연구될 수 있겠다.

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

I. Introduction

Colorectal cancer (CRC) is the third among the most predominant cancers worldwide. In South Korea, CRC was reported to be the third most common cancer type except thyroid cancer in 2015. Approximately 80% of CRCs are localized in the bowel wall and/or its regional lymph nodes. In early-stage CRC (stage I-III) cases, surgical resection is the primary curative therapy and aimed to completely remove the tumor along with the adjacent vasculature and lymphatic drainage system of the affected colon. The most important prognostic factors to assess the post-surgery survival rate are the pathological stage and molecular as well as histological features [1-3].

The late-diagnosed patients (remaining 20%) with *de novo* unresectable metastatic CRC and patients with stageII/III CRC (approximately40% of total CRC patients) experience recurrence despite undergoing curative surgery.The predominant metastatic sites include the liver, lungs, lymph nodes, and peritoneum. Unresectable and metastatic CRC is incurable and requires palliative systemic chemotherapy. However, the major clinical challenge regarding chemotherapeutic treatment is the primary or acquired resistance, and several theories were proposed concerning its suppression [4-6].

Apurinic-apyrimidinic endonuclease-1 (APEX1) is one of the proteins essential for base excision repair and is correlated with cancer progression in various human solid malignancies[7-13]. Furthermore, APEX1 was reported to contribute to colon cancer (CC) progression through the upstream activation of the Jagged-1/Notch signaling pathway[14]. Moreover, a study reported that Jagged-1 activated by APEX1 acts as an anti-cancer drug-resistance factor in advanced biliary cancer[15].

In this study, we investigated the role of Jagged-1 activated by APEX1 as an chemoresistance factor in CRC.

II. Materials and Methods

1. Cell culture

Human CC cell lines (HCT-15, SW620, HCT-116, DLD-1, SW480, and LoVo) were cultured in RPMI1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS, Lonza), 100 units/mL penicillin, and 100 μg/mL streptomycin (Invitrogen), while Caco-2 cells were grown in MEM medium with 20% FBS. All the cell lines were acquired from the American Type Culture Collection (ATCC) and maintained in a humidified incubator with an atmosphere of 5% CO_2 at 37 °C.

2. Preparation of drug solutions to perform *invitro*assays

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

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

Cell viability was determined rming MTT assay according to the standard protocol. Cells were seeded in a 96-well plate and incubated for 24 h. These cells were treated with either oxaliplatin or 5-FU for 24 h. After treatment, 10 μL MTT (1 mg/mL) in PBS was added and the cells were incubated for 4 h at 37 °C. Subsequently, the medium containing MTT was removed, 100 μL

dimethyl sulfoxide (DMSO) added, and the cells incubated for another 20 min at 37 °C with gentle shaking. The absorbance was read using an ELISA plate reader with a 570-nm filter. Cell viability was calculated from relative color intensity in treated compared to that in untreated samples.

4. small interfering RNA (siRNA)-mediated APEX1 knockdown

To knockdown APEX1 expression, the cells were transiently transfected with specific siRNA, using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions. The sequence used to target APEX1 was 5′-AAGTCTGGTACGACTGGAGTA-3′, while that for the negative control siRNA (Bioneer) was 5′-CCUACGCCACCAAUUUCGUdTdT-3′. The cells were transfected with either pSilencer2.1-U6-neo control shRNA or pSilencer2.1-U6-neo APEX1 shRNA, using Lipofectamine 2000 (Invitrogen) and cultured in selection medium containing 500 μg/mL neomycin for 2-3 weeks.

5. Immunoblotting

Cells were washed with 1× PBS and lysed in lysis buffer (20 mM HEPES (pH 7.4), 2 mM EGTA, 50 mM glycerol phosphate, 1% Triton X-100, 10% glycerol, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 10 μg/mL leupeptin, 10 μg/mL aprotinin, 1 mM Na_3VO_4, and 5mM NaF) and the concentration of protein was determined using a dye-binding microassay (Bio-Rad, Hercules, CA, USA).

Equal concentrations of cell-or tissue- extracts were resolved by 8-12% SDS-PAGE followed by the electrophoretic transfer of protein bands onto a

polyvinylidene difluoride membrane (PALL life sciences). The membranes were blocked for 1 h with TBS-t (10 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 0.1% Tween-20) that contains 5% non-fat milk and incubated with specific primary antibodies overnight at 4 °C. The blots were washed four times for 15 min/wash with TBS-t and incubated for 1 h with corresponding peroxidase-conjugated secondary antibodies (1:4000, Jackson Immuno Research Inc.). The blots were again washed four times with TBS-t and developed using an enhanced chemiluminescence detection system (ECL; iNtRON Biotechnology, South Korea). The antibodies used in our study were mouse anti-APEX1 (sc-17774) and mouse anti-Jagged-1 (sc-390177) purchased from Santa Cruz Biotechnology.

6. Statistical analysis

Data in all the experiments are represented as the mean ± standard deviation. Statistical comparisons were performed using two-tailed paired Student's t-test. The p values < 0.01 were considered to indicate statistically significant differences. Analyses were performed using GraphPad Prism (GraphPad) and Excel (Microsoft) softwares.

III. Results

1. Estimation of APEX1 expression in CC cell lines

In this study, we utilized seven CC cell lines. Initially, we measured the APEX1 and Jagged-1 constitutional expression levels in HCT-15, SW620, HCT-116, Caco-2, DLD-1, SW480, and LoVo cell lines. APEX1 and Jagged-1 expressions were detected by western blotting using α-tubulin as a loading control. The western blot analysis revealed that all the CC cell lines expressed a high level of APEX1; however, four of the CC cell lines (HCT-116, Caco-2, DLD-1, and LoVo). Particularly, DLD-1 cells co-expressed high levels of APEX1 and Jagged-1 (Fig.1). Therefore, we selected two celllines for the further experiments : the DLD-1 cellline as it strongly expresses APEX1 as well as Jagged-1, and SW480 as it strongly expresses only APEX1.

2. Efficiency of chemotherapeutic drugs (oxaliplatin and 5-FU)

We performed MTT assay to assess the sensitivity of DLD-1 and SW480 cell lines toward effective well-known chemotherapeutic agents, namely, oxaliplatin and 5-FU (Fig. 2). The DLD-1 cell line was more resistant to both the chemotherapeutic agents than SW480 cell line. Additionally, we estimated the IC50 values and demonstrated that the IC_{50} values of both the drugs were higher in the resistant DLD-1 cellline (2.2-fold and 1.7-fold for oxaliplatin and 5-FU, respectively) than in the sensitive SW480 cell line. These results indicated that the simultaneous expression of APEX1 and Jagged-1 might be associated with chemoresistance toward oxaliplatin and

5-FU (Table 1).

3. Jagged-1 expression after APEX1 knockdown in CC cell lines.

We estimated the variation in APEX1 and Jagged-1 expression levels in DLD-1 and SW480 cell lines after APEX1 knockdown. To perform APEX1 knockdown experiments, either APEX1 or control siRNA was transfected into the DLD-1 and SW480 cells. The western blot results revealed that the DLD-1 and SW480 cells transfected with APEX1-siRNA exhibited a reduction in endogenous APEX1 level by approximately 80% compared to the control-siRNA transfected cells.

In DLD-1 cells, Jagged-1 expression was clearly decreased after APEX1 knockdown, which suggested that Jagged-1 was activated by APEX1 (Fig. 3).

4. Efficiency of the chemotherapeutic drugs (oxaliplatin and 5-FU) after APEX1 knockdown

Finally, we assessed the variation in the drug sensitivity of DLD-1 and SW480 cells after APEX1 knockdown. APEX1 is believed to be a chemoresistance factor that enhances DNA repair against oxaliplatin and 5-FU treatment. After APEX1 knockdown, the chemoresistant DLD-1 cells were rendered sensitive to both the drugs and exhibited a remarkable decrease in IC50 values compared to cells with constitutional APEX1 expression (approximately by 50% and 44% for oxaliplatin and 5-FU, respectively). However, the inherently chemosensitive SW480 cells exhibited a minimal decrease in IC50 values after APEX1 knockdown compared to normal

cells (approximately by 3.4% and 6.7% for oxaliplatin and 5-FU, respectively) (Fig 4).

These results suggested that Jagged-1 activation through APEX1 stimulation might be a major chemoresistance pathway compared to APEX1 expression alone during oxaliplatin and 5-FU treatment in colon cancer.

In conclusion, the co-expression of APEX1 and Jagged-1 might be a major anti-cancer drug resistance factor, and the main chemoresistance mechanism involved in this condition is Jagged-1 activation by APEX1.

IV. Discussion

1. History of chemotherapy development in Colorectal cancer

The development of chemotherapy to treat CRC initiated after the identification of 5-FU in 1957 [16]. The subsequent 5-FU-based chemotherapy development was attributed to the finding that the inhibition of thymidylate synthase by 5-FU is strengthened in the presence of the reduced form of folate leucovorin (LV). After this discovery, the combinatorial use of 5-FU and LV was established to increase the efficacy of chemotherapy through several studies in human CC cell lines. These data regarding the synergic cytotoxic effect of 5-FU and LV combination led to numerous phase III clinical studies until the 1990s and the median overall survival rate (OSR) improved to approximately > 12 months in 5-FU/LV-treated CC patients compared to 7-8 months in CC patients treated with 5-FU alone [17-21].

In the early 2000s, the novel combinations of cytotoxic chemotherapeutic agents to treat CRC were further developed owing to the development of novel drugs such as irinotecan (topoisomerase I inhibitor) and oxaliplatin (third-generation platinum agent), regarding which two major studies were published in 2004 [22-23].

A study using irinotecan with bolus 5-FU/LV termed as IFL regimen to treat CRC patients indicated that IFL treatment significantly increased the progression-free survival rate (PFSR) (7.0 vs. 4.3 months) and OSR (14.8 vs. 12.6 months) compared to 5-FU/LV treatment. According to the N9741 inter group clinical trial, the treatment using FOLFOX (Infusional 5-FU/LV with oxaliplatin) regimen revealed significantly improved OSR (19.5 vs. 15.0 months) compared to that achieved by the IFL regimen[24-25]..

Finally, clinical trials named Gruppo Oncologico Italia Meridionale (GOIM) and Groupe Coopérateur Multidisciplinaire en Oncologie (GERCOR) indicated similar treatment efficacy in FOLFOX as well as FOLFIRI (Infusional 5-FU/LV with irinotecan) regimens. Treatments including FOLFOX followed by FOLFIRI or vice versa were major standard chemotherapy strategies with similar OSR (20.6 vs. 21.5 months)[26-27].

Since the 2000s, anti-cancer therapy drastically changed owing to the advent of targeted therapies. As the knowledge regarding the genetic profiles and pathogenesis of specific cancers increased, treatments that target molecules or signaling cascades involved in the tumor growth or suppression gradually improved the OSR in cancer patients. Currently, well-known targets for CRC treatment are vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) [28-29].

Bevacizumab is a humanized monoclonal antibody of VEGFA and representative drug that targets VEGF. According to several reports, addition of bevacizumab to standard combination cytotoxic chemotherapy (CCC) significantly increased the OSR and PFSR independent of the response rate [30-32].

Cetuximab is a well-known biological drug that inhibits EGFR. In case of RAS and BRAF wild type tumors, especially in patients with a primary tumor on the left side of the colon, the combinations of cetuximab and standard cytotoxic doublet regimens effectively improve OSR and PFSR as well as response rate. Therefore, this combination is used as a primary option to treat specific CRC cases. The addition of these agents that target EGFR to CCC increased the OSR by > 4-6 months compared to that achieved by the CCC [33-37].

2. Current standard systemic treatment for unresectable, advanced or metastatic CRC

The current standard treatment of unresectable or metastatic CRC involves the chemotherapeutic agents either in combination or alone that include cytotoxic chemotherapy agents (5-FU/LV, capecitabine, irinotecan, and oxaliplatin), agents that target signaling molecules (bevacizumab, cetuximab, panitumumab, ziv-aflibercept, and regorafenib), and immune checkpoint drugs (pembrolizumab and nivolumab). The mechanisms of action of these drugs are distinct and the chemotherapy regimens are chosen by considering the treatment objectives, type of previous treatment, mutational profiles, and other toxic profiles. Currently, the standard chemotherapy regimen to treat CRC is a fluoropyrimidine doublet including FOLFOX or FOLFIRI along with biological agent that targets VEGF (bevacizumab, ramucirumab, or ziv-aflibercept)) or (cetuximab or panitumumab) [38-42].

3. Expression of APEX1 and Jagged-1 in CRC

APEX1 is a multifunctional protein that is essential to perform base excision repair and its major function is to repair the single-strand DNA cleavage and apurinic/apyrimidinic (AP) sites. Moreover, it stimulates the DNA-binding ability of several transcription factors related to cancer progression, such as AP1, p53, and early growth response 1 (EGR1). Clinically, the abnormal APEX1 overexpression was reported in various solid tumors. Multiple studies revealed APEX1 association with cancer progression and poor prognosis[7-13].

The Notch signaling plays a major role in the cell fate determination and progenitor cell population maintenance during the embryonic development. The Notch signaling activation emerged as an important aspect of research and recent reports suggest that it plays an important role in the development and progression of numerous human malignant solid tumors. Jagged-1 is one of the five Notch receptor ligands and enhances the Notch signaling that affects the growth of various cancers by the regulation of cancer stem cell survival rate. The survival of cancer stem cells might improve survival rate, inhibit apoptosis, and stimulate proliferation and metastasis of cancer cells. Clinically, a high Jagged-1 expression level was reported as a poor prognostic factor in many cancer groups[43-50].

A report revealed an association between the CC progression and APEX1 mediated Jagged-1 upregulation. According to the aforementioned study, APEX1 stimulates tumorigenesis in noncancerous as well as CC cell lines. The researchers demonstrated that APEX1 activates the Notch signaling through Jagged-1 activation and this signal promotes CC progression. Furthermore, the reports indicated that APEX1 expression is positively correlated with the expression of Jagged-1 and cleaved Notch in the human

CC tissue [14,51,52].

4. Chemoresistance mechanism

The development of combination chemotherapy and targeted therapy increased the response and survival rates in CC patients. However, the main mechanism of this treatment is cytotoxic chemotherapy. The individual administration of either of the representative cytotoxic combination therapies (FOLFOX or FOLFIRI regimen) revealed an OSR < 2 years owing to chemotherapy resistance in CC patients. Furthermore, despite the advancements in chemotherapy, a better combination of chemotherapy regimen remains to be developed until date. The major challenge of cytotoxic chemotherapy is development of resistance against chemotherapeutic agents. For a long time, chemoresistance was studied and various theories were proposed; however, as the chemoresistance mechanism involves complex processes it is not understood through single process[53-56].

FOLFOX regimen is a major chemotherapy strategy administered to treat most of the CRC patients. Oxaliplatin is a third-generation platinum agent that induces DNA damage by interfering with DNA replication and finally causes the death of rapidly proliferating cancer cells[57-59]. 5-FU affects several pathways; however, it primarily interrupts the thymidylate synthase activity and blocks the synthesis of pyrimidine thymidine, which is an essential nucleoside for DNA replication[60].

Generally, chemotherapy drugs such as oxaliplatin and 5-FU induce cancer cell death by increasing their DNA damage. However, the improvement of DNA repair capacity through the activation of pathways such as the base excision repair (BER) pathway results in chemoresistance[61-63].

5. Role of APEX1 and Jagged-1 as chemoresistance factors

APEX1 is one of the major proteins involved in the BER pathway. Several reports suggested that APEX1 overexpression in various cancers is associated with chemoresistance. Therefore, APEX1 might act as a major factor in chemoresistance[7-13].

In Notch signaling pathway, several cancers with Jagged-1 overexpression are reported to exhibit a poor prognosis. However, a report indicated no correlation between Notch signaling and prognosis. Therefore, in this point of view, Notch signaling is not highly studied. As previously mentioned, APEX1 activates the Notch signal via Jagged-1 and promotes CC progression [43-50]. Recently, studies reported that APEX1 and Jagged-1 are associated with chemoresistance in biliary cancer and Jagged-1 was reported to be activated by AEPX1[15]. However, the role of APEX1 or Notch signaling through the Jagged-1 activation in chemoresistance was not investigated in CRC.

Our results indicated that APEX1 expression in absence of Jagged-1 expression is mostly unrelated to chemoresistance in CRC. However, the simultaneous expression of APEX1 and Jagged-1 might be a major chemoresistance factor and the Jagged-1 activation by APEX1 is one of the major chemoresistance pathways during oxaliplatin and 5-FU treatment.

V. Conclution

The development of chemotherapeutic agents including cytotoxic and biological agents that target cancers has increased the treatment efficacy and survival rate. Most of the effective combination therapies merely improved the OSR by approximately 2-3 years owing to tumor resistance against chemotherapy. Therefore, chemoresistance remains a major challenge in cancer therapy.

Our results indicate that the simultaneous overexpression of APEX1 and Jagged-1 might be a major anti-cancer drug-resistance factor during oxaliplatin and 5-FU treatment of CRC patients.

Concordant with the results of previous studies, our results demonstrated the Notch signaling activation via Jagged-1 activation by APEX1 as one of the major chemoresistance pathways against oxaliplatin and 5-FU treatment in CRC.

Therefore, the co-expression of APEX1 and Jagged-1 might be used as a potential biomarker to predict poor response to chemotherapy in CRC. The pathway that involves Notch signaling activation via Jagged-1 activation by APEX1 might act as a major therapeutic target in chemoresistant CRC. However, further clinical studies are essential to establish and implement this therapeutic strategy.

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	DLD-1	SW480
Oxaliplatin(IC50) ug/ml	**3.86**	1.72
5-FU (IC50) ug/ml	9.08	5.31
	DLD-1/siAPEX1	SW480/siAPEX1
Oxaliplatin(IC50) ug/ml	**1.92**	1.67
5-FU (IC50) ug/ml	5.08	4.95

Table 1. MTT assay to assess the efficiency of chemotherapeutic drugs in CC cell lines.

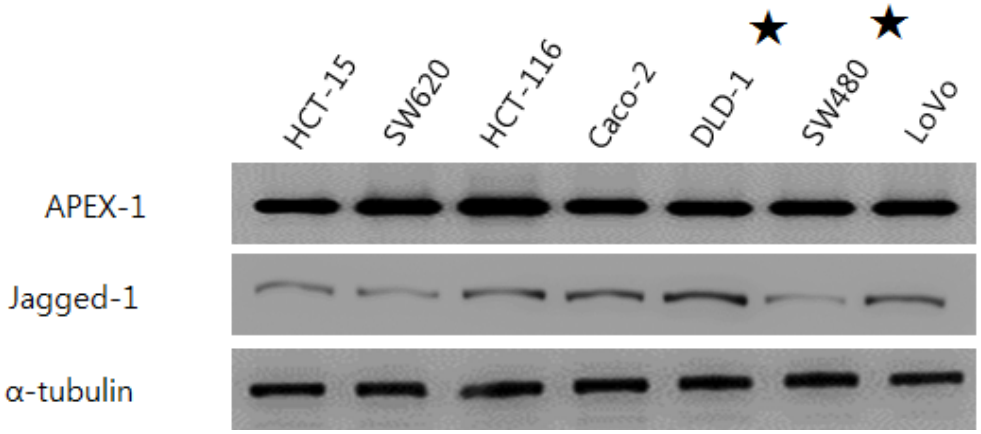

Figure 1. APEX1 expression was high in all the CC cell lines, as shown by western blotting. However, HCT-116, Caco-2, DLD-1, and LoVo cells expressed high Jagged-1 levels. We selected two cell lines to perform the further experiments: DLD-1 cells exhibited strong expression of both the proteins, and SW480 cells exhibited strong expression of APEX1 but not Jagged-1.

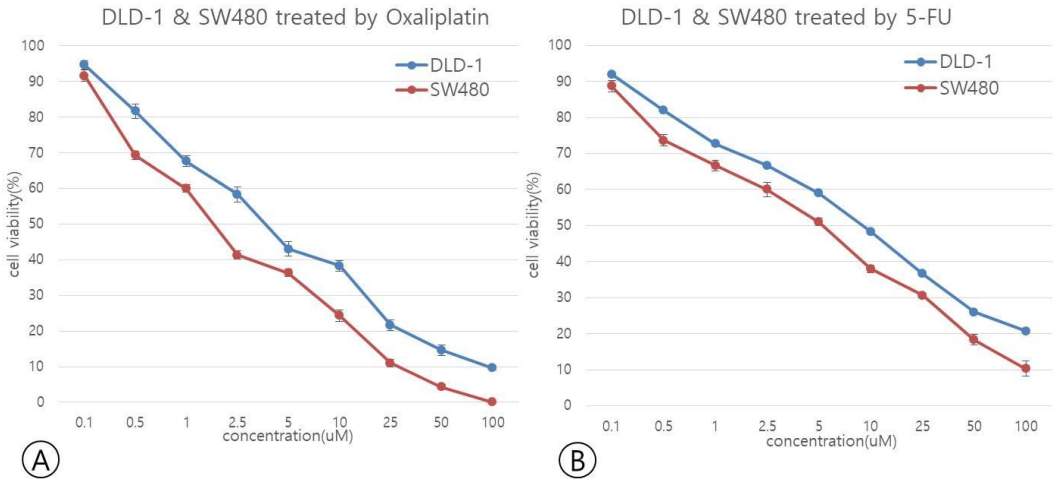

Figure 2. MTT assay to assess the efficiency of chemotherapeutic drugs in CC cells (A: oxaliplatin, B: 5-FU). Cells were cultured in 96-well plates and treated with oxaliplatin or 5-FU. The IC_{50} values were 2.2-fold (oxaliplatin) and 1.7-fold (5-FU) higher in DLD-1 cells than in SW480 cells.

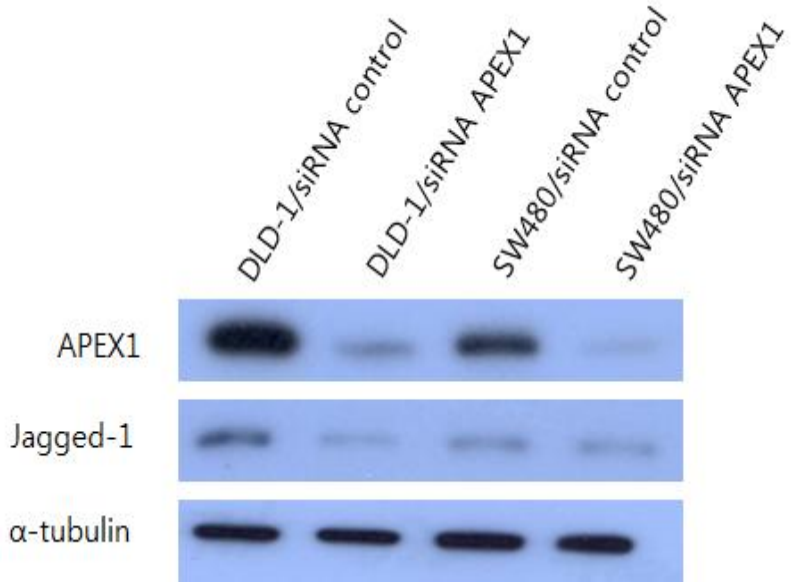

Figure 3. Western blotting of APEX1 and Jagged-1 expression in CC cell lines DLD-1 and SW480 after APEX1 knockdown. Jagged-1 expression in DLD-1 cells was prominently decreased after APEX1 knockdown.

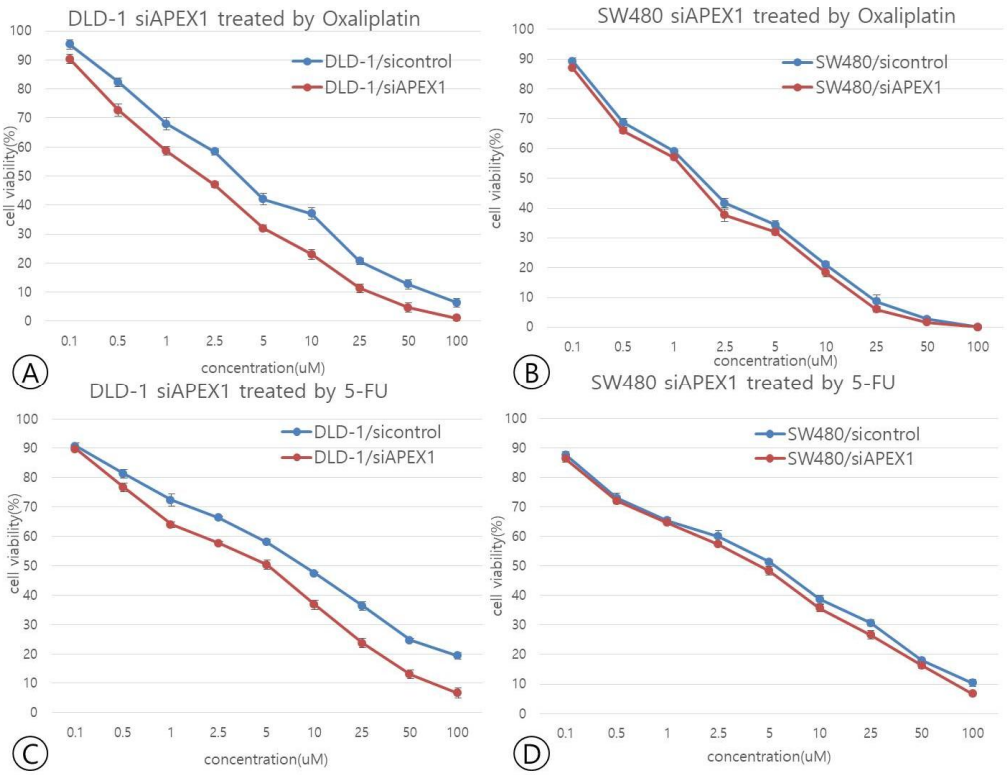

Figure 4. MTT assay to assess the efficiency of chemotherapeutic drugs in CC cells. Cells were seeded in 96-well plates and treated with oxaliplatin or 5-FU. After APEX1 knockdown, MTT assay performed using chemosensitive DLD-1 cell line indicated a prominent decrease in the IC_{50} values of chemotherapeuticagents (approximately 50% and 44% for oxaliplatin(A) and 5-FU(B),respectively).The chemoresistant SW480 cell line exhibited a minimal decrease in the IC_{50} values of chemotherapeuticagents (approximately 3.4% and 6.7% for oxaliplatin(C) and 5-FU(D), respectively).

Abstract

Background

Colorectal cancer is one of the most common cancer in the world and the frequency is also rising rapidly in Korea. Surgical resection is the only method of cure. In case of inoperable advanced metastatic or recurrent cancer, chemotherapy combined with target therapy is the only treatment option. However, the expected life expectancy of inoperable advanced colorectal cancer is within three years, and the biggest problem is anticancer drug resistance. It is known that Jagged-1-activated Norch signaling by APEX1 in colorectal cancer promotes colorectal cancer, but its role as an anticancer drug resistance factor is unknown.

The aim of this study was to investigate the clinical role of APEX1 and Jagged-1 as anti-cancer drug resistance factors.

Method

We checked the expression of APEX1 and Jagged-1 in 7 human colon cancer cell lines using Western blot. The cell line in which both APEX1 and Jagged-1 are strongly expressed simultaneously and the cell line in which only APEX1 is strongly expressed are selected. 5-Dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) assay was used to evaluate the anticancer susceptibility of 5-Fluorouracil (5-FU) and Oxaliplatin.

Result

The IC_{50} of coexpression of APEX1 and Jagged-1 cells were higher than those expressing APEX1 only cellline. that indicated coexpression of APEX1 and Jagged-1 is chemoresistance factor. Meanwhile, inhibition of APEX1

expression in cell lines expressing APEX1 and Jagged-1 simultaneously inhibited Jagged-1. the inhibition of APEX1 and the MTT assay revealed that the IC_{50} of APEX1 and Jagged-1 and IC_{50} of APEX1 alone were almost similar.

Conclusion

Jagged-1-activated colon cancer by APEX1 is resistant to the standard chemotherapy regimens(5-FU and Oxaliplatin). Overexpression of Jagged-1 by APEX1 may be one of the predictors of the response to chemotherapy and may further be studied as a therapeutic target for chemotherapy of advanced colorectal cancer.