HGF overexpression as a potential predictive marker of MET inhibitor in gastric cancer

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Directed by Professor Sun Young Rha

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ABSTRACT

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Cancer is the leading cause of death and gastric cancer (GC) is the second most common cancer in Korea. Recently, receptor tyrosine kinase (RTK) targeted agents are developing in many types of cancer including GC, however, HER2 targeting antibody, trastuzumab is the only approved drug in GC. Exploring other novel RTKs is progressed.

MET is a proto-oncogene that encodes a protein known as hepatocyte growth factor receptor (HGFR), involved in proliferative, survival and invasive/metastatic abilities of cancer cells. MET has received considerable attention as a potential target for cancer therapy. However, there is no approved agent targeting MET in clinic. Onartuzumab, one of the most studied MET target agents in clinical trials, also failed in phase III to select proper patients with proper biomarker criteria.

Exploring new biomarkers for the clinical success of MET inhibitor is needed. In this study, we investigated the predictive marker of Sym015, a mixture of 2 monoclonal antibodies against MET. First, we profiled MET and HGF status in 49 GC cell lines. Then, we screened the sensitivity of Sym015 and explored the factors that were associated with sensitivity. As a result, we observed that HGF overexpression cell lines were sensitive to Sym015. To demonstrate HGF overexpression as a predictive marker, gene silencing experiment was conducted with siRNA transfection followed by cell viability and invasion assay.

Key words: gastric cancer, HGF, MET inhibitor, predictive marker

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I. INTRODUCTION

According to cause-of-death statistics (2014) in Korea, cancer is the first cause of death and gastric cancer is the second most common cancer[1,2]. Patients with metastatic gastric cancer have a very poor prognosis with 15-17 wks median survival despite increased use of chemotherapy[3]. Therefore, targeted agents are being developed to improve prognosis and survival rates of patients.

Targeted agents are developed in various cancer types and especially targeting

receptor tyrosine kinases (RTKs) has actively studied. Because RTKs including HER2, EGFR, FGFR and MET are overexpressed or amplified in cancer cells and constitutive activation of RTKs causes the signaling pathway related cell survival, proliferation and invasion[4]. Although trastuzumab, HER2 targeted agent, is commonly treated to HER2 positive patients, the incidence of HER2 positive is 10 % in gastric cancer. Therefore, novel molecular targets are required to treat more patients with targeted agents[5].

MET is a proto-oncogene that encodes a protein known as hepatocyte growth factor receptor (HGFR), involved in proliferative, survival and invasive/metastatic abilities of cancer cells. MET has received considerable attention as a potential target for cancer therapy. MET amplification is present in 4–5 % of gastric cancer patients, and associated with poor outcomes and significantly shorter median survival[6-8].

Hepatocyte growth factor (HGF), the ligand of MET is also associated with poor outcomes and significantly shorter overall survival in gastric cancer[9]. Especially, circulating HGF can be measured as a pharmacodynamic biomarker of onartuzumab, a monoclonal antibody against MET, in advanced solid tumors, suggesting that HGF might be used as a biomarker to predict the sensitivity of MET inhibitor[10].

MET inhibitors have shown to be sensitive to MET amplification. For example, foretinib, PHA-665752, and crizotinib are sensitive to MET amplification in preclinical studies[11,12]. However, none of MET inhibitors has succeeded in clinical trials across all cancer types.[13,14] Onartuzumab, which is one of the most studied MET inhibitors, also failed in clinical trial because of difficulties in screening proper patients. Therefore, novel MET inhibitors are being developed and Sym015, a mixture of two monoclonal antibodies directed at MET receptor, is one of the leading agents in the pipeline.

To explore the novel marker to predict the sensitivity of MET inhibitor, we profiled MET and HGF status in 49 gastric cancer cell lines and screened the

sensitivity of Sym015. We confirmed that 6 MET amplified cell lines were all sensitive to Sym015. Interestingly, HGF overexpression cell lines were sensitive to Sym015. To confirm the HGF overexpression as a predictive marker of Sym015, we inhibited the expression of HGF by siRNA. After knockdown of HGF, phenotype changes were validated by cell viability assay and invasion assay. Viability and invasion related signaling pathway molecules were also analyzed.

Consequently, we suggest that HGF overexpression as a potential predictive marker of MET inhibitor in gastric cancer.

II. MATERIALS AND METHODS

1. Drug

MET inhibitor, Sym015 (a mixture of two monoclonal antibodies against MET) was given by Symphogen (Symphogen A/S, Ballerup, Denmark).

2. Cell culture

Forty-nine gastric cancer cell lines were used in this study. Three cell lines were obtained from the American Type Culture Collection (ATCC, Virginia, USA), 11 cell lines were obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea), 10 cell lines were purchased from the Japanese Cancer Research Resources Bank (JCRB, Osaka, Japan), and 25 cell lines were established by the Cancer Metastasis Research Center , Yonsei University College of Medicine (CMRC, Seoul, Korea) and Songdang Institute for Cancer Research, Yonsei University College of Medicine (SICR, Seoul, Korea) from metastatic gastric cancer patients through isolation of ascites or pleural fluids from 1989 to 2013. Cells were cultured in Eagle's Minimum Essential Medium (EMEM), Roswell Park Memorial Institute-1640 (RPMI-1640), Dulbecco Modified Eagle's Media (DMEM) containing 10 % fetal bovine serum (Lonza, Basel, Switzerland), 100 units/ml penicillin and 100 μg/ml streptomycin (Lonza, Basel, Switzerland). Cultured cells were incubated at 37 ˚C in an atmosphere with 5 % CO_2.

3. Cell viability assay

Cells (8×10^3) were seeded into a 96 well plate. After 24 hr, Sym015 was treated at specific doses (4, 20, 100, 250, 500 nM). Following 3 days of incubation, CCK-8 (Dojindo, Kumamoto, Japan) solution was added and the plates and further incubated at 37 ˚C for 2 hr. The absorbance was read at a wavelength of 450 nm and analyzed for Calcusyn (Biosoft, Cambridge, UK) software.

4. Whole exome sequencing and RNA sequencing data analysis

Whole exome sequencing (WES) and RNA sequencing data of 49 GC cell lines were obtained from the genome database of Songdang Institute for Cancer Research (SICR), Yonsei University College of Medicine (Seoul, Korea). Briefly, copy number variants (CNVs) and single nucleotide variants (SNVs) were evaluated using WES data. The mRNA expression levels were measured in fragments per kilobase of exon model per million mapped reads (FPKM) without normalization.

5. Western blot analysis

Total protein (20 ug) was used and separated by SDS-PAGE (10 % polyacrylamide gel), then transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were incubated in blocking solution consisting of 5 % skim-milk in TBST at room temperature for 1 hr before being incubated 4 ℃ overnight with primary antibodies specific for c-Met, p-ERK (Santa Cruz, CA, USA), p-Met, p-AKT, p-ERK, FAK, p-FAK (Cell signaling Technology, Beverly, MA, USA) and HGF (Abcam, Cambridge, MA, USA). Peroxidase-conjugated anti-mouse or anti-rabbit were used as secondary. The protein expressions were developed on X-ray film using the enhanced chemiluminence reagent (ECL;

Amersham, Buckinghamshire UK). Data were normalized to α-tubulin (Sigma-Aldrich, St Louis, MO, USA) and the intensity of protein was semi-quantified using Image J software (NIH, Bethesda, MD, USA).

6. Quantitative real-time PCR (qPCR) analysis

Genomic DNA (gDNA) of the cells was extracted using phenol-chloroform-isoamyl alcohol (25:24:1). To determine the relative quantification of copy number variants of MET in 49 GC cell lines, we conducted TaqMan® Gene Expression Assays (Applied Biosystems, Foster City, CA, USA). The real-time PCR was run under the following conditions: the reactions containing Taqman probe, Master mix, gDNA, RNase. Cycling conditions were 10 min at 95 ℃, followed by 40 cycles at 30 sec at 95℃, 1 min at 60 ℃. TaqMan probe sequence was designed as follows: 5'-FAM-CCG AGC TAC TTT TCC-3'. The Ct value was calibrated with normal gDNA.

7. Enzyme-linked immunosorbent assay (ELISA)

The lysate and conditioned media were prepared as following instructions. Cells (1×10^6) were seeded into a 6 well plate. After 24 hr, cells were washed with PBS and replaced into serum free media. The next day, lysate and conditioned media were harvested to measure the concentration of HGF. It was measured by using the Quantikine HGF Immunoassay from R&D Systems (Minneapolis, MN, USA) according to the manufacturer's instructions. The concentration of cell lysate was 20 ug and conditioned media was 5 ug.

8. Invasion assay

To measure invasiveness of SNU484 and IM95m, invasion assay was performed using 8-um pore size trans-well filters (BD Biosciences, San Jose, CA, USA). The inner sides of the trans-well were coated with 50 ng/ul Matrigel (Corning, Tewksbury, MA, USA) and 4 x 10^5 cells were plated into each trans-well filters, and then placed on a 24-well culture plate. After 24 hr incubation, non-migrated cells on the upper surface of membrane were washed with cotton swabs and migrated cells on the bottom side of the membrane were stained crystal violet for 5 min. Migrated cells were visualized under the microscope and quantified by counting the number of cells at each point. Then, the invaded cells were eluted with 33 % acetic acid and added to 96 well plates. The absorbance was read at a wavelength of 570 nm.

9. Knockdown of HGF expression using small interfering RNA

Twenty-four hours prior to transfection, various numbers of cells were seeded in culture medium without antibiotics and seeded in a plate. Transfection of siRNA was carried out with Lipofectamine RNAiMAX (Life Technologies, Carlsbad, CA, USA). Silencer Select Predesigned siRNA targeting HGF (cat No. s6529) and Silencer Select Negative Control were purchased from Life Technologies. siRNA targeting HGF mixed with Opti-MEM I Reduced Serum Medium (Life Technologies, Carlsbad, CA). This mixture was mixed with an equal volume of Lipofectamine RNAiMAX in Opti-MEM I Reduced Serum Medium. After 10 minutes of incubation, the final mixture was added to each well of the plate for a final siRNA concentration of 10 nM.

For siRNA induced viability assay, SNU484 and IM95m ($4x10^5$) were seeded into a 6 well plate. After 24 hr, HGF siRNA was added to a final concentration of 10 nM and incubated for 48 hr. Then, cells were trypsinized and seeded into a 96 well plate.

Afeter 24 hr, Sym015 was treated (4, 20, 100, 250, 500 nM) and viability assay was performed after 72 hr of incubation with Sym015.

For siRNA induced invasion assay, SNU484 and IM95m ($2x10^5$) cells were plated into each well of a 6 well plate. After 24 hr, siRNA was added to a final concentration of 10 nM and incubated for 48 hr. Then, cells were trypsinized and the invaded cells were analyzed by the same method mentioned above.

10. Statistical analysis

Student's t-tests and one-way ANOVA were conducted to analyze the findings of the in vitro assay using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). *p*-value of less than 0.05 was considered statistically significant.

III. RESULTS

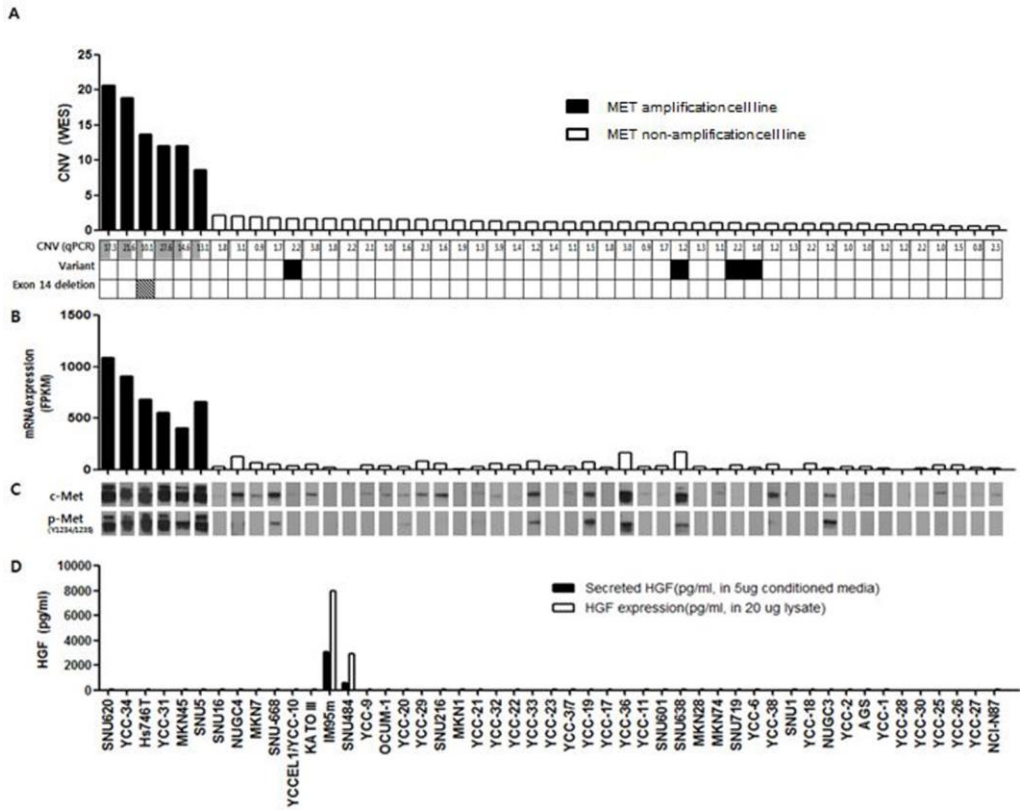

Figure 1. **MET and HGF profiling in 49 gastric cancer cell lines.** (A) MET copy number was analyzed by WES and confirmed by qPCR. Six cell lines were MET amplified (Black columns). Variants were detected in 4 cell lines. And exon 14 deletion was detected only 1 cell line, Hs746T. (B) mRNA expression was analyzed by RNA sequencing data. mRNA was overexpressed in 6 MET amplified cell lines. (C) c-Met and p-Met (Y1234/1235) expression was determined by western blot assay. The c-Met and p-Met were overexpressed in MET amplified cell lines. Also, YCC-36 and SNU638 were c-Met overexpressed cell lines. (D) Hepatocyte growth factor (HGF) in lysate and secreted HGF were measured by ELISA and 2 cell lines, IM95m and SNU484 were overexpressed.

To characterize MET status in 49 gastric cancer cell lines, we first identified the cell lines at the DNA level. 49 GC cell lines were listed in the order of MET copy number variation by WES data. Amplification of MET was analyzed using WES data and confirmed by qPCR. Amplification was observed in 6/49 (12.2 %) cell lines. Copy numbers of 6 amplified cell lines varied from 8.6 to 20.6 copies with median 13 copies; SNU620 (20.6 copies), YCC-34 (18.8 copies), Hs746T (13.6 copies), YCC-31(12.1 copies), MKN45 (12.1 copies), SNU5 (8.6 copies). Copy number of MET confirmed in 6 amplified cell lines with median 15.9 copies (10.1-27.6) by qPCR. Next, using WES data, we detected genetic variants of MET in 4/49 (8.2 %) cell lines including YCCEL1/YCC-10 (H58L), SNU638 (N375S), SNU719 (D153A) and YCC-6 (B1382). We also detected exon 14 deletion in Hs746T (1/49 (2 %)). MET amplification and variant were mutually exclusive except Hs746T (Figure 1A).

Then, we used RNA sequencing data to analyze mRNA expression of MET. The mRNA levels correlated with DNA amplification in the cell lines (R^2=0.7651, p<0.0001) (Figure 1B). In protein level, we measured c-Met and p-Met (Y1234/1235) expression. The MET amplified cell lines showed the c-Met and p-Met overexpression. Except of 6 MET amplified cell lines, 2 cell lines (YCC-36, SNU638) showed MET overexpression (Figure 1C). We measured the expression in lysate and conditioned media of HGF in 49 GC cell lines. HGF was overexpressed in lysate and conditioned media in 2/49 (4.1 %) cell lines, SNU484 and IM95m. Interestingly, these 2 cell lines were neither MET amplified nor overexpressed (Figure 1D).

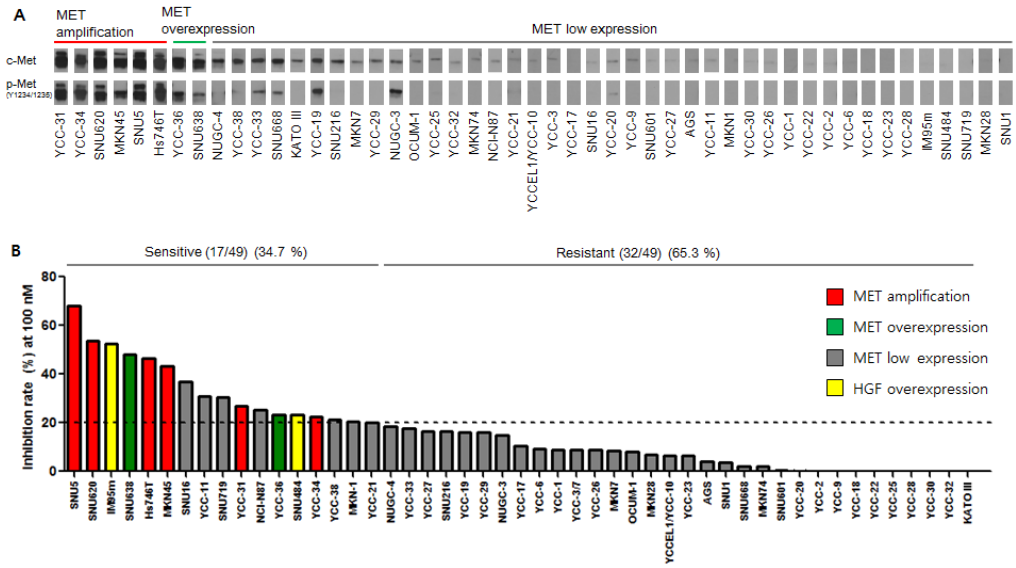

Figure 2. Sensitivity of Sym015 in 49 gastric cancer cell lines. (A) c-Met and p-Met expression in 49 GC cell lines listed in order of c-Met intensity. (B) Growth inhibition rate (%) of 49 GC cell line at Sym015 100 nM. Forty-nine GC cell lines were divided into 6 MET amplified (red columns), 2 HGF overexpressed (yellow columns), 2 c-MET overexpressed (green columns) and the rest of cell lines were MET negative because they were neither MET amplified nor overexpressed (grey columns) according to Figure 1.

Forty-nine GC cell lines were listed in order of c-Met intensity. We considered YCC-36 and SNU638 as c-MET overexpressed cell lines because their c-MET expressions were similar to that of MET amplified cell lines and they also overexpressed p-Met (Figure 2A). Sensitivity of Sym015 was analyzed by growth inhibition rate (%) at Sym015 100 nM. At a cutoff inhibition rate of 20 %, 17/49 (34.7 %) cell lines were sensitive and 32/49 (65.3 %) cell lines were resistant to Sym015[15]. Six MET amplified cell lines were all sensitive. Interestingly, HGF

overexpression cell lines (SNU484, IM95m) were all sensitive to Sym015. SNU484 and IM95m were not MET amplified or overexpressed. Regardless of the status of MET, HGF overexpression might predict the sensitivity of MET inhibitor (Figure 2B). Therefore, we confirmed the importance of HGF overexpression by inhibition of it.

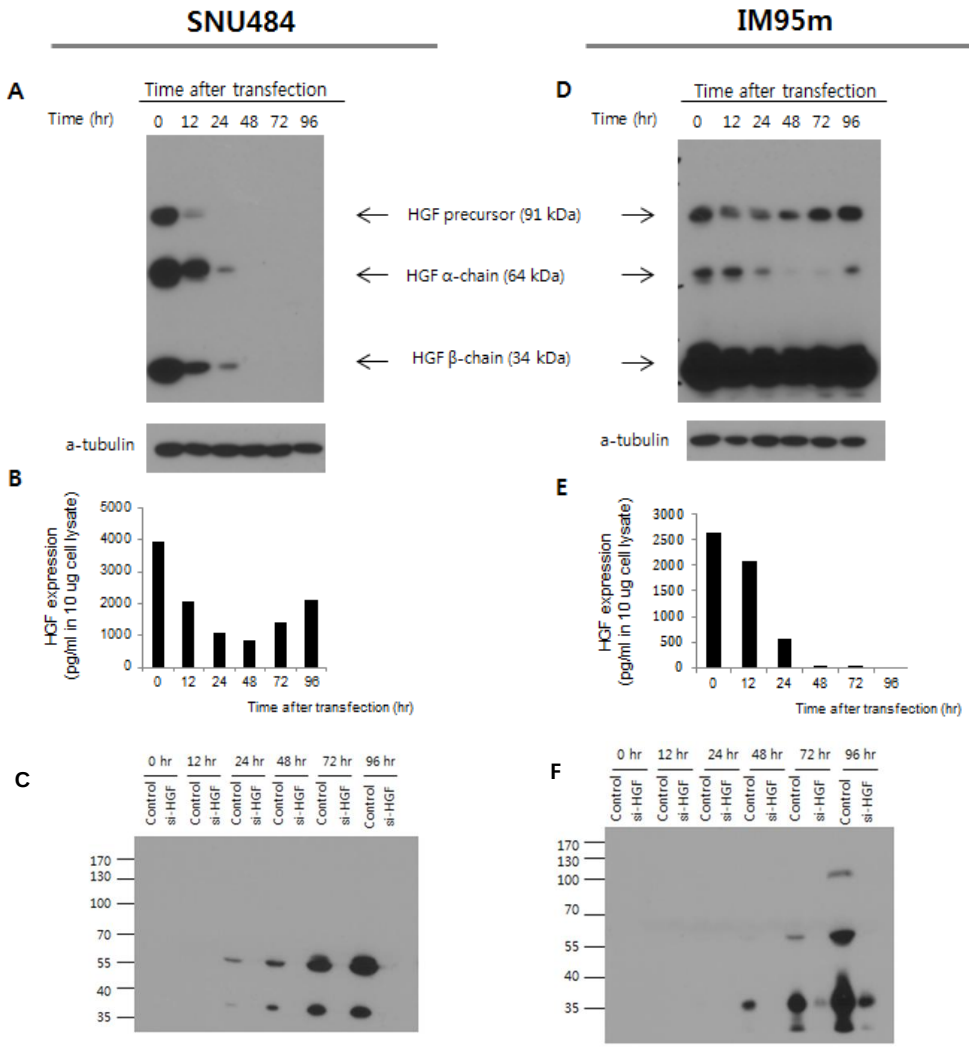

Figure 3. Knockdown of HGF expression in HGF overexpression cell lines. (A) HGF expression was measured in cell lysate by western blot assay. In SNU484, HGF expression in lysate was blocked after 48 hr of treatment of siRNA against HGF (siHGF). (B) Knockdown of HGF expression in lysate was quantified by ELISA. The expression of HGF in lysate was mostly reduced after 48 hr of treatment likewise Figure 3A. (C) Secreted HGF in conditioned media was

measured by western blot assay. Secreted HGF was reduced by siRNA in comparison to control of each time. (D) In IM95m, HGF expression was measured in cell lysate by western blot assay. HGF expression in lyastae blocked after 48 hr of treatment. (E) Knockdown of HGF expression in lysate was quantified by ELISA. HGF expression in lysate blocked after 48 hr of treatment likewise Figure 3D. (F) Secreted HGF was reduced by siRNA in comparison to control each time.

To elucidate the role of HGF in SNU484 and IM95m, we conducted knockdown of HGF by siRNA. The protein in lysate and secreted protein was extracted after 12,24,48,72 and 96 hr of incubation with HGF siRNA. HGF is secreted as a precursor form (pro-HGF). In certain condition, pro-HGF is converted to active form that has alpha and beta chains[16]. We detected pro-, alpha- and beta-form of HGF in western blot analysis. In SNU484, HGF expression in lysate was reduced after 48 hr after treatment (Figure 3A). Likewise, total HGF expression in lysate was quantified by ELISA and confirmed the same results. The HGF expression was inhibited at 48 hr and maintained the reduced expression at 72 and 96 hr (Figure 3B). Also, secreted HGF expression was inhibited compared to the same time point of control (Figure 3C). In IM95m, HGF expression in lysate blocked after 48 hr of treatment (Figure 3D). Total HGF expression in lysate quantified by ELISA and showed that the HGF expression was reduced mostly after 48 hr of treatment siRNA compared to control. However, HGF expression in lysate was recovered as time goes on (Figure 3E). Secreted HGF expression was blocked compared to the same time point of control (Figure 3F). As a result of HGF knockdown, Sym015 was treated after 48 hr of siRNA treatment.

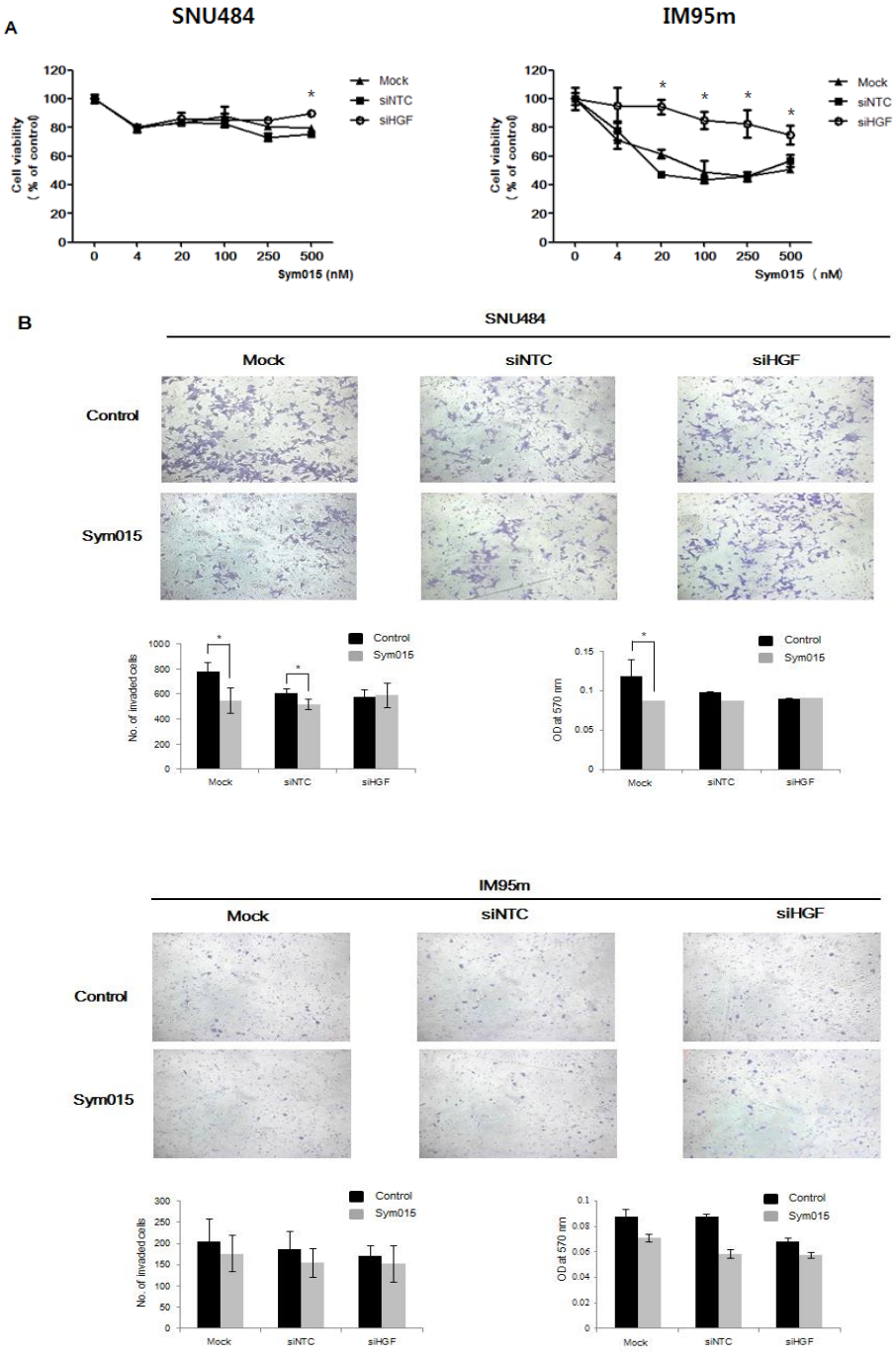

Figure 4. HGF knockdown induced influence of sensitivity and invasiveness with treatment of Sym015. (A) The influence of sensitivity to Sym015 according to HGF knockdown in SNU484 and IM95m. Although there was no effect in sensitivity to Sym015 by HGF knockdown in SNU484, the sensitivity of Sym015 was reduced in IM95m by HGF knockdown. * $p<0.05$ compared to the mock or si-negative control (siNTC). (B) The influence of invasiveness to Sym015 according to HGF knockdown. Although there was no effect in invasiveness in IM95m, the ability to reduce the invasiveness of Sym015 was decreased in SNU484.

To identify whether a knockdown of HGF could influence the sensitivity of Sym015, we repressed the expression of HGF and treated Sym015. In SNU484, there was no difference of sensitivity compared to the mock or siNTC groups. However there was significant difference in IM95m. Cell viability (%) was increased, which means the sensitivity of Sym015 was decreased in HGF knockdown group compared to mock and siNTC groups ($p<0.05$). These results indicated that inhibition of HGF influence the sensitivity of Sym015 in IM95m (Figure 4A).

To examine whether a knockdown of HGF could influence the invasiveness of Sym015, SNU484 and IM95m were transfected with siRNA and seeded into trans-well with Sym015. In SNU484, the number of invaded cells of mock with Sym015 treated group was reduced compared to control group and the siNTC group showed similar pattern. However, there was no reduction of the number of invaded cells with Sym015 treated group compared to Sym015 non-treated group when the expression of HGF was repressed. Unlike SNU484, there was no influence of decreased HGF to invasiveness of Sym015 in IM95m. The number of invaded cells was confirmed by elution the stained crystal violet. These results indicated that inhibition of HGF influence the invasiveness of SNU484 when treated with Sym015 (Figure 4B).

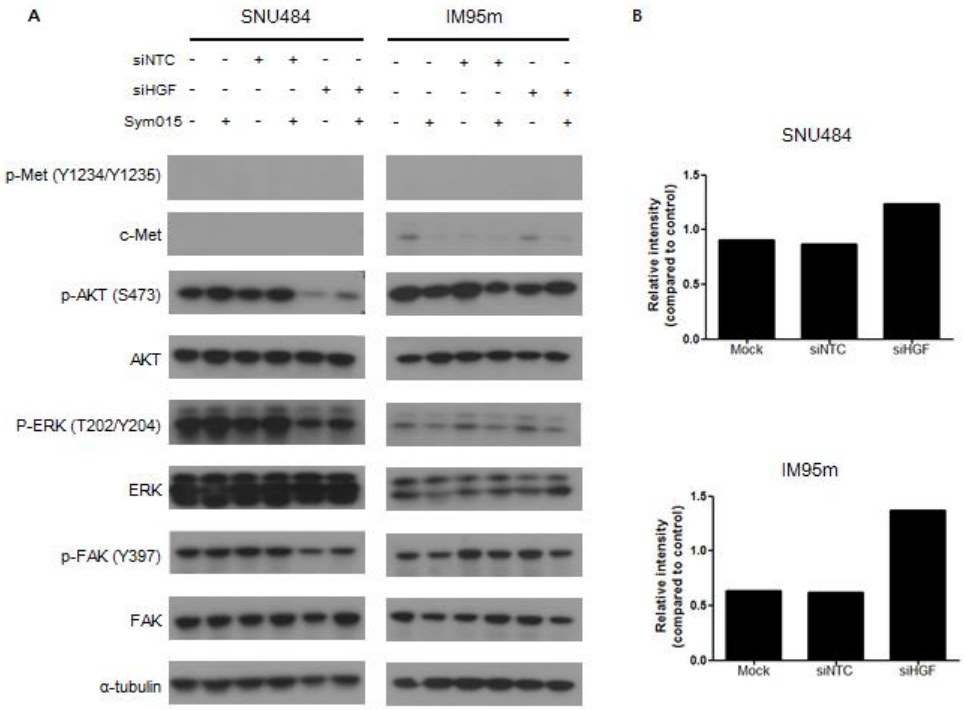

Figure 5. HGF knockdown induced influence of downstream signaling molecules of MET pathway with treatment of Sym015. (A) Downstream signaling molecule was determined by western blot. (B) When compared to control (intensity=1), the intensity of p-AKT/AKT was analyzed by image J. The activation of AKT was increased when Sym015 was treated in HGF knockdown group.

To investigate the pathway of phenotypic change, we examined the change of downstream signaling pathways in each group, and the activation status of downstream molecules was analyzed by measuring the intensity of phosphorylation-form and total-form of molecules. Three major pathway molecules (AKT, ERK and FAK) were analyzed that related to cell proliferation, survival, invasion and migration. In SNU484, we observed that inhibition of HGF reduced the ability of

reducing invasiveness of Sym015 (Figure 4B). However, there was no significant change of AKT, ERK and FAK pathway (Figure 5A). In IM95m, we observed that inhibition of HGF reduced the ability of reducing cell viability of Sym015 (Figure 4A). This could be explained by activation of AKT pathway. The intensity of p-AKT/AKT was increased despite of Sym015 treatment in HGF knockdown condition, in SNU484 and IM95m. Relative intensity was defined as the intensity of Sym015 treatment group normalized to control group. In mock and siNTC group, the intensity of p-AKT/AKT was decreased when they were treated with Sym015, which means the AKT pathway was inhibited. By activating the AKT pathway, IM95m showed relatively resistance to Sym015 when HGF was repressed compared to other mock and siNTC groups (Figure 5 A,B).

IV. DISCUSSION

Targeting receptor tyrosine kinase (RTK) is actively developed for cancer therapies including HER2, MET, EGFR and so on. HER2, a member of EGFR family, is used as a therapeutic target of trastuzumab in gastric cancer patients. However, the incidence of HER2 positive patients is approximately 10 % in gastric cancer. To treat more gastric cancer patients, more molecular targets are needed to be explored.

MET is amplified 4-5 % in gastric cancer patients and associated with cancer proliferation, survival, invasion and metastasis. Many MET targeted agents are being developed and MET amplification considered as predictive marker of MET inhibitor. However, it was difficult to figure out MET-positive biomarkers for MET inhibitor in patients and no MET inhibitors have succeeded in clinical trials. Without considering only MET status as a predictive marker, we suggested that hepatocyte growth factor (HGF) overexpression, a ligand of MET, might be a potential predictive marker of MET inhibitor.

Mature HGF is comprised of two-chains, 69 kDa of α-chain and 34 kDa of β-chain. Two chains are disulfied-linked. The HGF α-chain binds to MET with high affinity whereas the binding of α-chain does not activate MET. In contrast, HGF β-chain, linked to the c-terminal domain of the HGF α-chain, binds to MET with lower affinity than the α-chain, but is mandatory for ligand-induced MET activation[16,17]. HGF β-chain binds to sema domain 2,3 of MET and Sym015 binds to same position. Therefore, inhibition of HGF β-chain is more important than α-chain in HGF knockdown induced phenotype change of Sym015. Our data showed that β-chain of HGF was inhibited at each time point in SNU484, but that of IM95m was less inhibited than SNU484. Although we expected SNU484 would be affected by knockdown of HGF and shown more phenotype change than IM95m, the result did not match as we expected.

First, we profiled MET and HGF status in 49 GC cell lines and screened Sym015 sensitivity in 49 gastric cancer cell lines. As expected, MET amplified cell lines were sensitive to Sym015. However, the sensitivity of MET overexpression cell lines was varied. Interestingly, HGF overexpression cell lines, SNU484 and IM95m, were sensitive to Sym015 and we investigated whether HGF overexpression might be a predictive marker of MET inhibitor. We inhibited the HGF expression and the influences of major phenotype, cell viability and invasion[18] by Sym015 were conducted. Interestingly, SNU484 was highly invasive cells and IM95m was highly proliferative cells through MET pathway. These two cell lines showed different phenotypes in the baseline. The HGF knockdown induced influence of sensitivity to Sym015 was shown in IM95m and the influence of invasiveness to Sym015 was shown in SNU484. The downstream pathway molecules were analyzed to find out the pathway of change of phenotypes. The AKT pathway was activated when the Sym015 was treated in HGF knockdown group in IM95m. This explained the result that HGF knockdown induced change of sensitivity to Sym015 in IM95m. However, there was no activation of downstream molecules in SNU484. Further studies are needed that other downstream pathway might affect this change of phenotype.

In gastric cancer patients, the positive rate (%) of HGF expression varied from 34.7 to 87.5 in tissue and serum.[8,19,20] There is no elucidate cut off value of HGF expression and further exploration is needed to find out the criteria of HGF overexpression for using it as a predictive marker of MET inhibitors.

Consequently, we suggest that HGF overexpression might be a potential predictive marker of MET inhibitor in GC.

V. CONCLUSION

Targeting MET has received considerable attention for cancer therapy. As already known, MET amplification showed sensitivity to MET inhibitor. Except MET amplification, we explored another predictive marker for selecting more GC patients that would be sensitive to MET inhibitor. In this study, we suggested that HGF overexpression might be the predictive marker of MET inhibitor.

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ABSTRACT (IN KOREAN)

위암에서 MET inhibitor의 potential predictive marker로써 HGF overexpression의 영향

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한국에서 위암은 발생률 2위의 흔히 나타나는 암 종이고 전 세계 암 사망의 수위를 차지하는 중요한 암이다. 최근 위암을 포함한 다양한 암 종에서 receptor tyrosine kinase (RTK)를 표적으로 하는 치료제들이 개발되고 있으며 임상시험에서도 효과를 보이고 있다. 하지만, 위암에서 임상적으로 효과가 입증 된 표적치료제는 HER2 표적치료제인 trastuzumab 뿐이라 새로운 표적의 발굴이 필요하다. 최근, 암세포의 survival, growth, invasion, metastasis등에 중요한 역할을 한다고 알려져 있는 MET을 표적으로 하는 치료제들이 개발되고 있고 전임상시험에서 효과를 보이고 있지만 최종적으로 임상시험을 성공한 치료제는 없다. 임상시험에서 가장 많이 연구 된 MET 표적치료제 중 하나인 onartuzumab 또한 정확한 biomarker의 기준을 잡아 환자를 선별하는데 어려움을 겪어 임상 3기에서 실패했다. MET 표적치료제의 임상에서의 성공을 위해 새로운 biomarker의 탐색이 필요한 시점이다. 본 실험에서는 현재 개발되고 있는 MET 표적치료제 중 하나인 Sym015를 통해 MET 표적치료제의 효과를 예측할 수 있는 marker를

찾아보고자 한다. 우선 49개 위암 세포주에서 MET의 status와 MET의 ligand인 HGF의 expression을 profiling했고 Sym015 sensitivity와 관련 지어 효과를 나타내는 factor들을 찾아보았다. 그 결과, HGF overexpression 된 세포주에서 Sym015가 sensitive한 것을 알 수 있었고 이를 증명하기 위해, HGF overexpression 세포주에서 HGF의 발현을 inhibition시킨 뒤 cell viability assay와 invasion assay를 수행하여 phenotype 및 관련 molecule의 변화를 확인했다. HGF를 knockdown 시킴으로써 Sym015에 의한 cell viability 및 invasiveness 억제 효과가 감소함을 확인하였다. 이로써 HGF overexpression이 Sym015의 효과를 예측할 수 있는 predictive marker로 가능성이 있음을 제시하고자 한다.

핵심되는 말: 위암, HGF, MET inhibitor, predictive marker