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Resistance mechanism to Trastuzumab in HER2-positive cancer cells and its overcome by Src inhibition

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서울대학교 대학원

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이 논문을 의학석사 학위논문으로 제출함

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Resistance mechanism to Trastuzumab in HER2-positive cancer cells and its overcome by Src inhibition

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Approved by Thesis Committee:

Professor _____ Chairman

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Abstract

Resistance mechanism to Trastuzumab in HER2-positive cancer cells and its overcome by Src inhibition

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Background: Trastuzumab in combination with chemotherapy is a standard of care for patients with HER2-positive breast and gastric cancer. Resistance mechanism to trastuzumab, anti-HER2 therapy, includes multiple pathways. Among them, Src is not well known especially in HER2-positive gastric and

biliary tract cancers. We investigated the role of Src involved in trastuzumab resistance and explored the potential of Src inhibition as a trastuzumab resistance overcoming strategy.

Methods: Four trastuzumab-resistant (HR) cells (SNU216HR, N87HR, SNU2670HR, SNU2773HR) were established from 2 HER2-amplified gastric cancer cells (SNU216, NCI-N87) and 2 HER2-amplified biliary tract cancer cells (SNU2670, SNU2773). For Src inhibition, bosutinib was used. MTT assay, colony formation assay, cell cycle analysis by FACS Calibur flow cytometer, and cell migration assay were done. Animal experiments were conducted to test anti-tumor effect of bosutinib using SNU2670 and SNU2670HR xenograft models.

Results: SNU2670HR/NCI-N87HR cells showed pSrc activation, in contrast, SNU216HR/SNU2773HR cells exhibited decreased pSrc expression. In these pSrc decreased HR cells, pFAK was elevated. Bosutinib downregulated Src-FAK signaling more obviously in Src activated HR cells than parental cells. In pSrc decreased HR cells, bosutinib reduced Src-dependent FAK phosphorylation to affect cell fate. Bosutinib inhibited the growth of both parental cells and HR cells, and induced apoptosis and G1 arrest in HR cells. Bosutinib suppressed HR cell migration more effectively compared with parental cells. Bosutinib exhibited potent tumor growth inhibition in both SNU2670 and SNU2670HR xenograft models and more significantly suppressed tumor growth in HR models.

Conclusion: Src activation may contribute to trastuzumab resistance in part in HER2-positive gastric and biliary tract cancer cells. Targeting Src might be a candidate strategy to overcome trastuzumab resistance in HER2-positive cancers.

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Introduction

The human epidermal growth factor receptor 2(ERBB2) is overexpressed or amplified in approximately 15-20% of breast cancer and 10-15% of gastric cancer and 10-20% of biliary tract cancer [1-3]. As a member of human epidermal growth factor receptor (HER) family, amplification of the HER2 gene leads to HER2 homodimerization or heterodimerization with other receptor tyrosine kinase (RTK) members [4]. As a result, the multiple downstream signal pathways are activated to regulate tumor cell proliferation, survival and cell cycle progression**.**

Trastuzumab (Herceptin) is a humanized monoclonal antibody that interferes with the HER2 receptor [5]. Currently, trastuzumab plus chemotherapy is a standard of care in HER2-positive breast and gastric cancers. However, the resistance to anti-HER2 therapy is eventually developed as acquired resistance or primary resistance. Many researchers demonstrated that trastuzumab resistance mechanisms, but none of them has pragmatic implication in clinical practice.

Src is a proto-oncogene and coordinates multiple signaling pathways known to be involved in tumor progression [6]. High levels of pSrc expression was found in various cancers, like colon, liver, lung, breast, and pancreas [7]. As is well known, Src can be activated by numerous factors, for example, receptor tyrosine kinases, G protein coupled receptors, adhesion receptors, cytokine receptors [6]. Src has two major phosphorylation sites, tyr416 and tyr527, while Src is in activating status, tyr416 is phosphorylated and tyr527 is dephosphorylated [8]. Once Src is activated, it can upregulate multiple

downstream molecules, such as AKT, ERK or STAT3, leading to increase cell proliferation and cell survival. Extensive preclinical evidence indicates that Src activation facilitates cell motility and Src also participates in stimulating angiogenesis under hypoxic conditions [11]. Moreover, Src interacts with focal adhesion kinase (FAK) to accelerate cell migration, cell invasion, cell cycle progression [8-10]. Promoting metastasis is one of the most principal roles of Src activation [12], indeed, in many cancers increased pSrc directly influences tumor growth and metastasis. Recently Src inhibitor has been used in clinical trials for metastatic breast cancer [13]. Additionally, Src mediates E-cadherin or integrin, which may cause activation of various signaling networks [14-15].

So far, Src has been considered as a promising molecular target for anticancer therapy, moreover several studies have revealed that Src contributes to trastuzumab resistance in HER2-positive breast cancer [8, 11, 16-19]. Among these research, the mechanism of Src activation can be explained as follows. First, PTEN mutation directly induces Src activation [8]. Next, TGF-β integrates HER2 receptor and integrin, leading to Src activation [20]. Furthermore, CUB domain containing protein 1(CDCP1) interacts with HER2, promoting Src activation [19]. In breast cancer, except trastuzumab resistance, Src activation has a crucial role in other targeting therapeutic resistance including lapatinib [21], cetuximab [22]. However, Src activation has not been well studied in HER2-positive gastric and biliary tract cancer. In terms of the

significant functions of Src, it is not surprising that targeting Src would improve response to trastuzumab resistant patients.

Bosutinib (SKI-606) is a Src family kinases inhibitor (including Src, Lyn, Hck) and ATP-competitive Bcr-Abl tyrosine kinase inhibitor [23]. Following studies revealed that bosutinib has excellent effect on reducing tumor growth, invasion, metastasis [24-26]. In this study, we use bosutinib to explore the effect of antitumor survival.

In order to ascertain whether Src plays an important role in trastuzumab-resistant HER2 positive cancers, we established four kinds of trastuzumab-resistant HER2-positive cancer cells and used bosutinib to inhibit Src. In this study, our purpose was to uncover role of Src in trastuzumab-resistant cells and further evaluate the effect of bosutinib in overcoming trastuzumab resistance in HER2-positive cancer cells.

Materials and Methods

1. Cell lines and reagents

We established 2 kinds of patient-derived HER2-amplified biliary tract cancer cell lines, SNU2670 and SNU2773. SNU216, HER2-amplified gastric cancer cell line, was purchased from Korean Cell Line Bank. NCI-N87, another HER2-amplified gastric cancer cell line, was purchased from American Type Culture Collection. All of them were cultured in RPMI-1640 media (Welgene Inc. Daegu, Korea) containing 10% FBS and 10ug/ml gentamicin at 37℃ in a 5% CO2 atmosphere. Trastuzumab was purchased from Roche (Korea) and bosutinib was provided by Pfizer Inc. (New York, NY, USA)

2. Generation of trastuzumab resistant cell lines

Trastuzumab-resistant (HR) cells were established using four HER2-amplified cells. SNU216HR, NCI-N87HR, SNU2670HR, SNU2773HR cells were derived from original cells and maintained in the condition of 20ug/ml or 50ug/ml of trastuzumab. After approximately one year culture, HR cells were defined.

3. Cell growth inhibition assay

Cells were seeded in 96-well plates at density of $(0.8{\sim}8) \times 10^3$ cells per well and incubated overnight at 37℃. Cells were exposed to increasing concentrations of bosutinib alone or combination with trastuzumab for 72 hours. Then, 50ul of 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolim bromide solution(Sigma Aldrich) was added to each well and incubated at 37℃. Four hours later, the media was removed and 150uL of DMSO was added. After 5 minutes, the absorbance of each well was measured at 540nm. The experiments

were done in triplicates.

4. Colony formation assay

Cells ($0.5 \sim 4 \times 10^3$) were seeded in 6-well plates and exposed in different concentrations of trastuzumab or bosutinib. After 10~16 days, colonies were stained with coomassie blue for 2 hours and counted by program (Gel doc). Each experiment was repeated three times.

5. Western blot

Cells ($3 \sim 8 \times 10^5$) were seeded in 100mm dishes and treated with trastuzumab (10μg/ml), or bosutinib (0.01, 0.1, 1 μM) or trastuzumab (10 μg/ml) plus bosutinib (0.01, 0.1, 1 μM) for 24 hours, then cells were harvested and lysed. The following primary antibodies were purchased from Cell Signaling Technology (Beverley, MA, USA), including: P-EGFR(Tyr1068), EGFR, P-HER2(Tyr1248), HER2, P-HER3(Tyr1289), HER3, P-Src(Tyr416), Src, P-AKT(Ser473), AKT, P-FAK(Tyr925), FAK, P-STAT3(Tyr705), STAT3, P-ERK, ERK. β-Actin was purchased from Sigma Aldrich (St. Louis, MO, USA).

6. Cell cycle analysis

Cells ($0.5 \sim 2.5 \times 10^5$) were seeded in 60mm dishes and treated with different concentrations of bosutinib (0.01, 0.1, 1 μM) for 24 hours, 48 hours, and 72 hours, then, cells were harvested and fixed with 70% ethanol at -20℃. After two days, 7ul of RNAse (20 μg/ml) was added for 10 minutes at 37℃ and the samples stained with 13ul of propidium iodide. Results were detected by FACS caliber flow cytometer. Each experiment was repeated three times.

7. Migration assay

Cells ($3{\sim}8\,\mathrm{X}\,10^5$) were seeded in 6-well plates and incubated at 37℃. After 24 hours, cells were scratched with 200ul pipet yellow tips and further incubated in different doses of bosutinib (0.01, 0.1, 1, 10 μM) or trastuzumab (0.01, 0.1, 1, 10, 100 μg/ml) or combination. The images were captured using image J program at 0 hours, 6 hours, 24 hours, 48 hours and 72 hours. All experiments were done triplicates**.**

8. In vivo study

Animal experiments were performed at the Biomedical Center for Animal Resource Development of Seoul National University, Seoul, Korea, according to the institutional guidelines with prior approval from the institutional animal care and use committee. Four-week-old female athymic nude mice were purchased from Central Lab Animal Inc. (Seoul, South Korea) and each mouse was injected subcutaneously with $2\,\mathrm{X}\,10^7$ SNU2670 or SNU2670HR cells. When tumor volume reached 200mm^3, mice were divided into four groups (4 mice per group). Bosutinib was administered orally once a day at a dose of 150mg/kg for three weeks, the control groups were treated with vehicle containing 0.5% methylcellulose and 0.4% Tween 80 through oral gavage. The body weights and tumor size were measured every other day and the volume was calculated utilizing the formula: Tumor volume = [(width)^2 X height] /2

Results

1. Characterization of trastuzumab resistant (HR) cells.

To explore the unclear resistant mechanism to trastuzumab in HER2-positive cancers, we generated four trastuzumab resistant (HR) cell lines (SNU216HR, NCI-N87HR, SNU2670HR, SNU2773HR). HR cell lines showed the reduced sensitivity to trastuzumab treatment than the parental cell lines (Fig. 1A). To exam the differences between parental and HR cell lines, we observed the basal expression levels of molecules involved in signal transduction (Fig. 1B). We found that four HR cell lines could be divided into two groups, according to the Src activation level. The phosphorylation of Src at Tyr416 was reduced in SNU216HR and SNU2773HR cell lines compared with their parental cells. On the contrary, pSrc at Tyr416 was activated in SNU2670HR and NCI-N87HR cell lines. But no change in the levels of total Src was detected in all HR cell lines. In addition, the phosphorylation levels of HER2/ HER3/ AKT/ FAK were significantly enhanced in SNU216HR and SNU2773HR. The levels of pHER3 and pAKT were downregulated in both of SNU2670HR and NCI-N87HR cells, however, the phosphorylation of FAK and STAT3 were increased in these cells. In breast cancer, HR cells exhibited HER2 reduction versus parental cells [8], in our study, we observed only two HR cells (SNU2773HR/SNU2670HR) showed decreased HER2 levels. We also found EGFR was prominent upregulated in four HR cells than parental cells. The above signal changes may be modulated by Src. Src might be the key player to acquire trastuzumab resistance in our study.
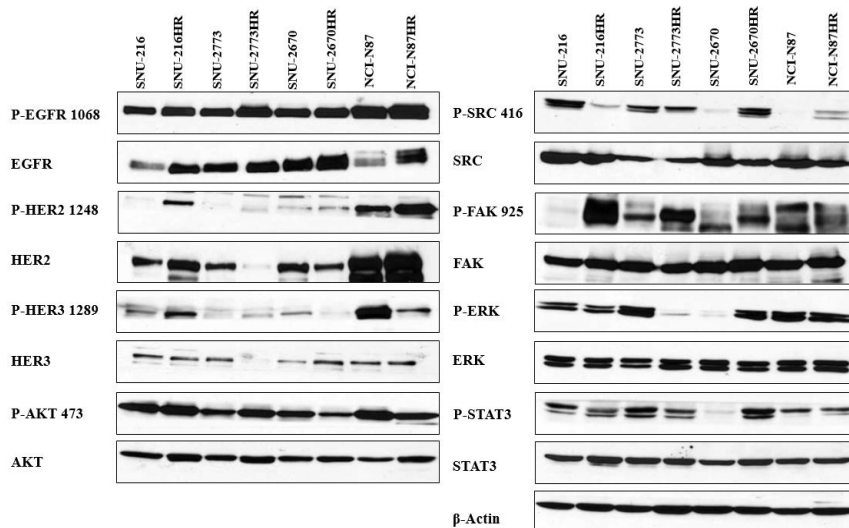
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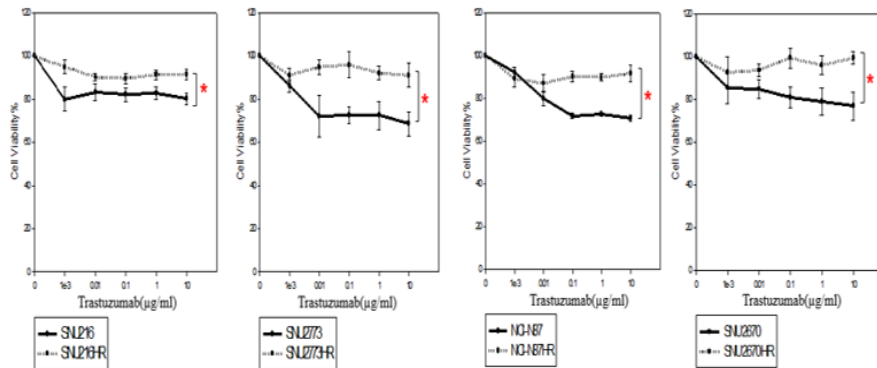
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Figure 1. **Characterization of trastuzumab-resistant (HR) cells**

(A) Both of parental and HR cells were incubated with increasing concentrations of trastuzumab for 72 hours. Cell viability was measured by MTT Assay. * P <0.05

(B) Basal expression levels displayed major signaling changes between parental and HR cells.

2. Bosutinib inhibits the growth of both parental and HR cells

To evaluate the anti-proliferation effect of bosutinib in HER2 amplified cancer cells, we measured cell viabilities following treatment of bosutinib for 72 hours by MTT assay. We observed all 8 kinds of cells were inhibited by the treatment of bosutinib alone, and SNU2670HR was most sensitive one to bosutinib (Fig 2A). We calculated IC50 of eight cells after treatment with bosutinib alone (Table 1). Src activated NCI-N87HR and SNU2670HR were more sensitive to bosutinib compared with parental cells, but SNU216HR was less inhibited by bosutinib with respect to parental cell and there was no significant difference between SNU2773 and SNU2773HR.

We then utilized Colony Formation Assay to identify the long-term anti-proliferative effects of bosutinib (Fig 2B). Our data indicated that six kinds of cells showed growth inhibition by bosutinib (Fig 2B and Table 1). More specifically, SNU216HR and NCI-N87HR cells were more sensitive to bosutinib than the parental cells. Significant inhibitory effects of bosutinib were observed in SNU2773 / SNU2773HR / SNU2670 / SNU2670HR cells.

These data suggested that bosutinib had anti-proliferative effects in both parental and HR cells, especially in Src activated cells such as SNU2670HR and NCI-N87HR.

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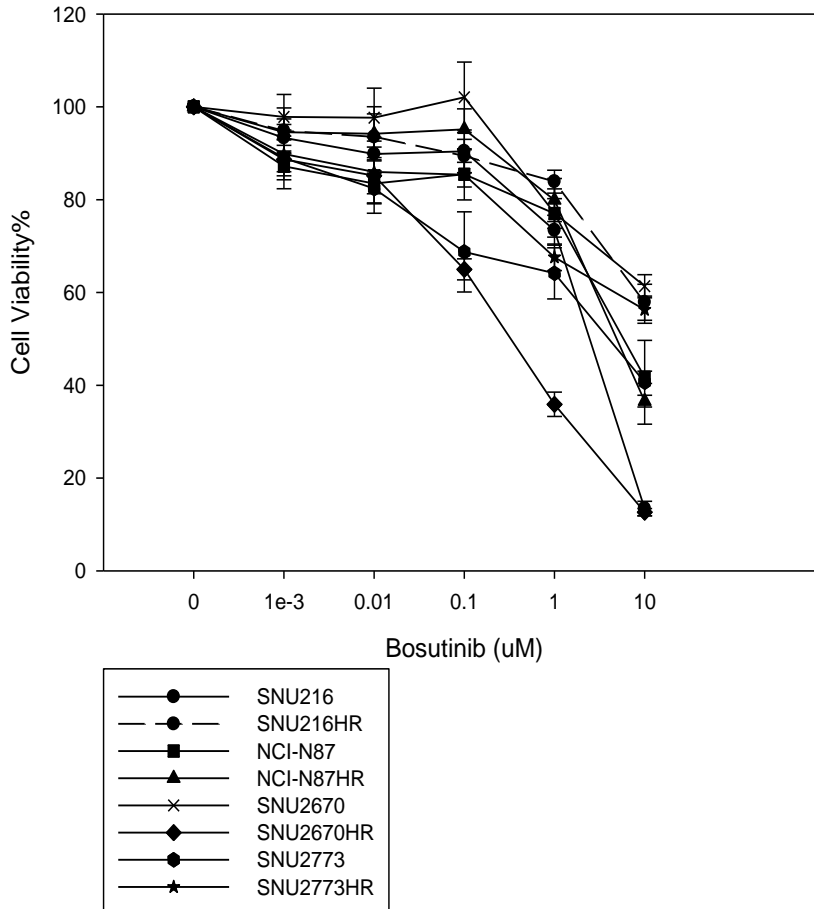
Figure 2. Anti-proliferation effect of bosutinib in parental cells and HR cells.

(A) Eight cells were exposed at different concentrations of bosutinib (0.001, 0.01, 0.1, 1, 10μM) for 72 hours. Error bars represent three independent experiments.

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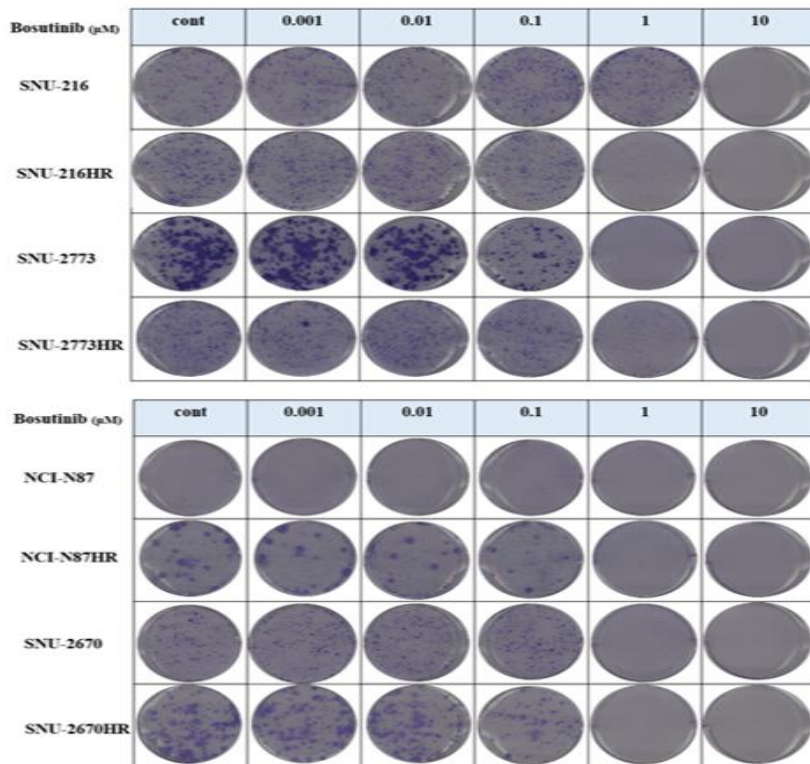
Figure 2. Anti-proliferation effect of bosutinib in parental cells and HR cells.

(B) Colony Formation Assay. All the cells were treated with various doses of bosutinib (0.001, 0.01, 0.1, 1 10 μM) for 10-16 days.

Cell lines	IC50 (μM)	
	MTT	CFA
SNU216	2.6	>10
SNU216HR	>10	0.69
SNU2773	>10	0.05
SNU2773HR	>10	0.31
NCI-N87	>10	>10
NCI-N87HR	4.7	1.1
SNU2670	>10	0.26
SNU2670HR	0.47	0.15

Table 1. MTT Assay and Colony Formation Assay IC50 of
bosutinib treatment

3. Src contributes to trastuzumab resistance and Src inhibitor downregulates the signaling pathway

To validate the effect of bosutinib on HER2 signaling pathway, we detected Src related molecules after treatment with bosutinib for 24 hours. Practically, bosutinib inhibited all signals in both parental cells and HR cells (Fig 3). EGFR has been reported to activate Src in HER2 amplified cancer cells. Phosphorylation of EGFR was increased in all HR cells versus parental cells, but elevated pSrc was observed in NCI-N87HR and SNU2670HR cells not in SNU216HR and SNU2773HR cells. Bosutinib inhibited EGFR and HER2 phosphorylation more effectively in NCI-N87HR and SNU2670HR cells. pAKT, pERK, and pSTAT3 were also more inhibited in NCI-N87HR and SNU2670HR cells by bosutinib with respect to parental cells. In SNU216HR and SNU2773HR cells, although pAKT and pERK were less inhibited by bosutinib compared to parental cells, Src-dependent FAK phosphorylation which involved in cell cycle progression and migration was significantly downregulated.

These results support the important role of Src activation and it may contribute to trastuzumab resistance.
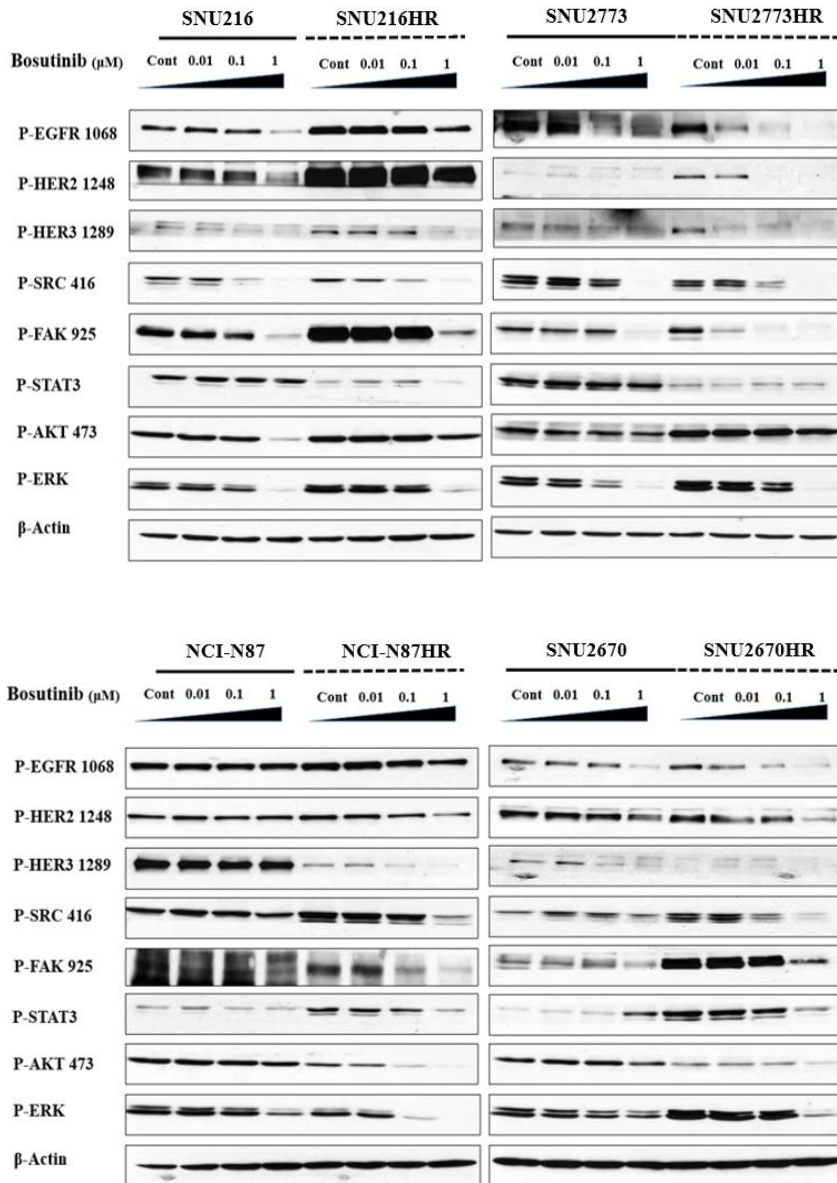
Figure 3. Src inhibitor downregulates the related signaling pathway.

Both parental and acquired HR cells were treated with bosutinib (0.01, 0.1, 1 μM) for 24 hours. Src related signaling pathway molecules were exhibited.

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4. Bosutinib induces G1 arrest and apoptosis.

To further evaluate the effect of bosutinib on cell cycle progression, eight cells were treated with bosutinib for 24, 48 and 72 hours. Here, we displayed 48 hours or 72 hours. As shown in Figure 4, five cells exhibited G1 arrest by bosutinib at 1μM except SNU2773, NCI-N87 and NCI-N87HR cells (Fig 4). In SNU2773 cell bosutinib induced G1 arrest at 24 hours (data not shown). In contrast, trastuzumab did not induce G1 arrest or apoptosis up to the concentration of 10μg/ml (data not shown). Following treatment with bosutinib for 48 hours or 72 hours, sub-G1 increased more obviously in HR cells (SNU216HR/SNU2773HR/SNU2670HR) than parental cells except NCI-N87HR cells.
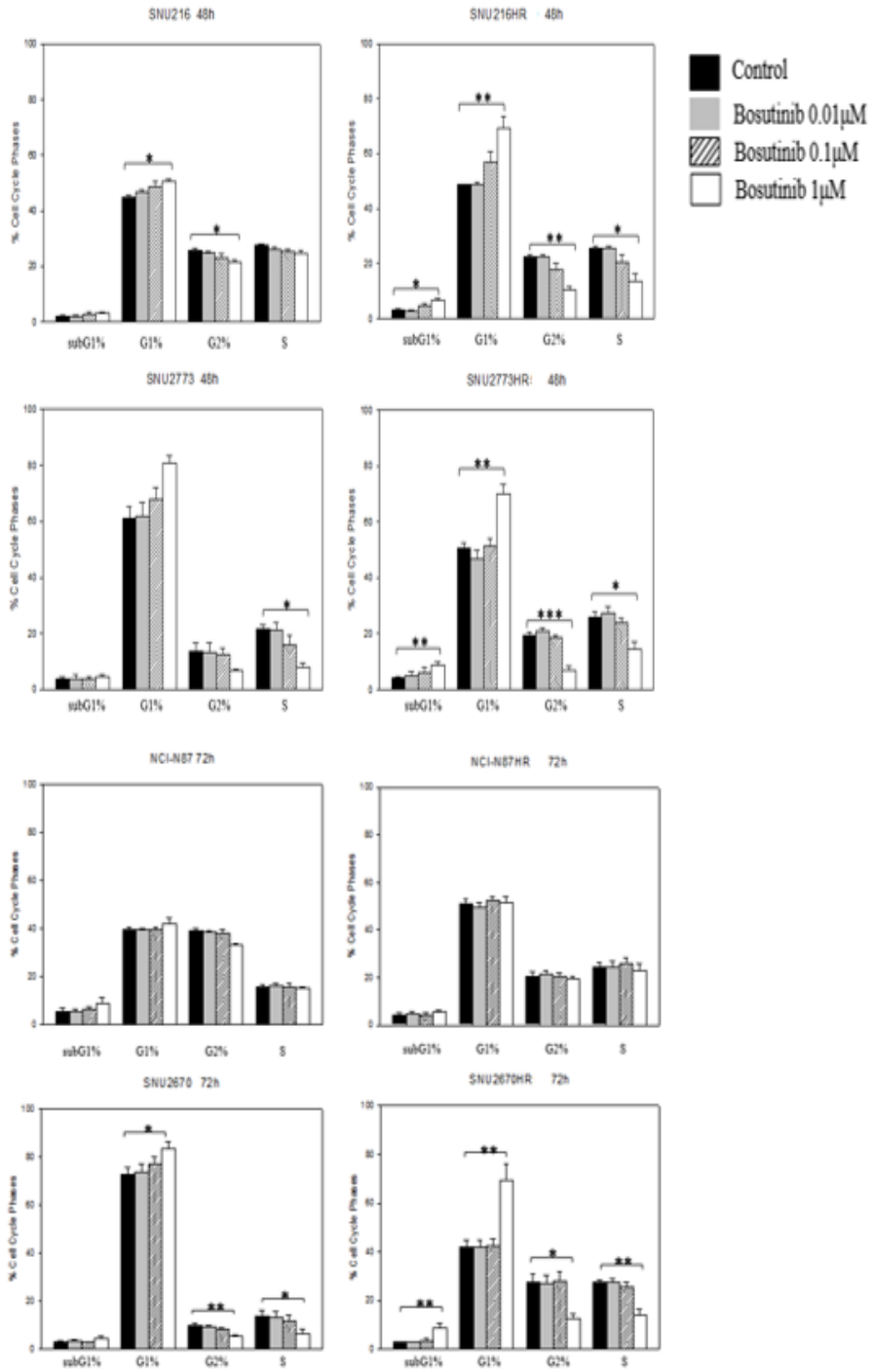
Figure 4. Bosutinib induces G1 arrest and enhances apoptosis.

After treatment with bosutinib (0.01, 0.1, 1 μM) for 48 hours or 72 hours, the cell cycle analysis was conducted. Error bars represent three independent experiments. * P value<0.05, ** P value<0.01, *** p value< 0.001.

5. Bosutinib inhibits HR cell migration more effectively compared with parental cells.

Src plays an important role in cell migration. To evaluate the effect of bosutinib on cell migration, Migration Assay was conducted. Eight cells were treated with bosutinib for 6 hours, 24 hours, 48 hours and 72 hours. Here, the images were captured after treatment with bosutinib alone for 48 hours (Fig 5). We found that HR cells showed faster migration than parental cells and bosutinib showed potent anti-migratory effect in both of parental and HR cells. Migration of all cells except NCI-N87 was inhibited by bosutinib (1 µM) significantly (Fig 5). Our results suggested that bosutinib inhibited cell migration very effectively and it worked more obviously in HR cells.
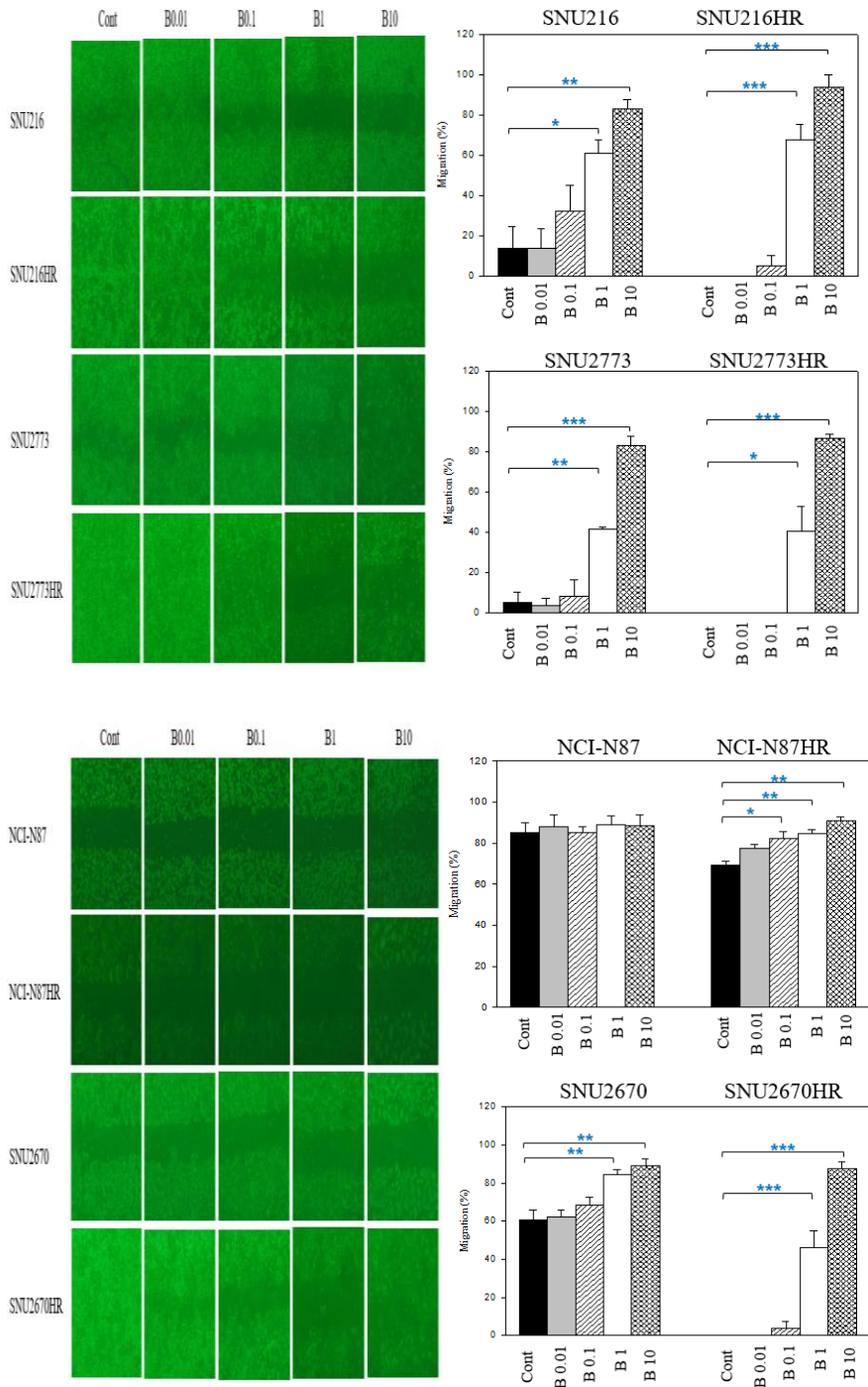
Figure 5. Bosutinib inhibits HR cell migration more effectively compared with parental cells.

All Cell lines were incubated with indicated concentrations of bosutinib (0.01, 0.1, 1, 10μM) for 48 hours.

# 6. In vitro effects of combination of trastuzumab and bosutinib.

In the present study, we observed the potential anti-tumor effect of bosutinib in Src activated HR cells with respect to parental cells. However, in eight cell lines bosutinib was not always been displayed high sensitivity, therefore we conducted combination treatments of trastuzumab and bosutinib by MTT Assay/ Colony Formation Assay/ Western Blot/ Migration Assay.

We measured cell viabilities following treatment with trastuzumab alone or bosuitnib alone or both of them for 72 hours, unfortunately there was no synergistic effect (Fig 6A). In SNU2773HR/NCI-N87HR/SNU2670HR cells, although there was no significant difference between bosutinib alone and combination, compared with trastuzumab alone bosutinib still effectively inhibited cell viability. Then Colony Formation Assay was conducted, the data exhibited similar results with MTT Assay (Fig 6B). After all cells were exposed at trastuzumab plus bosutinib for 24 hours, Western Blot was conducted. We observed that all signals were blocked by combined treatment or bosutinib at 1μM (Fig 6C). Furthermore, to evaluate the anti-migration effect of combination of trastuzumab and bosutinib, we captured the migration images after 48 hours. Trastuzumab (10 μg/ml) did not inhibit cell migration in any of them and combination of trastuzumab and bosutinib had no synergism (Fig 6D).

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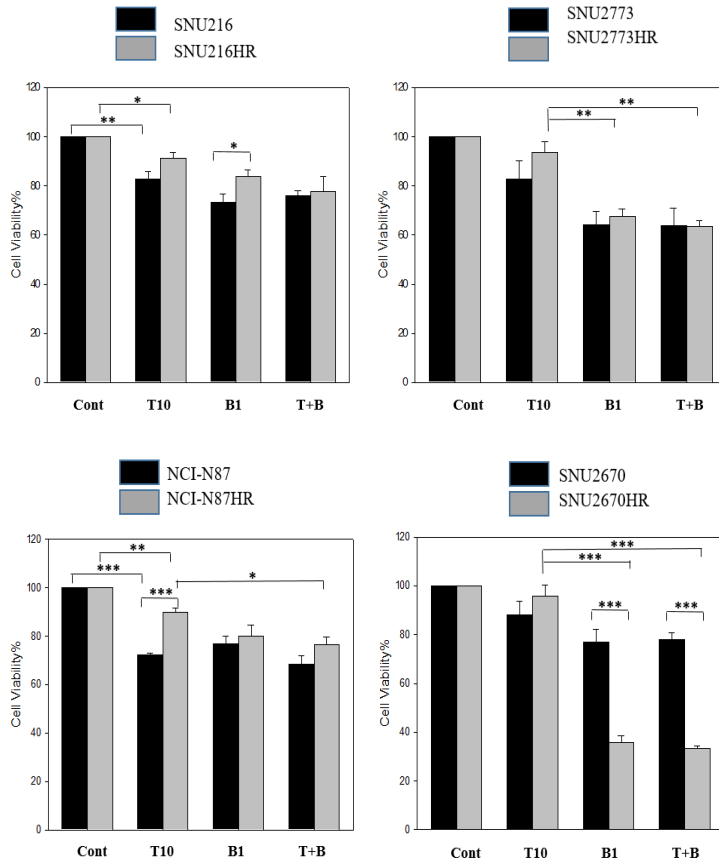
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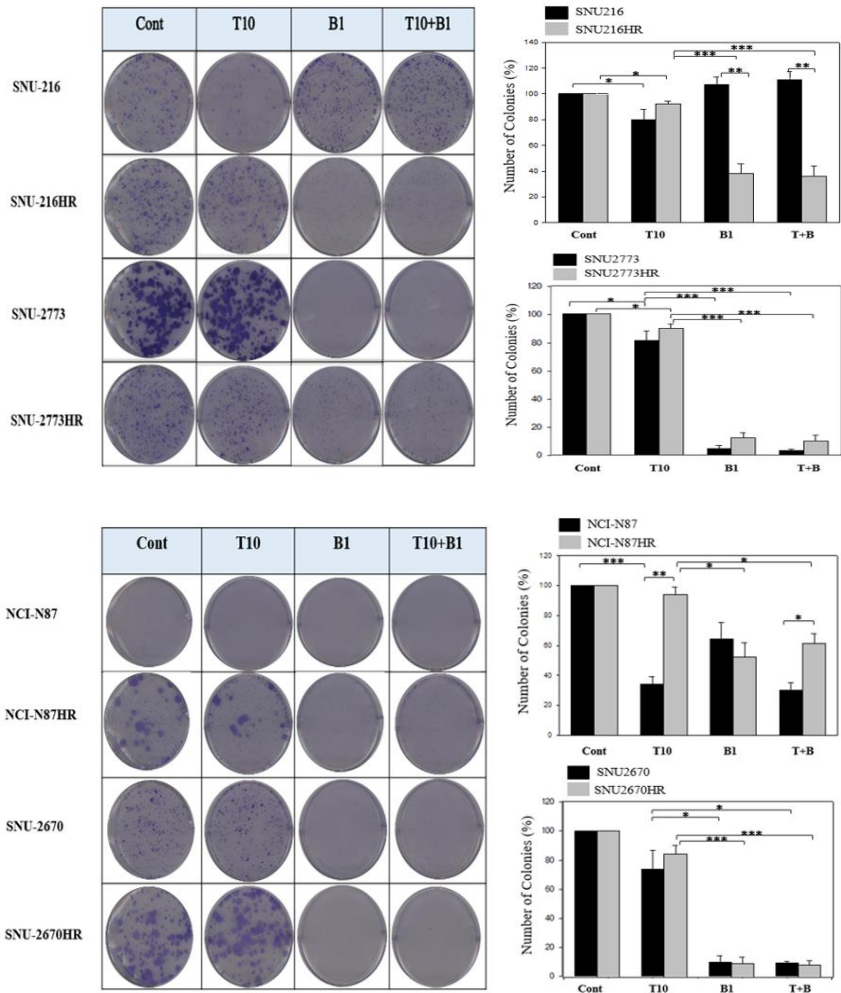
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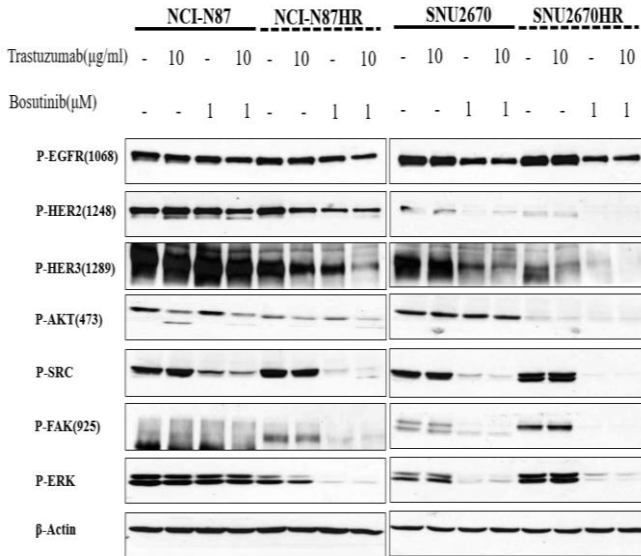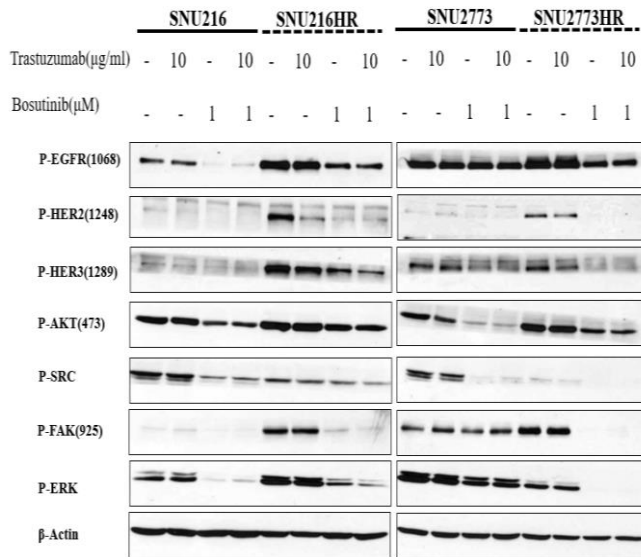
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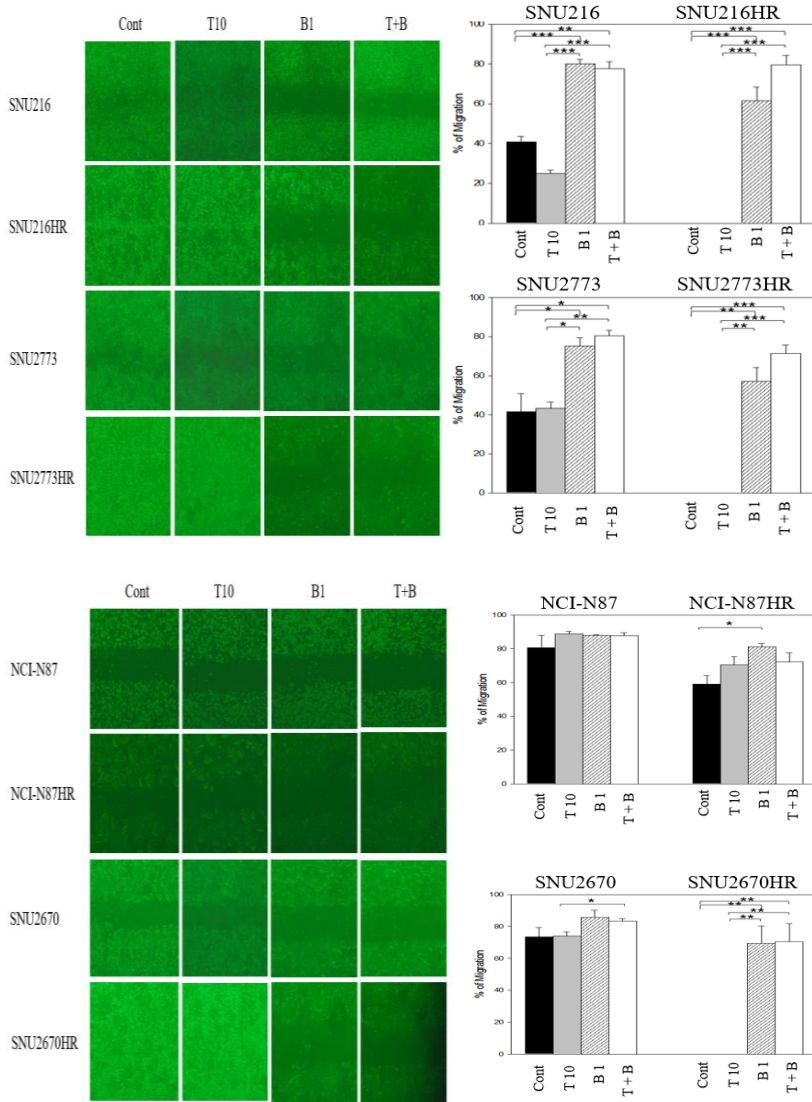
Figure 6. The combination effect of trastuzumab and bosutinib.

(A) Effect of trastuzumab (10µg/ml) and bosutinib (1µM) combination on cell viability for 24 hours. (B) Effect of trastuzumab (10µg/ml) and bosutinib (1µM) combination on cell proliferation for 10-14 days. (C) Eight cells were treated with trastuzumab (10µg/ml) alone or bosutinib (1µM) alone or combination for 24 hours. (D) Eight cells were exposed at trastuzumab (10µg/ml), bosutinib (1µM) or combination, after 48 hours the images were captured.

7. Bosutinib overcomes trastuzumab resistance in vivo

In vitro data displayed that bosutinib had potent effect in both parental cells and HR cells, especially in Src activated HR cells. To validate targeting Src could overcome trastuzumab resistance in vivo, we made xenograft models using SNU2670 and SNU2670HR cells, mice were divided into four groups, which contained two control groups and two bosutinib treatment groups. During treatment course, the effect of bosutinib therapy did not influence the body weight, it presented well tolerated in xenograft models [Fig 7A]. Consistent with in vitro observations, the obvious anti-tumor effect of bosutinib was exhibited in both parental (P<0.05) and HR (P<0.01) mice xenografts compared with each control group, not only that, bosutinib more effectively suppressed tumor growth in HR models than parental (P<0.05) (Fig 7B). Taken together, these data suggest that bosutinib enough to overcome trastuzumab resistance in vivo

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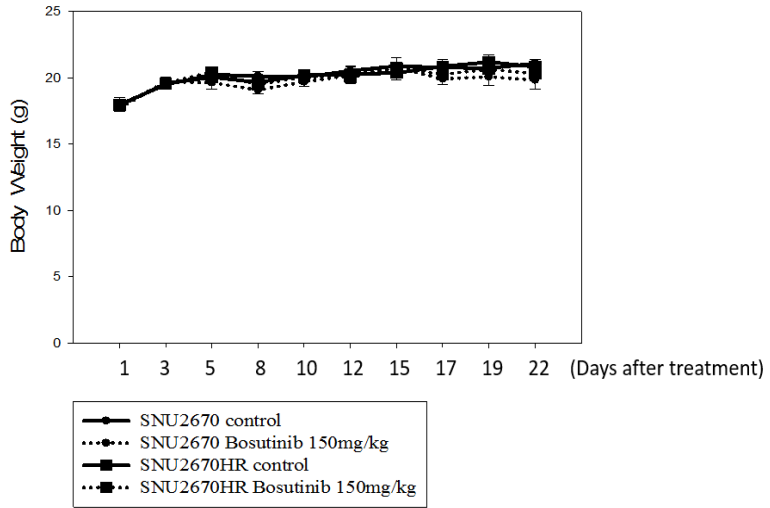
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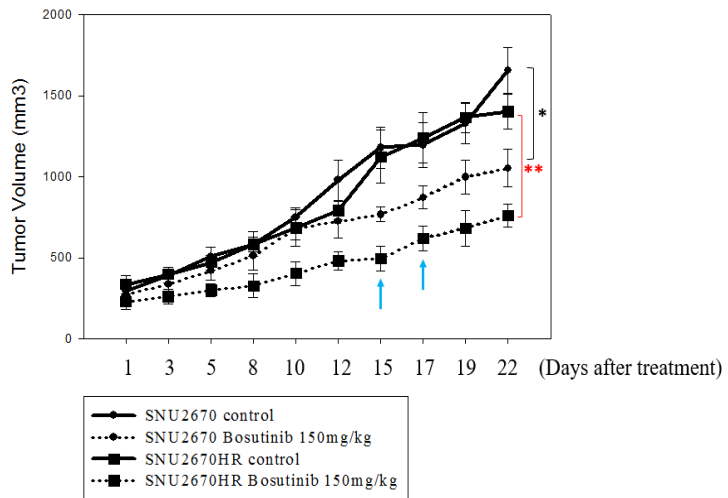
Figure 7. In vivo anti-tumor study of bosutinib as a single agent in SNU2670 and SNU2670HR xenograft model

Both of SNU2670 and SNU2670HR cells (2×10^7) were injected and treatment by bosutinib at doses of 150mg/kg for 21 days. (A) The graph shows mice body weight curves for different groups. (B)The graph shows tumor growth curves for control (vehicle) groups and bosutinib groups. * P value<0.05, ** P value<0.01, *** p value< 0.001. Arrow means p value <0.05 between SNU2670 bosutinib treatment group and HR bosutinib treatment group.

Discussion

Many approaches to clarify the resistance mechanism to anti-HER2 agents in HER2-positive cancers have been attempted [19, 27-30], and several reports demonstrated that Src activation is one of the key factors [8, 17, 18]. In our study, the primary purpose was to explore Src activation involved in trastuzumab resistance and utilizing Src inhibition strategy to overcome trastuzumab resistance.

Here, we generated four trastuzumab-resistant (HR) gastric and biliary tract cancer cells. Interestingly, there were different characterization of these HR cells (Fig 1B). Despite under these circumstances, Src inhibitor still could overcome the resistance.

The previous studies indicated that multiple mechanisms driving trastuzumab resistance in HER2-positive cancers [4]. Deregulated HER2-AKT pathway is the most prevalent obstacle [30-33]. In parallel, SNU216HR and SNU2773HR cells exhibited increased expression levels of pAKT compared to parental, in contrast to SNU2670HR and NCI-N87HR. AKT as a downstream molecule of Src, the general case, Src activation accompanied by AKT phosphorylation, but in our case SNU2670HR and NCI-N87HR cells expressed elevated expression level of pSrc than parental and pAKT decreased, in SNU2670HR/NCI-N87HR cells AKT activation may be inhibited by certain molecules. Secondly, PTEN loss directly leads to Src activation, but the four HR cells did not showed any change in PTEN expression level. Thirdly, constitutive phosphorylated STAT3 was also a common reason for trastuzumab resistance [27]. The phosphorylation of STAT3 was enhanced in NCI-N87HR and SNU2670HR cells which harbored high level of Src phosphorylation. STAT3 is also a downstream of Src, in SNU216HR and SNU2773HR cells,

pSTAT3 level was downregulated. Moreover, Bcl-2 or CDK4/6 or ribosomal S6 have been considered as a resistant mechanism of trastuzumab in several research [28, 34, 35].

In this study, we confirmed Src was contributed to trastuzumab resistance but we have not discovered the reason why Src was activated in some HR cells but not the others. According to early studies, Src mutation could cause Src activation in lapatinib (dual EGFR and HER2 tyrosine kinase inhibitor) resistant cells [36]. Despite we did not detect Src mutation in our work, based on comprehensive understanding of Src, it is considered as a very rare event. The other major complex which facilitate Src activation including E-cadherin and integrin and transmembrane RTKs [11].Next we need to figure out the mechanism of Src activation in trastuzumab resistant cells, further studies should be warranted.

SNU216HR and SNU2773HR cells showed reduced expression levels of pSrc, however, we still believe that Src inhibition could overcome trastuzumab resistance. In Figure 3 bosutinib at 1μM blocked signaling pathways in SNU216HR and SNU2773HR, Figure 4 revealed that bosutinib induced G1 arrest and apoptosis in SNU216HR and SNU2773HR more effectively than parental. Figure 5 displayed SNU216HR and SNU2773HR migration were suppressed by bosutinib more sensitive compared to parental. As is well known, Src inhibitor is sensitive to Src activation, but in our study pSrc decreased HR cells could also be affected by Src inhibitor, we speculate that bosutinib inhibited Src dependent FAK Tyr925 phosphorylation to mediate the downstream molecules.

Today, more study reveal that Src has a universal role in regulating pathways involved in resistance and Src-targeting therapy is considered

3 1

promising [8]. Currently, different kinds of Src inhibitors have been developed and ongoing clinical trials, among them there are common reagents including dasatinib, saracatinib and bosutinib. As a monotherapy, these Src inhibitors are generally well tolerated. Distinct from the other compounds, bosutinib showed significant efficacy in solid tumor [37-38].

In conclusion, Src contributes to trastuzumab resistance in HER2-positive cancer cells and Src-targeting strategy could be interesting measure to overcome trastuzumab resistance.

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국문초록

Trastuzumab는 현재 HER2 양성 유방암과 위암에서 세포독성 항암제와 병합하여 표준 치료제로 사용되고 있다. 이러한 trastuzumab에 대한 내성기전으로는 다양한 것들이 보고되고 있다. 그 중에서도 Src은 특히 HER2 양성 위암과 담도 암에서 그 동안 역할이 알려진 바가 없었다. 본 연구에서 우리는 trastuzumab 내성에 관련된 Src의 역할을 규명하고 Src 억제제를 사용하여 trastuzumab 내성극복 전략이 될 수 있는 가능성에 대한 탐구를 진행하였다.

본 연구진은 HER2 양성 위암세포주(SNU216, NCI-N87)와 담도암 세포주(SNU2670, SNU2773) 로 부터 trastuzumab 내성세포주(HR) 네가지 종류를 수립하였다. Src 억제제인 bosutinib를 이용하여 MTT Assay, Colony Formation Assay, Cell Cycle Analysis, Migration Assay를 진행하였으며 in vitro 실험결과를 바탕으로 SNU2670 과 SNU2670HR를 이용해 구축한 in vivo 실험에서도 bosutinib의 항종양효과를 테스트하였다.

실험결과 basal expression level에서 SNU2670HR / NCI-N87HR 세포의 pSrc의 발현이 증가하였고 반면에 SNU216HR / SNU2773HR 세포의 pSrc의 발현이 감소하는 것을 확인하였다. 하지만 pSrc이 감소한 HR세포에서는 pFAK이 증가하는 것을 관찰하였다. Bosutinib은 모세포주보다 Src이 활성화 되어 있는 HR 세포주에서 Src-FAK 신호전달을 더 효과적으로 억제하는 것을 확인하였다. 또한 pSrc이 감소 된 HR 세포에서는 Src에 의한 pFAK의 발현을 감소시켜 세포에 영향을

준다. Bosutinib은 모세포주와 HR 세포주에서 모두 세포증식을 억제하고 cell cycle 분석에서는 HR세포주가 모세포주보다 G1 arrest가 증가하고 apoptosis가 유도되는 것을 관찰할 수 있었다. 뿐만 아니라, bosutinib을 처리하였을 때 cell migration이 HR 세포주에서 모세포주에 비해 더 의미 있게 감소하는 것을 관찰하였다. SNU2670과 SNU2670HR을 이용한 in vivo 모델에서도 bosutinib은 탁월한 항종양 효과를 보였고 이러한 효과는 모세포보다 HR 모델에서 더 큼을 발견하였다.

결론적으로 Src의 활성화는 HER2 양성 위암과 담도암 세포에서 trastuzumab의 내성에 기여를 한다는 것으로 확인되었다. 이 연구는 Src을 억제하는 것이 HER2양성 암에서 trastuzumab의 내성을 극복하기 위한 전략이 될 수 있음을 시사한다.

주요어: HER2, Trastuzumab, 내성, Src 활성화, Bosutinib

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