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Anti-tumor effect of NVP-BKM120 alone or in combination with MEK162 contra human biliary tract cancer

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의학과 내과학 전공

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Abstract

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Phosphoinositide-3-kinase (PI3K)/AKT/mTOR signaling is one of the most significant pathways regulating tremendous cell processes such as cell proliferation, cell cycle and cell metabolism in many types of cancer. Given that PI3 kinase is the outset of the whole signaling, an agent potently targeting PI3K, such as NVP-BKM120 was expected to bring phenomenal outcome in regulation of cell processes stated

previously. In our study, the investigation of NVP-BKM120 against biliary tract cancer was conducted under the hypothesis of that biliary tract cancer cell lines would display different sensitiveness according to the different mutation status of PIK3CA and K-RAS. Considering high frequency of the reports indicating that PI3K pathway inhibition may induce the activation of RAS/RAF/MEK axis, we also conducted the combination study of BKM120 along with MEK162, a MEK inhibitor. In our study, BKM120 inhibited cell proliferation of biliary tract cancer cells in the short term and also successfully stagnated anchorage-independent cell growth in the long term except the cell line with the co-mutation of PIK3CA and K-RAS (SNU-869). BKM120 successfully blockaded key molecules of PI3K pathway such as P-AKT, P-p70s6k and P-4E-BP1 in two wild-type cell lines (SNU-245 and SNU-1196). In K-RAS mutant cell line (HuCCT-1), BKM120 failed to block PI3K pathway signals, though it conduced to G2 arrest in this cell line in cell cycle analysis. In co-mutant PIK3CA/K-RAS cell line (SNU-869), BKM120 failed to induce cell cycle arrestment and in western blot assay it also failed to block PI3K pathway signals by inducing a restoration of P-4ebp1 at 48h. In migration assay, BKM120 could inhibit cell migration both in wild type and K-RAS mutant cell lines but not in the cell line carrying co-mutations. In combination study with MEK162, cell proliferation was inhibited synergistically in the selected cell lines except K-RAS mutant cell line (HuCCT-1).

Taken together, BKM120 has a potent anti-tumor effect in wild-type biliary tract cancer cells. Combination study of BKM120 with MEK162 showed an enhanced anti-tumor effect in the selected cell lines especially in one with co-mutation of PIK3CA and K-RAS, but not in K-RAS mutant cell. Our study strongly suggests that BKM120 can be a promising therapeutic agent alone or in combination in the treatment of biliary tract cancer.

Keywords: Target Drug, PI3K Inhibitor, Biliary Tract Cancer, MEK Inhibitor, K-RAS Mutation, PIK3CA Mutation.

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Introduction

Biliary tract cancers (BTC) include the cancers from gallbladder, the extrahepatic bile ducts, intrahepatic bile ducts, or the ampulla of the vater (1). BTC is the second most common primary hepatobiliary cancer after hepatocellular cancer in the United States while it is still one of the most common cancers also in the world. In Korea, biliary tract cancers possess 2.4 % among all the types of cancers. Biliary tract cancers have been indicated with poor prognosis to the patients despite the development of diagnostic and therapeutic techniques. This can be attributed to the lack of remarkable biomarkers in spite of quite an amount of researches regarding BTC treatment (2). Thus to find noble biomarkers can be an effective approach to improve the guideline of biliary tract cancer treatment.

The PI3K/AKT/mTOR pathway is one of the pivotal pathways along with RAS/RAF/MEK and JAK/STAT pathway regulating various cellular processes such as cell growth, cell cycle and cell metabolism (3). Activation of PI3K pathway is manipulated through several mechanisms, for instance, somatic mutations or amplifications in p110α and HER2, genetic amplifications of AKT and loss of PTEN which functions as a tumor suppressor (3). Amongst, the most frequent factors contributing to the PI3K pathway activation in BTC would be HER2 amplification and somatic mutations in P110α(4).

Previous studies indicated 3.6% of the biliary tract cancer cells carrying hot-spot mutations of P110α (5). This implicates PI3K inhibitors should be deemed as one of the first candidates in the treatment of BTC and targeting PI3K pathway with a potent PI3K inhibitor would be a nice approach to discover viable biomarkers in the BTC.

Different types of agents have been developed to regulate PI3K pathway including PI3K inhibitors such as Wartmanin, NVP-BYL719 (6)and NVP-BKM120(7), dual PI3K-mTOR inhibitor as NVP-BEZ235(8), AKT inhibitor as A6730 and mTOR inhibitor as Rapamycin. Due to numbers of human cancer cells harboring PI3K isoform mutations, arising isoform-specific PI3K inhibitors could play an important role in the treatment by suppressing PI3K pathway. For instance, BYL719 and BKM120 are promising agents in this regard. Unlike other PI3K inhibitors, which have strong potency on wild type cancer cells but not in PI3K isoform-mutant cells, BYL719 has an exclusive potency in P110α mutant kinase while NVP-BKM120 can target on all of the three isoforms of PI3K – P110α, P110-β and P110-Ɣ. As to BKM120, it is a pan-class PI3K inhibitor known to be capable of PI3K inhibition regardless of the harbored isoform mutations(9).

At the meantime, another remarkable pathway worth to be referred is RAS/RAF/MEK/ERK pathway, which been reported to be frequently induced by PI3K pathway inhibition(10). Furthermore there are frequent RAS mutations (especially K-RAS mutation) in human cancer

cells are demonstrated to be related with a poor prognosis in previous studies(11). Thus dual inhibition of PI3K pathway and RAS pathway would be worth to try in the treatment of cancer cells known to be in both of the PI3K and RAS pathway active status.

Anti-tumor effect of NVP-BKM120 contra biliary tract cancer cells would be revealed in this study for the first time. As previously stated NVP-BKM120, a pan-class PI3K inhibitor can effectively target on PI3 kinase and lead to a reverted status of activated PI3K pathway as hypothesized. Also a combination study with MEK162, a MEK inhibitor, has been undertaken to identify the feasibility of BKM120 in combination study considering biliary tract cancer cells with dual activation of PI3K/MEK pathways. In addition, this will also shed a light to further combination studies of BKM120 against biliary tract cancer cells.

Materials and Methods

Cell lines and culture

Human biliary tract cancer cell lines (SNU-245, SNU-308, SNU-478, SNU-869, SNU-1079 and SNU-1196) were purchased from the Korean Cell Line Bank, and there are published reports illustrating the characterizations of these cell lines. HuCCT-1 was purchased from the Japanese Cancer Research Resources Bank and TFK-1 was purchased from DSMZ (Braunschweig, Germany). All cell lines were cultured in RPMI-1640 media (WelGENE Inc. Daegu, Korea) containing 10% fetal bovine serum (FBS) in a circumstance of 5% CO_2 at 37°C .

Cell growth inhibition assay

Tetrazolium dye (MTT; Sigma-Aldrich) assays were used to access the cell growth inhibition. To evaluate the effects of either BKM120 alone or combination with MEK162, the cells were exposed to each drug with a series of increasing doses and to both of drugs synchronously with a fixed concentration of MEK162 added to an incrementing concentration of BKM120, cell viabilities were then measured after 72 h of incubation.

Colony-forming assay

Different numbers of cells were plated in 6-well plates and incubated at 37°C, 5% CO_2 in the absence or presence of BKM120 at 0.1μM,

0.5μM, 1μM concentrations for 10-14 days. Colonies were then stained with coomassie blue for 1 hour for colony visualization. Colony numbers were counted by Gel doc.

Western blot

Cells were incubated with BKM120 in the 10% FBS media for 24h or 48h before taken to the procedures of protein extraction with lysis buffer. Equal amount of protein from each cell was subjected to SDS-PAGE followed by transfer to the nitrocellulose membranes. Membrane then was incubated with primary antibodies at 4°C for overnight after the step of blocking with blocking buffer. Antibodies probing phosphorylated AKT (p-AKT, Ser473), p-p70s6K (T389) p-4E-BP1 (Thr70/46), PTEN, Actin were purchased from Cell Signaling Technology(Beverley, MA,USA).

Cell cycle analysis

Following the incubation with BKM120 at different concentrations for 48h, cells were centrifuged at 1600 rpm for 5 min and fixed in 70% alcohol for no less than 2 days at -20°C. Samples were then exposed to 10μl of RNAse(100μg/ml) for 10 min at 37°C and stained with propidium iodide right before the measure of DNA content of the cells(10,000 cells) using a FACS caliber flow cytometer(Becton Dickinson Biosciences) via ModFit LT program(Verity Software House).

Migration assay

Cells were seeded onto 6-well plates and were cultured in a 10% FBS media for 24h. 200μl pipet tips were used to create scratches and

cells were cultured for another 72h in the absence or presence of BKM120 at 0.1μM, 0.5μM, 1μM concentrations. Images were taken at the time point of 0h, 24h, 48h and 72h counting from the moment BKM120 was added. The diameters of the gaps were measured with Image J program.

Result

1.NVP-BKM120 inhibited cell proliferation in biliary tract cancer cells.

To evaluate anti-proliferation effect of NVP-BKM120 in human biliary tract cancer cells, we measured cell viabilities of 8 BTC cells following 72-hour exposure to the BKM120 by MTT assay. Cell proliferation of all tested cell lines were inhibited, with IC_{50} values ranging from 1.27 to 4.81μmol/L (Table1, Fig.1A), but no conspicuous difference of sensitivity in different cell lines could be observed in this short-term assay. We then performed colony formation assay to determine if BKM120 has inhibitory effect against biliary tract cancers in anchorage-independent cell growth. A more crucial outcome was observed with more widespread curves indicating different IC_{50} values ranging from (0.5 to 1μmol/L)(Fig.1B). Given that, we picked 4 cell lines, SNU-245, SNU-1196, HuCCT-1 and SNU-869, based on different sensitivities of each cell line for further studies (Fig.1B, 1C). SNU-245 and SNU-1196 were classified as sensitive cell lines with the smallest IC_{50} value and modest one respectively, HuCCT-1 as a moderately sensitive one possessing a moderate IC_{50} value and SNU-860 as a resistant one with the maximal IC_{50} value amongst (Fig 1C).

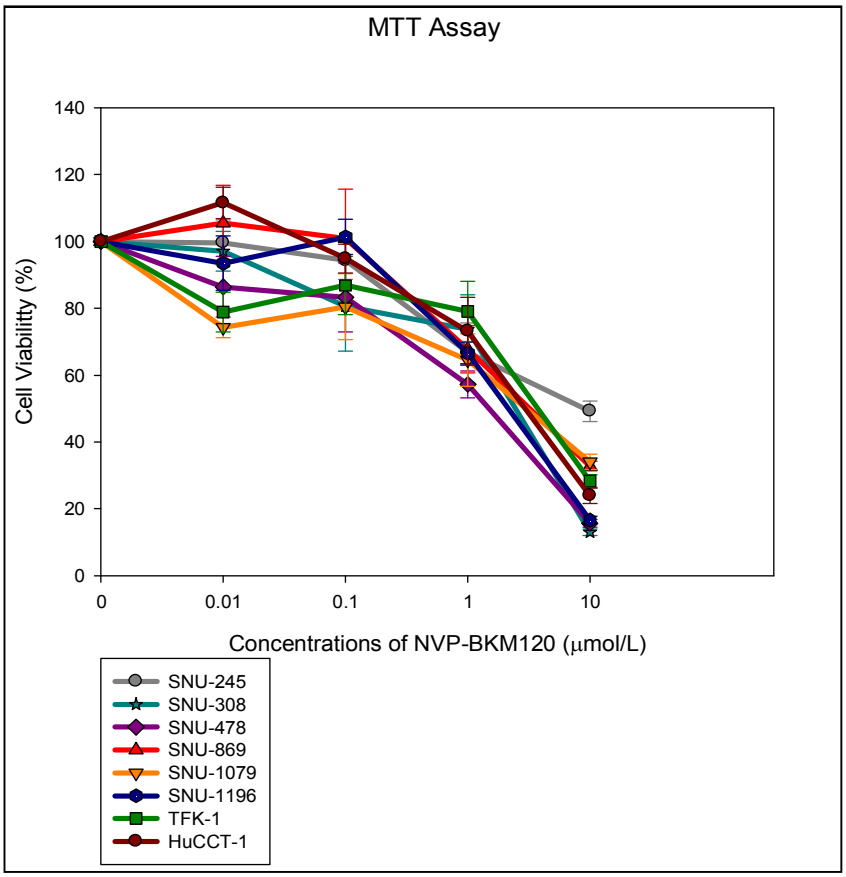


Figure 1. Anti-proliferation effect of NVP-BKM120 in 8 biliary tract cancer cell lines.

(A) Panels of 8 BTC cells were incubated with respective concentrations of BKM120 for 72 hours and cell proliferation was measured by MTT assay. Data shown is percentages to the vehicle control respectively. Each curve represents the mean of values from 3 independent experiments and is indicated with standard deviations.

Table 1.

Characteristics and MTT IC_{50} values of BTC Cell Lines.

Cell Line	EGFR	HER2	KRAS	BRAF	PIK3CA	PTEN	JAK2	MTT IC_{50} of BKM120(μM)
SNU-245	WT	WT	WT	WT	WT	WT	WT	3.92±1.65
SNU-478	WT	WT	WT	WT	WT	WT	WT	1.27±0.07
SNU-308	WT	WT	WT	WT	WT	WT	WT	2.47±0.04
SNU-869	WT	WT	p.G12D	WT	p.E545A	WT	WT	2.02±0.06
SNU-1079	WT	WT	WT	WT	WT	WT	WT	2.11±0.08
SNU-1196	WT	WT	WT	WT	WT	WT	WT	1.49±0.37
HuCCT1	WT	WT	p.G12D	WT	WT	WT	WT	2.24±±0.02
TFK-1	WT	WT	WT	WT	WT	WT	WT	4.81±6.78

Table1. Gene alterations of BTC cell lines. MTT IC_{50} values are indicated in the table.

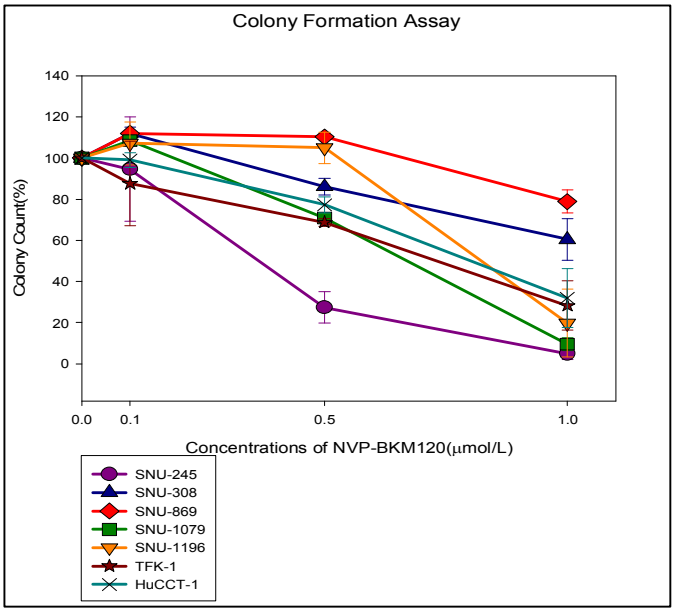


Figure 1. Anti-proliferation effect of NVP-BKM120 in 8 biliary tract cancer cell lines.

(B) 8 BTC cells were incubated with different concentrations of BKM120 for 10-14 days followed by coomassie blue staining on the last day of incubation and colony counting was manipulated via Gel Doc. Presented data in (B) is indicating percentages to the vehicle control. Each curve in the figure (B) represents the mean of values from 3 independent experiments and is indicated with standard deviations.

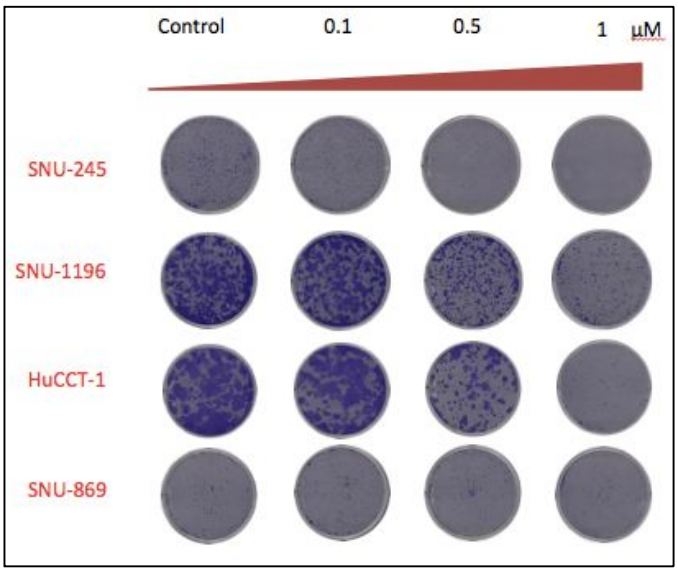


Figure 1. Anti-proliferation effect of NVP-BKM120 in 8 biliary tract cancer cell lines.

(C) 8 BTC cells were incubated with different concentrations of BKM120 for 10-14 days followed by coomassie blue staining on the last day of incubation.

2.NVP-BKM120 displayed a phenomenal blockade of PI3K pathway signaling in the sensitive cell lines.

To inspect the causal factors and investigate the mechanism leading to the different sensitivities, we conducted a series of trials starting with western blot. When incubated with increasing doses of BKM120 for 24 hours, 4 BTC displayed a similar tendency of PI3K pathway key molecules (Fig. 2A). Different extent of abatement of P-4ebp1 (T37/46) and a sharp diminution of P-p70s6k could be observed in all the cell lines excluding HuCCT-1, while mitigated P-AKT was also notable in the same cell lines (Fig. 2A). Albeit the generally concordant alterations of protein signals after 24-hour treatment with BKM120, double-timed (48-hour) treatment with BKM120 brought us completely new findings disclosing a restoration of P-4E-BP1 in the resistant cell line (Fig. 2B). P-4E-BP1 is a molecule negatively regulates protein translation by binding to EIF4E, which leads to the inhibition of its function as a protein translation factor, yet phosphorylated 4E-BP1 is depleted with such a function as a negative regulator which means a restoration of P-4E-BP1 might be one of the mechanisms attenuating BKM120 effect in resistant cell lines (12).

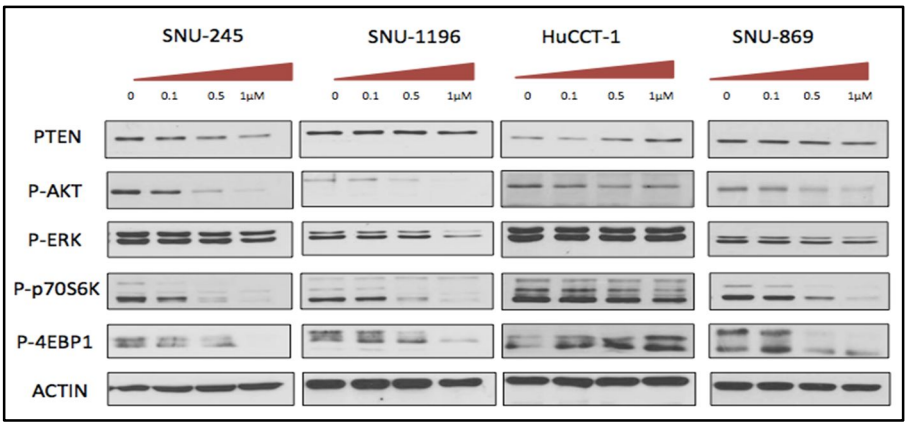


Figure 2. BKM120 blockaded PI3K pathway signaling in sensitive cell lines.

(A) 4 BTC cell lines were exposed to BKM120 at different concentrations for 24 hours and were probed with different antibodies as presented in the figure.

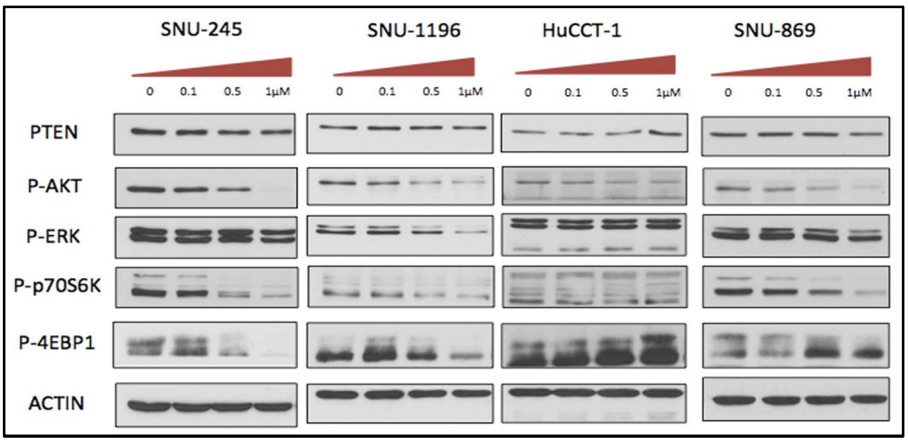
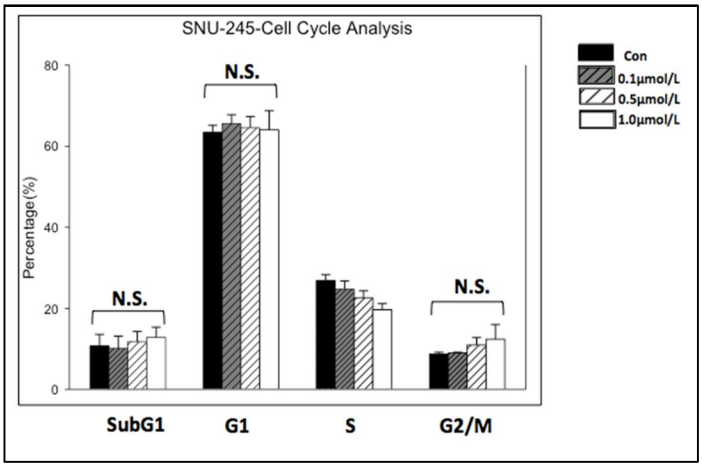


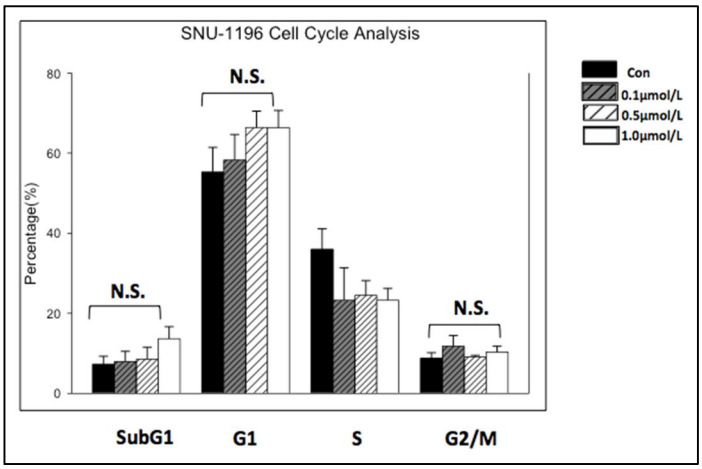
Figure 2. BKM120 blockaded PI3K pathway signaling in sensitive cell lines.

(B) 4 BTC cell lines were incubated with BKM120 at different concentrations for 48 hours and were probed with different antibodies as shown in the figure.

3.Cell cycle arrest was occurred in one cell line by the treatment of BKM120.

To examine if BKM120 has any effect in regulating cell cycle in biliary tract cancer cell, 4 cell lines were undertaken with the cell cycle analysis. SNU-245 and SNU-1196, which were determined as sensitive cell lines, showed no notable arrestment in G1 phase nor in G2/M phase while HuCCT-1, the one opined as a moderately resistant cell line, showed an arresting increment in G2/M phase. Reaching consensus to the previous view though, SNU-869 embodied no considerable changes under the same circumstances (Fig. 3). Cell cycle analysis served as a clue that different sensitivities of BTC cell lines to BKM120 may have little to do with cell cycle since only one cell line out of four, HuCCT-1, has responded with arrested G2 phase. Yet, taken all, it put us to an urgent situation to clarify other possible mechanisms giving rise to different responses of BTC cells to the BKM120.


SNU-245-Cell Cycle Analysis
Percentage(%)
80
60
40
20
0
N.S.
N.S.
N.S.
SubG1
G1
S
G2/M
Con
0.1μmol/L
0.5μmol/L
1.0μmol/L


SNU-1196 Cell Cycle Analysis
Percentage(%)
80
60
40
20
0
N.S.
N.S.
N.S.
SubG1
G1
S
G2/M
Con
0.1μmol/L
0.5μmol/L
1.0μmol/L

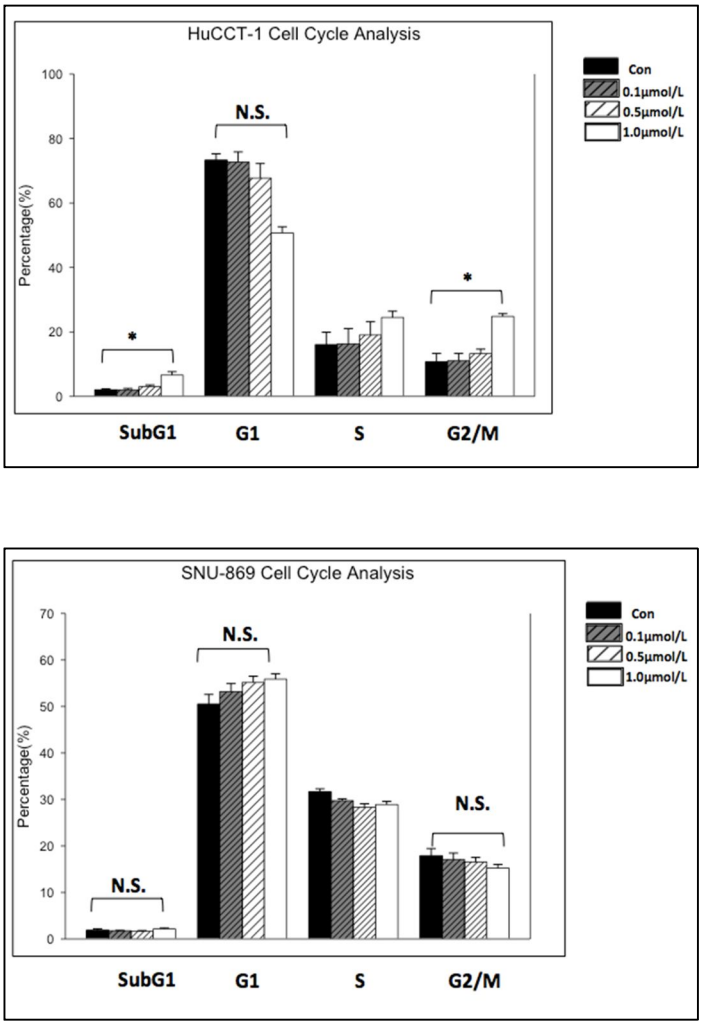
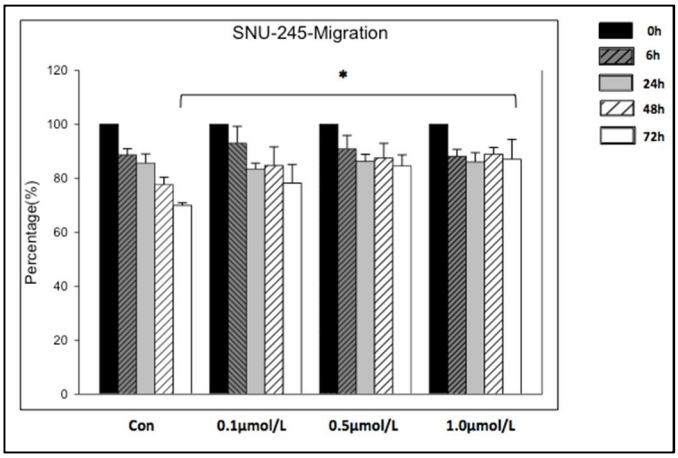


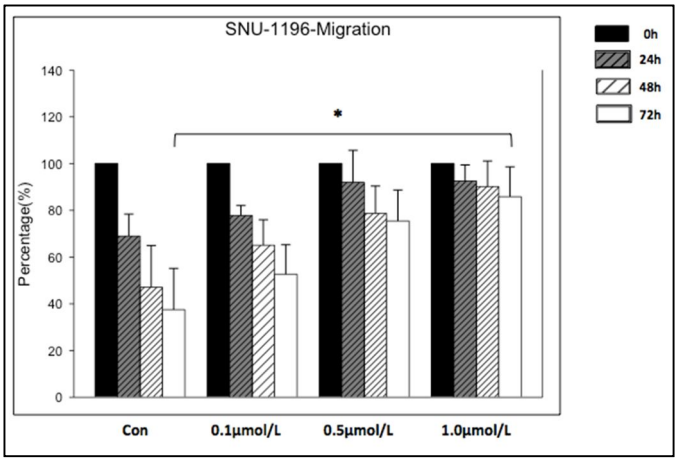
Figure 3. G2 arrest in one cell line was revealed via cell cycle analysis with BKM120.

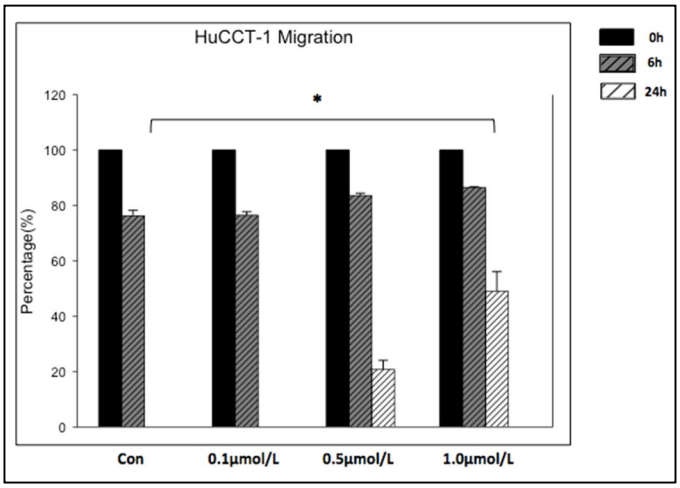
4 BTC cell lines were exposed to the indicated concentrations of BKM120 for 72 hours followed by the measurement of cell percentages in G1, S, G2/M phases and Sub G1 via cytometry analysis. Each column represents 3 independent experiments and is indicated with standard error. *,$p<0.01$.

4. BKM120 conspicuously inhibited cell migration in the sensitive cell lines but failed to inhibit migration in the resistant cell line.

Under the circumstance that activated Src, which is highly related to the cancer cell migration, can be one factor of the outset to the PI3K activation and co-stimulation of Src and PI3K are discovered in various types of cancers as indicated in the previous reports whereas highly activated Src was discovered in several biliary tract cancers, we were in need to know whether inhibiting PI3K pathway alone could inhibit cell migration (13-15). Migration assay was conducted to inspect if BKM120 could inhibit cell migration against different types of BTC cell lines selected previously. In line with our assumption, in SNU-245, SNU-1196 and HuCCT-1, BKM120 showed a noteworthy inhibitory effect in cell migration in time dependent and dose dependent fashion. It conforms a sharp contrast to the mitigated effect against SNU-869, which was sought to be resistant to BKM120, as we can observe that the gap distances in SNU-869 display similar tendency regardless of the presence or the absence of the drug treatment.


SNU-245-Migration
Percentage(%)
120
100
80
60
40
20
0
*
Con
0.1μmol/L
0.5μmol/L
1.0μmol/L
0h
6h
24h
48h
72h


SNU-1196-Migration
Percentage(%)
140
120
100
80
60
40
20
0
*
Con
0.1μmol/L
0.5μmol/L
1.0μmol/L
0h
24h
48h
72h



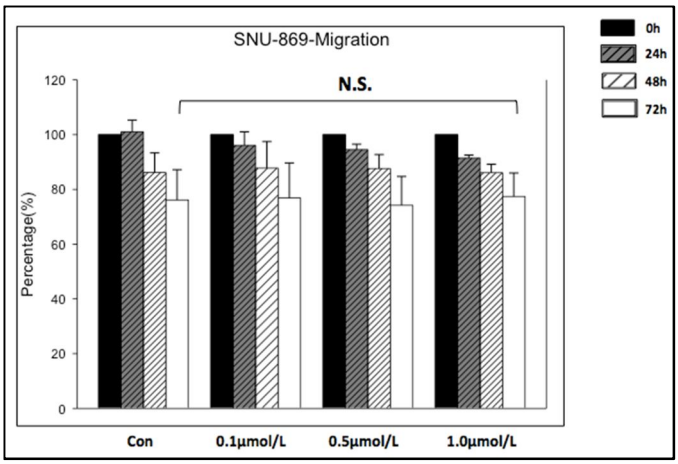
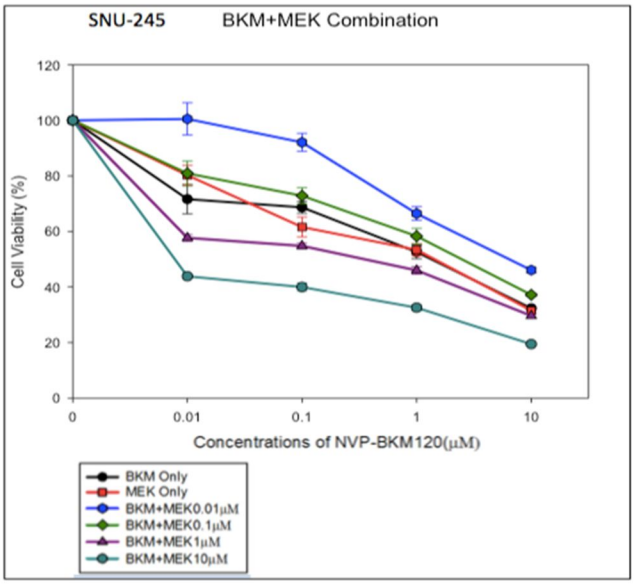


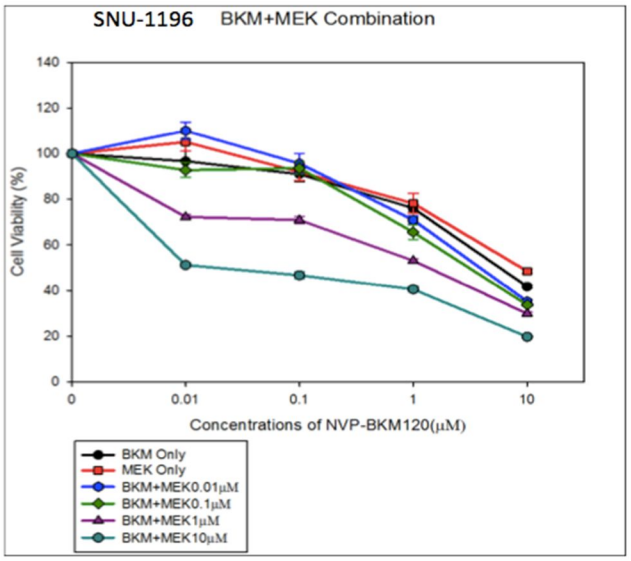
Figure 4. BKM120 notably inhibited cell migration in the sensitive cell lines.

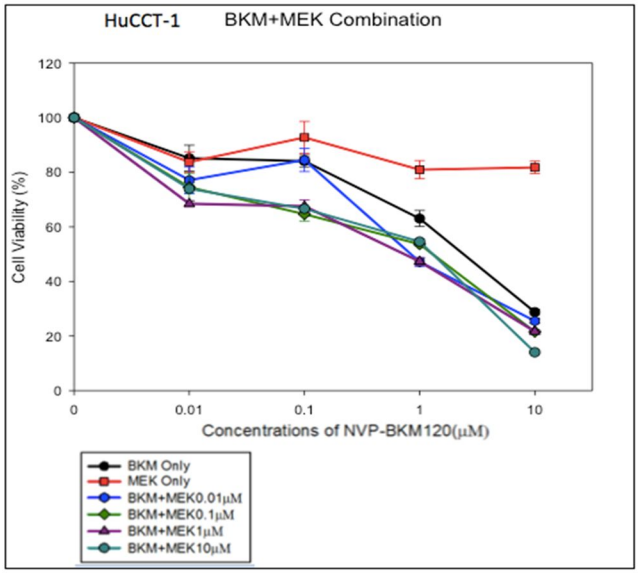
4 BTC cell lines were incubated with indicated concentrations of BKM120 for the times displayed in the figure then the diameters of the gap in each cell line was measured by image J. *,$p<0.05$.

5. Combining BKM120 with MEK162 was confirmed to have potentiated anti-proliferation effect in biliary tract cancer cells.

As 2 cell lines of biliary tract cancer were disclosed to be sensitive to the mono treatment of PI3K inhibitor while the other two drew out a weakened drug effect, we went on our research to find out if combined treatment of BKM120 with MEK inhibitor would effectively compensate the resistance to the PI3K inhibitor and if combination treatment would present an enhanced anti-tumor effect in sensitive cell lines as well. When conducted MTT assay with 4 cell lines all the cell lines except HuCCT-1 showed us shifting graphs which implies combined treatment has synergistic effect. IC_{50} values decreased as we increased the dose of the added MEK162 (Fig 5A, Table 2) Whereas HuCCT-1 did not follow the same pattern showing relatively clustered line patterns in the figure with relatively stable IC_{50} values (Fig 5A, Table 2). BKM120 at increasing doses (0.01μM, 0.1μM, 1μM, 10μM) with MEK162 at a fixed concentration of 0.1μM was indicated to have combination effect in SNU-869 while combining BKM120 with MEK162 at three different concentrations (0.01μM, 0.1μM, 1μM) respectively were all indicated to have synergism in SNU-1196 (Fig 5B). Thus, combining MEK inhibitor with BKM120 was unfolded here to have potentiated anti-proliferation effect against 4 selected cell lines to the different extents including sensitive and insensitive ones.


SNU-245
BKM+MEK Combination
Cell Viability (%)
120
100
80
60
40
20
0
0
0.01
0.1
1
10
Concentrations of NVP-BKM120(μM)
BKM Only
MEK Only
BKM+MEK0.01μM
BKM+MEK0.1μM
BKM+MEK1μM
BKM+MEK10μM


SNU-1196
BKM+MEK Combination
Cell Viability (%)
140
120
100
80
60
40
20
0
0
0.01
0.1
1
10
Concentrations of NVP-BKM120(μM)
BKM Only
MEK Only
BKM+MEK0.01μM
BKM+MEK0.1μM
BKM+MEK1μM
BKM+MEK10μM


HuCCT-1
BKM+MEK Combination
Cell Viability (%)
120
100
80
60
40
20
0
0
0.01
0.1
1
10
Concentrations of NVP-BKM120(μM)
BKM Only
MEK Only
BKM+MEK0.01μM
BKM+MEK0.1μM
BKM+MEK1μM
BKM+MEK10μM

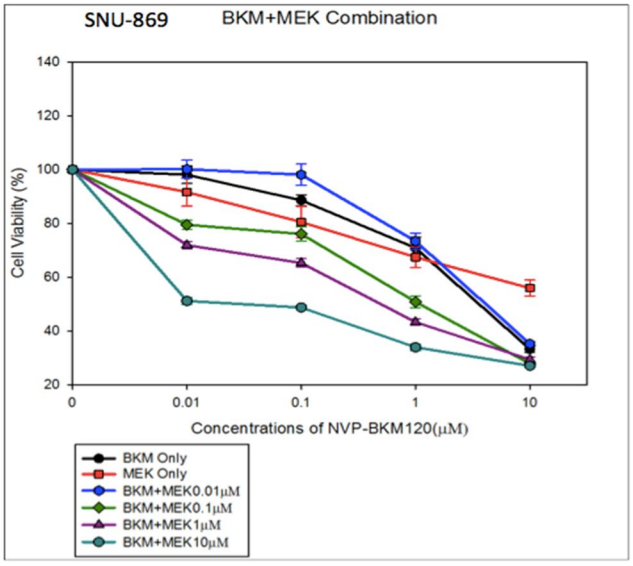
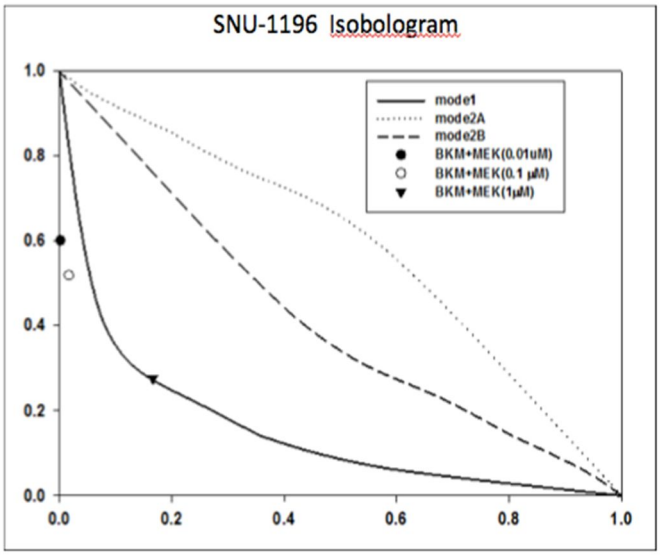

SNU-869
BKM+MEK Combination
Cell Viability (%)
140
120
100
80
60
40
20
0
0.01
0.1
1
10
Concentrations of NVP-BKM120(μM)
BKM Only
MEK Only
BKM+MEK0.01μM
BKM+MEK0.1μM
BKM+MEK1μM
BKM+MEK10μM

Figure 5. Enhanced anti-proliferation effect of BKM120 against biliary tract cancer cells was confirmed in the combination study with MEK162.

(A). Two 96-well plates of 4 cell lines were incubated with indicated concentrations of BKM120 and MEK162 respectively and 4 plates were exposed to the indicated concentrations of BKM120 with different fixed doses of MEK162 added to each plate respectively as displayed in the figure. Each curve represents three independent experiments and is indicated with standard error.


SNU-1196 Isobologram
mode1
mode2A
mode2B
BKM+MEK(0.01uM)
BKM+MEK(0.1 μM)
BKM+MEK(1μM)
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0

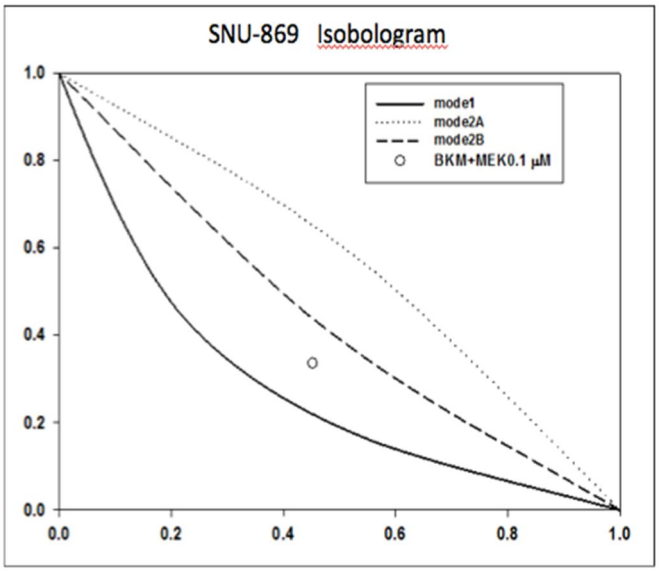

SNU-869 Isobologram
mode1
mode2A
mode2B
BKM+MEK0.1 μM
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0

Figure 5. Enhanced anti-proliferation effect of BKM120 against biliary tract cancer cells was confirmed in the combination study with MEK162.

(B). Isobologram of SNU-869 and SNU-1196 is displayed in this figure. Synergistic effect is indicated here when 0.1μM of MEK162 is added to diverse concentrations of BKM120 in the treatment of SNU-869 and when 0.01μM, 0.1μM and 1μM of MEK162 were added to diverse concentrations of BKM120 in SNU-1196. Isobologram was drawn with Sigma Plot.

Table 2 : Combination MTT IC_{50} Values(μM)

	Combination MTT IC50 Values(μM)					
	BKM Alone	MEK Alone	BKM MEK0.01μM	BKM MEK 0.1μM	BKM MEK 1μM	BKM MEK10μM
SNU-245	4.92	0.17	2.65	0.48	0.02	0.008
SNU-1196	3.83	6	2.3	1.99	1.06	0.01
HuCCT-1	1.73	N/A	0.89	0.19	0.79	1.14
SNU-869	2.43	N/A	2.77	0.96	0.2	0.01

Table 2: The MTT IC_{50} Values of different combinations of BKM120 and MEK162 in 4 cell lines are indicated in the table.

Discussion

Albeit there have been conspicuous advances in the treatment techniques regarding biliary tract cancer cells, it still remains as an urgency to seek more specified treatment. More narrowed-down targeting therapy, for example, targeted agents aiming to specific pathways are in need. In spite of tremendous targeted drugs developed in the past decades been disclosed to play prominent roles in the treatment of other solid tumors such as gastric cancer cells, breast cancer cells, evidences of targeted drugs proved to be eminent in the treatment of biliary tract cancers are yet numbered (16-20). In such a situation, we decided to unfold the anti-tumor effect of targeted drug against biliary tract cancer cells. And NVP-BKM120, a targeted drug that was known to have a strong impact on the cancer cells by targeting PI3K pathway, became one of the most prominent candidates suitable for our study(7, 9, 21-24). NVP-BKM120, a pan-class PI3K inhibitor, was given a series of investigation to inspect its anti-tumor effect in biliary tract cancer cells. All the biliary tract cancer cells responded to BKM120 similarly in short-term cell viability assay according to our data from MTT assay. 8 BTC showed relatively similar IC_{50} values ranging from 1 to 5 μmol/L (Fig 1A). As we continued our study, a much more notable difference could be observed from 8 BTC when undertaken with colony formation assay (Fig. 2B, 2C). As predicted, given a decent length of time to function, BKM120 treatment

led to the different responses of 8 BTC cells, and this is where we selected 4 cell lines possessing different sensitivities.

Continuously, western blots were performed to serve as evidences supporting previous findings followed by cell cycle analysis and migration assay. Typical molecules of the PI3K axis were given tested with treatment of BKM120 in the western blot. The probed signals such as P-AKT, P-p70s6k, P-4E-BP1 were observed to decrease in a dose dependent manner at the time point of 24h while at 48h with an occurrence of P-4E-BP1 restoration in SNU-869 (Fig. 2A, 2B). From what we know 4E-BP1 is a downstream protein of PI3K pathway playing pivotal role as a negative regulator of protein translation by binding to the eIF4E (eukaryotic translation initiation factor 4E). EIF4E is known to initiate protein translation via the interaction with eIF4G which can be obstructed by binding of 4E-BP1. Phosphorylation of 4E-BP1 though would deplete thus function of this molecule (12, 14). Given this, the increment of P-4E-BP1 can be an important factor attenuating the anti-tumor effect of BKM120 against biliary tract cancer cells yet more trials would be required to confirm this postulation. Continuously we ran some trials to analyze cell cycle changes of biliary tract cancer cells when treated with BKM120. As we assumed, SNU-869 did not show any changes while unexpected responses of the rest of cell lines were unfolded. (Fig. 3A) Only in HuCCT-1 could we observe an elevated G2/M phase but no significant changes could be seen in SNU-245 and SNU-1196.

To date, previous studies have given us valuable proofs of evident biomarkers of this agent including the worthwhile findings of K-RAS and PIK3CA mutations as predictive markers of BKM120. Previous findings imply cancer cells harboring PIK3CA tend to be more sensitive than wild type while K-RAS mutations are indicated as resistant biomarkers(22). Two cell lines selected to deputize the sensitive group, SNU-245 and SNU-1196 have no genetic alterations of K-RAS nor PIK3CA, while HuCCT-1, the moderately sensitive one has K-RAS mutation and SNU-869, the resistant cell line harbors both of K-RAS and PIK3CA mutation. Interestingly, SNU-869, which has PIK3CA mutation, exhibited a conversed result when it has been reported to be a sensitive biomarker of BKM120. Only then we figured the role of PIK3CA as a sensitive biomarker might be counteracted by the co-existence of K-RAS mutation in SNU-869(25, 26). Thus targeting RAS pathway concurrently could be an effective way to overcome the resistance, and there were previous researches reporting the synergistic effect of combination treatment in lung cancer cells those carry co-mutations of K-RAS and PIK3CA (26). Granted a reasonable explanation we then furthered our study of combination treatment complying BKM120 with MEK162, a MEK inhibitor to overcome the resistance of SNU-869.

Following our plan, MTT Assay was conducted to evaluate the combination effect in the selected 4 cell lines. In this cell viability assay, 3 cell lines out of 4 responded to our expectation showing a

notable synergism though HuCCT1 failed to drag out the combination effect of two agents. Co-targeting MEK pathway instead of PI3K pathway alone successfully addressed the resistance of SNU-869 to the PI3K inhibitor. The addition of MEK162 effectively counteracted the co-existence of PIK3CA and K-RAS mutations that weakened the anti-tumor function of BKM120. However, the combined treatment failed to draw out synergistic effect in K-RAS mutant cell line, HuCCT1. Interestingly if we refer to the previous publications, it is commentated that K-RAS mutation is a positive biomarker for MEK inhibitor(27, 28). Therefore we are in a demand to investigate the mechanism by which additional MEK inhibitor still led to nothing but failure to enhance anti-tumor effect of BKM120 in K-RAS mutant cell line. Besides, shall we carry on further study to uncover the signal changes at protein levels and find out other possible outcomes which manifesting synergistic effect of combination treatment in advance of performing more experiments to disclose the rationale hidden in the rear.

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국문초록

PI3K/AKT/mTOR signalin 은 많은 암종에서 cell proliferation, cell cycle arrest 와 apoptosis 를 조절하는 중요한 pathway 중 하나이다. 따라서 BKM120 같은 PI3K pathway 를 억제하는 표적항암제의 사용은 암세포의 증식을 억제하는데 효과적이다. 본 연구에서는 담도암에서 BKM120 에 대한 항종양효과 검증과 BKM120 에 대한 약제의 효과가 PIK3CA 와 K-RAS mutation 유무에 따라 다르게 나타나는 가설을 검증을 위해 실험을 진행하였다. 또한 많은 문헌들에서 PI3K pathway 를 억제하면 RAS/RAF/MEK Pathway 가 활성화 된다는 보고가 있어, MEK inhibitor 인 MEK162 와의 병용 연구도 진행하였다.

실험결과 BKM120 은 담도암 대부분의 세포주에서 세포증식을 억제하였으나 PIK3CA 와 K-RAS mutation 이 있는 SNU-869 에서는 짧은 시간 약 처리시 세포증식 억제 효과를 보였으나, 긴 시간 동안 약을 처리하였을 때는 억제효과를 보이지 못했다. PIK3CA 와 K-RAS 가 wild type 인 세포주 (SNU245, SNU1196) 에서는 BKM120 처리시 PI3K pathway 의 중요한 분자들인 P-AKT, P-p70s6k, P-4ebp1 의 level 이 효과적으로 감소되었다. K-RAS mutation (HuCCT-1) 이 있는 세포주에서는 위 분자들의 level 의 영향을 주지 못했으나, cell cycle 분석에서는 G2 arrest 를 관찰 할 수 있었다. PIK3CA 와 K-RAS mutation 이 있는 SNU-869 세포주에서는 중요분자들의 변화와 cell

cycle 의 변화가 없었으며, P-4ebp1 의 level 이 시간이 지날수록 다시 복원되었다. 세포이동실험에서도 다른 세포주들에 비해 SNU-869 세포주에는 영향을 주지 않았다.

MEK162 와의 병용연구에서 세포증식을 관찰하였을 때, wild-type 의 세포주들과 PIK3CA 와 K-RAS mutation 이 있는 SNU-869 세포주에서 항종양효과의 상승이 나타남을 확인하였다. 하지만 K-RAS mutation 만 있는 HuCCT-1 에서는 상승효과를 관찰하지 못했다.

결론적으로, BKM120 을 단독으로 처리하였을 때, mutation 이 없는 담도암 세포주에서 항종양 효과를 보였으며, PIK3CA 와 K-RAS mutation 이 있는 경우에는 MEK inhibitor 와의 병용이 효과가 있음을 확인하였다. 이 연구를 바탕으로 담도암에서 BMK120 의 단독 혹은 다른 약제와의 병용처리시 항종양효과의 가능성을 제시하였다.

주요어: 표적항암제, PI3K 억제제, 담도암, MEK 억제제, K-RAS Mutation, PIK3CA Mutation.

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