The PI3K–Akt mediates oncogenic Met-induced centrosome amplification and chromosome instability

Hyun-Ja Nam1,2, Sunyoung Chae1,2, Seung-Hoon Jang1,2, Hyeeseong Cho1,2 and Jae-Ho Lee1,2,*

1Department of Biochemistry and Molecular Biology, Ajou University School of Medicine and 2Department of Molecular Science and Technology, Graduate School of Ajou University, Suwon 443-721, Korea.

*To whom correspondence should be addressed. Department of Biochemistry and Molecular Biology, Ajou University School of Medicine, Suwon 443-721, Korea. Tel: +82 31 219 5053; Fax: +82 31 219 5059; Email: hjlee64@ajou.ac.kr

Correspondence may also be addressed to Hyeseong Cho. Tel: +82 31 219 5052; Fax: +82 31 219 5059; Email: hsho@ajou.ac.kr

The oncogenic ability of aberrant hepatocyte growth factor receptor (Met) signaling is thought to mainly rely on its mitogenic and anti-apoptotic effects. Recently, however, cumulating evidences suggest that genomic instability may be a crucial factor in tumorigenesis. Here, we address whether oncogenic Met receptor is linked to the centrosome abnormality and genomic instability. We showed that expression of the constitutive active Met (CA-Met) induced supernumerary centrosomes probably due to deregulated centrosome duplication, which was accompanied with multipolar spindle formation and aneuploidy. Interestingly, LY294002, a phosphoinositide 3-kinase (PI3K) inhibitor, significantly suppressed the appearance of supernumerary centrosomes. Moreover, knockdown of Akt with small interfering RNAs and overexpression of phosphatase and tensin homolog or dominant-negative Akt abrogated supernumerary centrosome formation, evidencing the involvement of PI3K signaling. We further showed that expression of CA-Met significantly increased aneuploidy in p53+/−/HCT116 cells but not in p53+/−/HCT116 cells, indicating that the ability of CA-Met to induce chromosomal instability (CIN) phenotype is related with p53 status. Together, our data demonstrate that aberrant hepatocyte growth factor/Met signaling induces centrosome amplification and CIN via the PI3K–Akt pathway, providing an example that oncogenic growth factor signals prevalent in a wide variety of cancers have cross talks to centrosome abnormality and CIN.

Introduction

The Met tyrosine kinase is a high-affinity receptor for hepatocyte growth factor (HGF) (1,2). Both Met and HGF are expressed in various tissues, and signaling via this receptor–ligand pair has been shown to affect a variety of biological activities, including cell growth (3,4), cellular motility (5) and angiogenesis (6). Aberrant activation of HGF/Met signaling via paracrine or autocrine activation has been implicated in the generation and spread of many types of human cancers, including major carcinomas as well as sarcomas (7). Notably, activating missense mutants of Met are found in human hereditary papillary renal carcinomas (8) and these mutants have been shown to possess constitutive kinase activity and transforming ability (9). p53-/- HCT116 cells, indicating that the ability of CA-Met to induce chromosomal instability (CIN) phenotype is related with p53 status. Together, our data demonstrate that aberrant hepatocyte growth factor/Met signaling induces centrosome amplification and CIN via the PI3K–Akt pathway, providing an example that oncogenic growth factor signals prevalent in a wide variety of cancers have cross talks to centrosome abnormality and CIN.

Materials and methods

Antibodies, plasmids and chemicals

Fetal bovine serum, trypsin–ethylenediaminetetraacetic acid, antibiotic–antimycotic containing penicillin G sodium, streptomycin sulfate and amphotericin B were obtained from Gibco BRL (Carlsbad, CA). Protease inhibitor cocktail was purchased from Roche Applied Science (Mannheim, Germany). Phosphatase inhibitors, nocodazole, U0126 and LY294002 were from Sigma Chemical Co. Antibody against the N-terminal half of Cep-170 (Carnegie Institution of Washington) and antibodies against p44/42 mitogen-activated protein kinase were purchased from Cell Signaling Technology (Beverly, MA): phopho-p44/42 mitogen-activated protein kinase (MAPK), p44/42 MAPK, phospho-p38 MAPK, p38 MAPK, phospho-stress-activated protein kinase/c-Jun N-terminal kinase (JNK), stress-activated protein kinase/JNK, phospho-Akt and Akt. Mouse monoclonal γ-tubulin antibody was purchased from Sigma Chemical Co., Antibody against the N-terminal half of Cep-170 was generously provided by Dr G.Guarguaglini (University of Rome, Rome, Italy). CA-Met construct, which is wild-type murine Met expression vector, was provided by Dr G.F.VandeWoude (Van Andel Research Institute), SRT
and SRT-tagged WT-Akt constructs were provided by Dr Myong-Joon Hanh (Sungkyunkwan University School of Medicine).

**Cell culture**

HeLa cells purchased from American Type Culture Collection (Manassas, VA) and HCT116 cells generously provided by Dr B Vogelstein (Johns Hopkins University, Baltimore, MD) were cultured in Dulbecco’s modified Eagle’s medium nutrient mixture F-12 HAM (Sigma Chemical Co.) supplemented with 10% (vol/vol) fetal bovine serum (Gibco BRL) in 37°C incubator with 5% CO2 in air. To generate HeLa cells overexpressing CA-Met Met, cells were co-transfected with a plasmid pSV2neo (28), which confers resistance to G418, and a plasmid encoding the pMB1-CA-Met Met. MOCK cells were transfected with plasmid pSVneo and pMB1 empty vector. Cells were selected for 2 weeks with 800 μg/ml G418 (Life Technologies, Carlsbad, CA) and then grown as pools with 500 μg/ml G418.

**Cell synchronization**

For mitosis arrest, cells were treated with 100 ng/ml nocodazole for 12 h. To arrest the cells at G1–S boundary, double thymidine block was performed. Cells were treated with 1 μM thymidine for the first synchronization. Twenty-four hours later, cells were washed with thymidine-free medium (first release) and cultured in complete medium for 8 h. Then, cells were cultured again in thymidine-containing medium for 14 h for the second round of synchronization. After that, cells were washed with thymidine-free medium and cultured in complete medium (second or final release).

**Cell cycle analysis by flow cytometry**

Cells synchronized at G1–S boundary by double thymidine block were trypsinized, centrifuged and fixed with 70% cold ethanol for 30 min. After samples were washed with phosphate-buffered saline (PBS) and then resuspended in a solution containing RNase A for 5 min. propidium iodide was added. After 5 min of incubation, samples were subjected to FACS analysis using a FACS Vantage flow cytometer (Becton Dickinson, Franklin Lakes, NJ).

**Giems staining**

MOCK and CA-Met cells were exposed to 100 ng/ml nocodazole for 12 h, and mitotic cells were then collected, incubated at 37°C for 15 min in 0.075 M hypotonic KCl, pelleted and fixed with methanol:acetic acid (3:1) solution. After washing three times with PBS, samples were spread on slide glass, dried at room temperature (RT) and then stained with 3% (wt/vol) Giemsa staining solution.

**Immunoblotting**

Conventional immunoblotting was performed as previously described (29) using corresponding antibodies. Briefly, cell lysates (50 μg) were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and then were transferred to nitrocellulose membrane. After blocking for 1 h at RT with PBS containing 0.05% (vol/vol) Tween-20 and 5% (wt/vol) nonfat milk, membranes were incubated with primary antibodies at 4°C, followed by washing with PBS containing 0.05% Tween-20 and incubated with horseradish peroxidase-conjugated anti-rabbit or anti-mouse antibody (Amersham Biosciences, Piscataway, NJ) for 1 h at RT. Detection was carried out using ECL reagents (Amersham Biosciences) and exposing them to x-ray film.

**Immunofluorescence analysis**

Cells were fixed in methanol:acetone (1:1) solution and permeabilized with 0.075% Triton X-100. Fixed cells were pre-incubated in blocking solution (1% bovine serum albumin in PBS), followed by incubation overnight with primary antibodies at 4°C. Cells were then washed three times with shaking and probed with a rhodamine-conjugated anti-rabbit antibody (1:1000; Jackson Immuno-Research Laboratories, West Grove, PA) or fluorescein isothiocyanate-conjugated anti-mouse antibody for 1 h at RT. After washing, cells were mounted in the mounting solution containing 4’,6-diamidino-2-phenylindole and examined by confocal microscope (LSM510; Carl Zeiss, Oberkochen, Germany).

**Knockdown experiment**

Pre-designed siRNA oligonucleotides targeting for Akt (Akt1 and Akt2) were purchased from Life Technologies. HeLa cells (1 x 10^5) were seeded in 35 mm dishes. After 24 h, cells were transfected with 200 nmol each of specific or non-silencing control siRNA oligonucleotides using Oligofectamine according to manufacturer’s instruction (Life Technologies).

**Results**

**Constitutive activation of Met signaling induced supernumerary centrosomes and multipolar spindle formation**

In order to address whether oncogenic Met tyrosine kinase receptor is linked to centrosome abnormality, we utilized the M1268T mutant, a strong constitutively activating form of Met that was originally found in human papillary renal cancer (8). Stable cell lines were established by transfecting pMB1-M1268T complementary DNA into HeLa cells, followed by selection with G418. Among >50 clones, different clones (#1, #3 and #5) expressing M1268T were selected and analyzed for the Met activation. To determine the status of tyrosine phosphorylation of Met in these clones, cell lysates were subjected to immunoprecipitation using anti-murine Met antibody, followed by western blot analysis with anti-phosphotyrosine antibody. All these clones exhibited significant increases in the levels of tyrosine phosphorylation of Met (Figure 1A), verifying constitutively active states of Met (CA-Met). Under microscope, all these clones also showed similar scattered growth pattern (data not shown), which is an indicator of cells with Met activation (30,31).

We then determined the number of centrosomes in asynchronously growing cells by staining for γ-tubulin and counting >200 cells in each experiment. We found that ~2% of control cells transfected with the pMB1 vector contained supernumerary centrosomes (n ≥ 3). In contrast, 6–9% of cells expressing CA-Met showed supernumerary centrosomes (Figure 1B), which was 3- to 4-fold higher than that of control cells (P < 0.005). Since different CA-Met clones did not yield differences in the activation of Met and in the formation of supernumerary centrosomes, we rationalized that these CA-Met clones can be pooled for further analysis.

At mitotic phase, centrosome duplication and segregation are completed and therefore, it is better to determine supernumerary centrosomes in mitotic cells. To count the number of centrosomes in mitotic phase, cells were arrested at prometaphase by nocodazole treatment and then released from the drug. At 60 min after release when most of cells remained at mitosis, numbers of centrosomes in each cell were determined by staining for γ-tubulin (Figure 1C, upper panel). Whereas ~5% of the control cells contained ≥2 centrosomes (supernumerary centrosomes), ~25% of cells expressing CA-Met contained supernumerary centrosomes, which was significantly higher than that of control cells (Figure 1C, lower panel). In addition, tripolar and tetrapolar mitotic spindles with multidirectional chromosomal staining were significantly increased in CA-Met cells (15.8 ± 2.7% versus 2.4 ± 0.5%, P < 0.005) as shown in Figure 1D. Furthermore, chromosome segregation defects such as bridge chromosomes in anaphase cells were often observed (Figure 1E, upper panel), and the frequency was significantly higher than that of control cells (P < 0.005) (Figure 1E, lower panel).

Supernumerary centrosomes can be generated either by multiple cycles of centrosome duplication in a single cell cycle or by cytokinesis failure itself without overduplication of centrosomes (20). Cells with supernumerary centrosomes often fail to undergo cytokinesis due to formation of aberrant spindles. In turn, the cytokinesis block plays a secondary role in generation of supernumerary centrosomes. To investigate the possibility of centrosome duplication, we analyzed Cep170 protein, which localizes to only one mature centrosome from G1 to early G2 phases, whereas two Cep170-positive centrosomes can be shown through late G2 to mitotic phase as two separated centrosomes (32). In cells showing four centrosomes due to S phase arrest by hydroxyurea treatment, most contained one to three Cep170-positive staining and no cells showed four Cep170-positive staining (supplementary Figure 1 is available at Carcinogenesis Online). In contrast, cytokinesis failure by itself induced by cytochalasin D treatment greatly increased cells with four Cep170-positive staining up to ~70%. Meanwhile, ~80% of CA-Met cells contained one to three Cep170-positive staining, whereas <20% of them displayed four Cep170-positive staining (supplementary Figure 1 is available at Carcinogenesis Online). These results indicate that supernumerary centrosomes in CA-Met cells are mainly driven by overduplication of centrosomes during cell cycle and partly by division failure. Together, this is the first indication that constitutive active Met signaling induces supernumerary centrosomes probably via deregulation of centrosome duplication cycle, which is probably to contribute to multipolar spindle formation and chromosomal bridge formation.
CA-Met enhanced CIN

It has been shown that cells with severe aberrant chromosomes do not survive through cell cycle progression, whereas cells with relatively mild alterations in chromosome survive, increasing the chance for accumulation of CIN (33). To address whether the cells with supernumerary centrosomes and multipolar spindle survived and revealed accumulation of CIN in interphase cells, we measured the frequency of multinuclei (Figure 2A, a') and micronuclei formation (Figure 2A, b') that would be derived from aberrant chromosomal segregation during mitosis. As shown in the right panel of Figure 2A, numbers of multilucleated cells and micronuclei-containing cells were significantly increased in CA-Met cells compared with control cells (P < 0.05). The same phenomenon was also observed in cells transiently transfected with M1268T expression vector (Figure 2B), showing supernumerary centrosomes and subsequent multinuclei/micronuclei formation. Moreover, we observed that repetitive exposure to HGF induces supernumerary centrosomes in WI-38 human fibroblast cells (supplementary Figure 2 is available at Carcinogenesis Online), evidencing that prolonged activation of HGF/Met signaling contributes to accumulation of CIN.

Increasing extent of aneuploidy is one of the most representative indicator of CIN (34). Using metaphase spread, we first determined numbers of chromosomes from vector control cells (n = 300) and then divided them into three categories—N1, N2 and N3 (Figure 2C). The average number of chromosomes per one cell was determined as 59.2 in parental HeLa cells. To quantitatively express the extent of newly generated aneuploidy, we formulated, so called, an 'aneuploidy index'. The 'N2' window was defined as the window around the peak that 95% of cell population from parental HeLa cells belonged to and the aneuploidy index was defined as \( \left( \frac{N1 + N3}{N1 + N2 + N3} \right) \times 100 \% \). Thus, the aneuploidy index from parental HeLa cells should be 5% and the same window
was applied to either vector control cells or CA-Met cells for the comparison. We observed that the average number of chromosomes in CA-Met cells (average 5.62.6) was similar to that in vector cells but the CA-Met cells displayed a broader range of chromosome numbers (Figure 2C, lower panel) as expected by their large standard deviation (SD 5.16.7). Notably, the aneuploidy index of CA-Met cells was determined as 19.7% that was significantly higher than that of vector control cells (7.44%). Collectively, CA-Met enhanced the extent of CIN probably through the formation of supernumerary centrosomes.

**Activation of the PI3K–Akt pathway was necessary for centrosome amplification**

Cdk2/cyclin E kinase is a major player initiating centrosome duplication at G1/S phase and constitutive activation of Cdk2/cyclin E often leads to centrosome amplification (35,36). We first determined whether Cdk2/cyclin E kinase activity remained elevated in CA-Met cells using in vitro kinase assay using S phase cell lysates by releasing cells from the second thymidine block. We found that Cdk2/cyclin E kinase activity was not different between control and CA-Met cells (supplementary Figure 3 is available at Carcinogenesis Online), indicating that Cdk2/cyclin E was not a causative factor for centrosome amplification in CA-Met cells. We next assumed that cellular signaling kinases downstream of CA-Met might be involved in centrosome amplification. As shown in Figure 3A, western blot analysis revealed increased levels of phospho-ERK and phospho-Akt but not of phospho-p38 or phospho-JNK in CA-Met cells. Densitometry quantification revealed ~8-fold increase of phospho-ERK and 2-fold increase of phospho-Akt (Figure 3A, right panel) compared with control cells. To address which signaling pathway is responsible, we next
applied chemical inhibitors against the ERK or PI3K to CA-Met cells to investigate their effects on the centrosome amplification. Synchronized cells were treated with each signaling inhibitor (U0126 for MEK1 and LY294002 for PI3K) and subjected to centrosome analysis. We analyzed only the cells in the mitotic phase (mainly metaphase) because these inhibitors may also interfere with cell cycle progression (37). Interestingly, pretreatment with LY294002, but not U0126, significantly reduced the number of CA-Met cells with multiple centrosomes (Figure 3B, \( P < 0.05 \)). Moreover, inhibition of the PI3K pathway by LY294002 abrogated multinucleated cell formation in CA-Met cells (Figure 3C, \( P < 0.005 \)). At the concentration used for the experiment, both signaling pathways were effectively blocked as shown in Figure 3D.

To verify the importance of Akt in centrosome amplification of CA-Met cells, we suppressed Akt activity by knockdown using siRNA or by overexpressing the DN-Akt or phosphatase and tensin homolog (PTEN), the phosphatase for PI3K products. Two different siRNAs specifically targeting Akt messenger RNA (Life Technologies) was applied to CA-Met cells 48 h before nocodazole treatment and resulted in a clear abrogation of centrosome amplification (Figure 4A, \( P < 0.05 \)) as well as multinucleated cell formation (Figure 4B, \( P < 0.05 \)). Consistently, transfection of DN-Akt or PTEN into CA-Met cells also significantly suppressed centrosome amplification as well as multinucleated cell formation (Figure 4C and D). At the concentration used for the experiment, both signaling pathways were effectively blocked as shown in Figure 3D.

Increase of aneuploidy by CA-Met was observed in p53\(^{-/-}\) cells, but not in p53\(^{+/+}\) cells
Loss of p53 tumor suppressor function in cells leads to severe impacts on centrosome duplication cycle (38) as well as maintenance of genomic integrity (39). The p53 level in HeLa cells is very low, if any, because E6 and E6AP actively ubiquitinates and induces...
degradation of the p53 protein (40). In order to clarify the role for p53 in CA-Met-mediated CIN, we employed HCT116 human colon cancer cells with the background of p53^+/+ or p53^−/− status. HCT116 p53^+/+ and HCT116 p53^−/− cells were transfected with CA-Met expression vector and treated with G418 (500 μg/ml). The resultant stable clones (~100) were pooled and further cultivated for 2 months. First, we compared the number of centrosomes in these cells. Notably, overexpression of CA-Met in HCT116 p53^+/+ cells did not increase supernumerary centrosomes (Figure 5A). In contrast, cells with supernumerary centrosomes were already abundant in HCT116 p53^−/− cells, reaching to ~30% and overexpression of CA-Met did not cause a significant increase in these cells (Figure 5A). Next, we examined for progression of karyotypic profiles upon cell passages. The cells at the time of antibiotics selection were indicated as 0 month and further collected after 1 and 2 months through continuous passages. The passage numbers of each cell were essentially the same. In each analysis, three hundred metaphase cells were analyzed for karyotypic profiles. Notably, we observed that expression of CA-Met did not increase aneuploidy in HCT116 p53^+/+ cells (Figure 5B) that is different from what we have observed in HeLa cells (Figure 2B), suggesting that lack of p53 activity in HeLa cells probably contributed to aneuploidy formation. Indeed, the aneuploidy indice of HCT116 p53^+/+ cells were significantly higher than those of HCT116 p53^−/− cells through cell passages (Figure 5B and C). Moreover, expression of CA-Met significantly increased aneuploidy in p53^−/− HCT116 cells (Figure 5C). CA-Met expression in HCT116 p53^−/− cells resulted in almost 3-fold higher aneuploidy index than that in vector control cells at 1 month (Figure 5C, middle panel). The increased aneuploidy index in HCT116 p53^−/− cells also remained high after 2 months. Increase in the aneuploidy index was even observed at 0 month (29.1% for CA-Met versus 17.0% for control) because several rounds of cell proliferation had already occurred during the selection process. However, the average numbers of chromosomes were not significantly different among any of the cells tested (data not shown), suggesting that these cells might undergo relatively equivalent gain and loss of chromosomes. Together, our data indicate that the increase of aneuploidy by CA-Met is related with the p53 status of the cell.

Given that the Akt activation is important for centrosome amplification and multinucleated cell formation in CA-Met cells, inhibition of Akt activation may reduce aneuploidy formation. To address that,
we transfected M1268T complementary DNA along with PTEN into HCT116 cells lacking p53 gene (HCT116 p53−/− cells) and survival clones were selected in the presence of G418 (500 μg/ml). Western blotting showed that CA-Met and PTEN proteins were properly expressed in these cells (Figure 6A). Notably, the aneuploidy index in HCT116 p53−/− cells was relatively high (Figure 6B), which is almost twice of HeLa cells (Figure 2C), suggesting that loss of p53 contributed to increased aneuploidy in these cells. Expression of CA-Met increased the aneuploidy index from 15 to 26%, whereas co-expression of PTEN partly suppressed the CA-Met-induced aneuploidy formation (Figure 6B). Collectively, these data suggest that CA-Met-induced CIN is at least partly mediated by the PI3K–Akt pathway.

Fig. 5. Aneuploidy induction by CA-Met is related with p53 status. (A) HCT116 p53+/− and p53−/− cells were transfected with vector or CA-Met and selected in the presence of G418 (500 μg/ml) to establish stable cell lines. The numbers of centrosomes were determined by staining with anti-γ-tubulin antibody. (B and C) The same cell lines were analyzed for the aneuploidy index. The cells at the time of selection were designated as cells at 0 month, and cell samples were collected 1 and 2 months later. Metaphase spread of indicated cells was prepared by treatment with nocodazole for 12 h, and Giemsa staining was performed to count the chromosome numbers in 100 cells. The numbers of cells with the designated chromosome number on the x-axis are presented. Western blot analysis was performed to verify the presence or absence of p53 and/or CA-Met from the indicated cell lysates (inset).
Furthermore, 4 of 10 tumor explants induced by injection of tetraploid chromosome numbers in 100 cells. The numbers of cells with the designated nocodazole for 12 h, and Giemsa staining was performed to count the

Expression of Met and PTEN were determined by western blotting.

Discussion

Oncogenesis is a complicated process that mediates many different cellular and physiological events. Met is overexpressed and mutated in a variety of human malignancies (15) and its pleiotropic actions on angiogenesis (41), invasion, cellular motility (5), cell growth (3,4) and cellular and physiological events. Met is overexpressed and mutated in a variety of human malignancies (15) and its pleiotropic actions on angiogenesis (41), invasion, cellular motility (5), cell growth (3,4) and cellular and physiological events. Met is overexpressed and mutated in a variety of human malignancies (15) and its pleiotropic actions on angiogenesis (41), invasion, cellular motility (5), cell growth (3,4) and cellular and physiological events.

Fig. 6. Reduced aneuploidy formation by overexpression of PTEN in CA-Met cells. (A) HCT 116 p53−/− cells were transfected with CA-Met along with PTEN followed by selection in the presence of G418 (500 µg/ml). Expression of Met and PTEN were determined by western blotting. (B) Metaphase spread of indicated cells was prepared by treatment with nocodazole for 12 h, and Giemsa staining was performed to count the chromosome numbers in 100 cells. The numbers of cells with the designated chromosome number on the x-axis are presented. Aneuploidy indices were calculated as described in the Materials and Methods.

Reduced aneuploidy formation by overexpression of PTEN in CA-Met cells. (A) HCT 116 p53−/− cells were transfected with CA-Met along with PTEN followed by selection in the presence of G418 (500 µg/ml). Expression of Met and PTEN were determined by western blotting. (B) Metaphase spread of indicated cells was prepared by treatment with nocodazole for 12 h, and Giemsa staining was performed to count the chromosome numbers in 100 cells. The numbers of cells with the designated chromosome number on the x-axis are presented. Aneuploidy indices were calculated as described in the Materials and Methods.

Discussion

Oncogenesis is a complicated process that mediates many different cellular and physiological events. Met is overexpressed and mutated in a variety of human malignancies (15) and its pleiotropic actions on angiogenesis (41), invasion, cellular motility (5), cell growth (3,4) and cellular and physiological events. Met is overexpressed and mutated in a variety of human malignancies (15) and its pleiotropic actions on angiogenesis (41), invasion, cellular motility (5), cell growth (3,4) and cellular and physiological events.
cells. Therefore, it is probable that the critical role of p53 in prevention of CIN in our system is due to its dual role, namely prevention of centrosome amplification and removal of cells with CIN.

In summary, our data show that HGF/Met signaling induces centrosome amplification and CIN via the PI3K-Akt pathway. Thus, it seems that oncogenic growth factor/growth factor receptor not only control cell cycle progression but also clearly have cross talk to regulate mitosis and that the widespread appearance of CIN in human cancers might be due to the widespread abnormalities in growth factor signaling in human cancers.

**Supplementary material**

Supplementary Figures 1–3 can be found at http://carcin.oxfordjournals.org/

**Funding**

Korea Science and Engineering Foundation through Chronic Inflammatory Disease Research Center (R13-2003-019).

**Acknowledgements**

We thank Dr Woon Ki Paik (Korea University, Seoul, Korea) for his critical review of this manuscript, Dr G.F.Vande Woude (Van Andel Research Institute, MI 49503, USA) for CA-Met cDNA, Dr Myong-Joon Hanh (Sungkyunkwan University School of Medicine, Suwon, Korea) for SRT antibody and SRT-tagged WT-Akt constructs, Dr G.Guarguaglini (University of Rome, Rome, Italy) for anti-Cep170 antibody and Dr B. Vogelstein (Johns Hopkins Oncology, Baltimore, USA) for HCT116 cells.

**Conflict of Interest Statement:** None declared.

**References**


Received July 10, 2009; revised June 10, 2010; accepted June 13, 2010