

ARQ 197 a highly selective inhibitor of c-Met inhibits invasive and metastatic growth of cancer cells

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Abstract

Invasive growth and metastasis is the ultimate cause of death in cancer patients. The c-Met receptor tyrosine kinase is a key molecular driver of such malignant processes. The activation of c-Met mediates various cellular responses including proliferation, survival, motility, invasion and metastasis. c-Met expression has been shown to correlate with poor clinical outcome and likelihood of metastasis. Therefore, inhibitors of c-Met tyrosine kinase may block invasive and metastatic growth of cancer cells. ARQ 197 is a potent and selective c-Met kinase inhibitor that has been shown to inhibit HGF/c-met induced cellular responses *in vitro*. To investigate a potential therapeutic role for the c-Met receptor in metastasis and invasive growth, we examined the effect of ARQ 197 on migration and invasion in NCI-H441 cells. Activation of c-Met with HGF triggered signaling via the ERK cascade mediated by sequential phosphorylation of MEK1/2 and MAPK and induction of cell invasion in NCI-H441 cells. Treatment with ARQ 197 resulted in a decrease in phosphorylation of the MAPK signaling cascade and inhibition of invasion and migration. Furthermore, when c-Met siRNA was stably transfected in NCI-H441 cells, the cell migration was completely abrogated. Ectopic expression of c-Met in NCI-H661, a cell line having no endogenous expression of c-Met, caused it to acquire an invasive phenotype that was inhibited by ARQ 197. Taken together, these findings suggest that ARQ 197, by virtue of its selective inhibition of c-Met, may block the invasive growth and metastasis cancer cells.

Figure 1

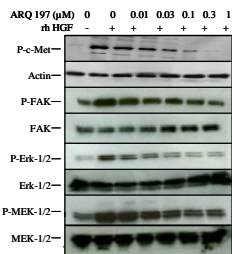


Figure 1. Characterization of the Effect of ARQ 197 on Met Signaling and its Downstream Targets in Cancer Cells: The effect of ARQ 197 on the phosphorylation of the Met receptor and representative downstream signaling molecules was examined in NCI H441 cells. NCI-H441 is a cell line derived from a papillary lung adenocarcinoma, is known to express high levels of c-met but does not demonstrate constitutive phosphorylation of c-Met in our hands. Cells were grown to confluence in full serum medium and were then starved in 0.5% FBS medium in the absence or in the presence of indicated concentrations of ARQ 197 for 16 hours and then either left unstimulated (-) or stimulated (+) with 100 ng/ml rhHGF for 10 minutes. The phosphorylation levels of c-Met and its downstream signaling molecules such as MEK1/2, ERK1/2 and FAK were assessed by immunoblotting using specific antibodies. ARQ 197 was able to inhibit HGF-induced phosphorylation of c-Met with an approximate IC₅₀ of 0.1 μM. In addition, ARQ 197 was able to inhibit HGF-induced downstream targets such as ERK1/2, MEK1/2 and FAK.

Table 1 The Ability of ARQ 197 to Inhibit Cancer Cell Proliferation

Cell Line	Tissue	Expression of c-Met	Invasion	IC ₅₀ (μM)
A549	Lung (NSCLC)	Yes	Yes	0.375
DBTRG	Brain	Yes	Yes	0.452
NCI-H441	Lung (NSCLC)	Yes	Yes	0.295
NCI-H661	Lung (NSCLC)	No	No	5.42

Figure 2

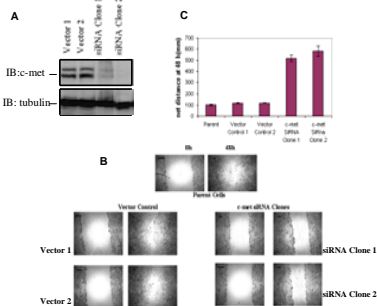


Figure 2. Inhibition of c-Met Alters the Migration of NCI-H441 Cells: (A) NCI-H441 cells were stably transfected with short-hairpin RNA constructs targeting the c-Met gene. Three types of c-Met RNAi constructs were used. Each construct contained a 21 bp sequence targeting different regions of Met and was cloned into the pRNAT-U6.1/Neo vector. The clones were selected by supplementing the medium with G418 and the c-Met knockdown expression was detected by Western blotting (B). The effect of stable c-Met siRNA expression in NCI-H441 cells on migration was assessed. NCI-H441 cells with c-Met siRNA expression clones 1 & 2 exhibited a significantly lower rate of migration compared with either the vector alone (clones 1 & 2) or the parental cells (Fig 2B). (C) The cell migration was quantified by taking pictures at fixed positions and by using Adobe Photoshop tool. These results suggest that inhibition of the HGF/c-Met pathway can affect cell migration.

Figure 3

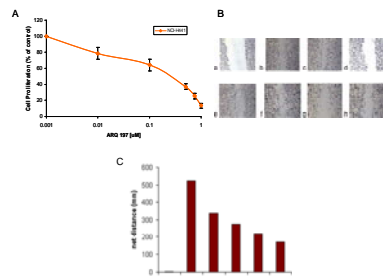


Figure 3. ARQ 197 Inhibits Cell Proliferation of Cancer Cells and Migration of NCI-H441 Cells: In order to correlate the genetic knockdown of c-met siRNA with ARQ 197 phenotype, we examined the effect of ARQ 197 on cell proliferation and migration of NCI-H441 cells. (A) 5000 cells were seeded onto 96-well plates in RPMI 1640 medium containing 10% FBS. The cells were allowed to attach and next day fresh complete medium containing varying concentrations of ARQ 197 was added. After incubation for 48h, the cell proliferation reagent WST-1 was added. The amount of WST-1 formazan produced was measured at 450 nm using an enzyme-linked immunosorbent assay reader. ARQ 197 significantly inhibited cell growth at an approximate IC₅₀ of 0.1 μM. (B) To test the effect of ARQ 197 on cell motility, a scratch wound migration assay was performed. Cells were seeded in 6 well tissue culture plates and cultured in complete serum conditions. The cells were allowed to grow to confluence. At confluence a gap was created by scraping cells with P200 pipette tip. Cells were then treated with HGF (100 ng/ml) to allow the migration of cells across the gap in the presence of increasing concentrations of ARQ 197. The medium was washed off at 24 hours and the cells were stained with Geimsa stain. (a) Untreated at 0 hour, (b) Untreated at 24 hours, (c) HGF (100 ng/ml) for 24 hours, (d) ARQ 197 at 1 μM alone for 24 hours, (e-h) HGF + ARQ 197 at 0.1, 0.5, 0.75, 1 μM for 24 hours. The plates were photographed at indicated time period. (C) Cell migration was quantified by taking pictures at fixed positions and by using Adobe Photoshop tool. As shown in the figure the treatment with ARQ 197 significantly reduced the rate of migration in NCI-H441 cells.

Figure 4

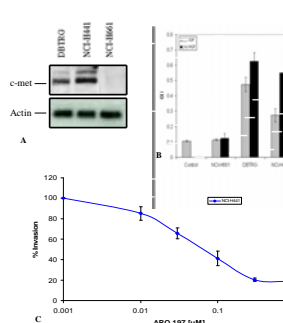


Figure 4: ARQ 197 Inhibits Invasion of c-Met Expressing Cancer Cells. (A) NCI-H441, NCI-H661, and DBTRG were assessed for expression of c-met by western blot. (B) 300 μl cell suspensions were placed in the invasion assay inserts with or without HGF (100 ng/ml). The cells were allowed to invade through a polycarbonate membrane coated with extracellular matrix proteins for 24 hours. (C) NCI-H441 cells were pretreated with indicated concentrations of ARQ 197 for 16 hours. 300 μl of each cell suspension (at a concentration 0.5 × 10⁶ cells/mL in serum free medium) was placed in individual inserts and incubated for 24 hours at 37°C.

Figure 5

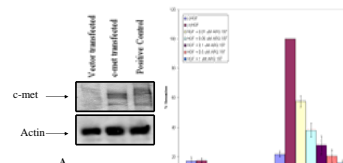


Figure 5. Overexpression of c-Met in NCI-H661 Cells Induces an Invasive Phenotype which is Pharmacologically Reversed by ARQ 197: (A) c-Met was transiently transfected into a c-Met null cell line (NCI-H661), and 24 hours after transfection, the cells were harvested, assessed for protein expression (B) NCI-H661 cells transiently transfected with either control vector or c-Met expressing vector and subjected to a matrigel invasion assay and the effect of increasing concentrations of ARQ 197 was assessed. NCI-H661 cancer cells lacking c-Met protein expression were unable to show an invasive phenotype. Overexpression of c-Met in NCI-H661 cells produced an invasive phenotype in the presence of HGF. ARQ 197 strongly inhibited the invasion of the c-Met over expressing NCI-H661 cell line. These data support the hypothesis that the anti-metastatic activity of ARQ 197 may be ascribable to the inhibition of c-Met kinase recapitulated in these *in vitro* experimental systems.

Figure 6

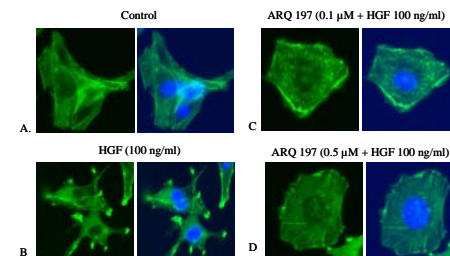


Figure 6. Effect of ARQ 197 on Actin Cytoskeleton in NCI-H441 cells: Serum starved cells (untreated cells) showed low levels of actin polymerization (A). After treatment with 100 ng/ml HGF, NCI-H441 cells displayed high levels of actin polymerization in the periphery of the cells with a distinct pseudopodia formation (B). Treatment of cells with ARQ 197 prior to the addition of HGF caused abrogation of actin polymerization (C & D)

Conclusions

In the present study, we examined *in vitro* effects of inhibiting the c-Met receptor using both a specific c-Met tyrosine kinase inhibitor (ARQ 197) and RNAi technology.

We assessed the ability of ARQ 197 to inhibit c-Met kinase in cells. Our results show that ARQ 197 was able to inhibit HGF induced phosphorylation of c-Met and its downstream targets in a dose dependent manner in NCI-H441 cells.

ARQ 197 significantly blocked a variety of HGF/Met dependent cellular processes including cell proliferation, motility, and invasion. Inhibition of c-Met signaling with either ARQ 197 or c-Met siRNA significantly decreased migration of NCI-H441 cells.

To further understand the role of c-Met in metastasis, we used a cancer cell line that lacks expression of c-met (NCI-H661), which were unable to invade in a matrigel assay. When the same cells overexpressed full length c-Met, the cells gained an invasive phenotype. This phenotype was abrogated by ARQ 197 in a dose dependent manner.

Treatment of NCI-H441 cells with HGF resulted in the increase of actin polymerization. Inhibition of c-Met by ARQ 197 resulted in a significant decrease in actin polymerization.

Taken together, our data demonstrate the effect of a selective c-Met inhibitor, ARQ 197, on a variety of c-Met driven biological functions and signaling events. Targeting c-Met signaling, therefore is potentially attractive as it may not only inhibit cell growth but also lower the rate of migration and invasion in a disease that is clinically characterized by metastasis. Our results indicate that the novel small molecule ARQ 197 provides the therapeutic potential of specifically targeting and inhibiting c-Met in cancers where the c-Met/HGF signaling network plays a central role in tumor growth and/or metastasis.