A New Anti-c-Met Antibody Selected by a Mechanism-Based Dual-Screening Method: Therapeutic Potential in Cancer

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c-Met, the high affinity receptor for hepatocyte growth factor (HGF), is one of the most frequently activated tyrosine kinases in many human cancers and a target for cancer therapy. However, inhibitory targeting of c-Met with antibodies has proven difficult, because most antibodies have intrinsic agonist activity. Therefore, the strategy for reducing the agonism is critical for successful development of cancer therapies based on anti-c-Met antibodies. Here we developed a mechanism-based assay method for rapid screening of anti-c-Met antibodies, involving the determination of Akt phosphorylation and c-Met degradation for agonism and efficacy, respectively. Using the method, we identified an antibody, F46, that binds to human c-Met with high affinity ($K_d = 2.56$ nM) and specificity, and induces the degradation of c-Met in multiple cancer cells (including MKN45, a gastric cancer cell line) with minimal activation of c-Met signaling. F46 induced c-Met internalization in both HGF-dependent and HGF-independent cells, suggesting that the degradation of c-Met results from antibody-mediated receptor internalization. Furthermore, F46 competed with HGF for binding to c-Met, resulting in the inhibition of both HGF-mediated invasion and angiogenesis. Consistently, F46 inhibited the proliferation of MKN45 cells, in which c-Met is constitutively activated in an HGF-independent manner. Xenograft analysis revealed that F46 markedly inhibits the growth of subcutaneously implanted gastric and lung tumors. These results indicate that F46, identified by a novel mechanism-based assay, induces c-Met degradation with minimal agonism, implicating a potential role of F46 in therapy of human cancers.

INTRODUCTION

Receptor tyrosine kinases (RTKs) are often dysregulated in

human cancer cells associated with genetic alternations, such as mutations, translocations, and amplifications (Cragg et al., 1999; Ferrara et al., 2004; Gschwind et al., 2004; Li et al., 2005). The *MET* oncogene and its transforming activity were first identified in a human osteosarcoma cell line. The *c-met* proto-oncogene product, c-Met tyrosine kinase, is the high affinity receptor for scatter factor/hepatocyte growth factor (SF/HGF), which regulates a wide array of biological activities, including cell proliferation, survival, motility, and differentiation. c-Met is mainly expressed in cells of epithelial origin, while the c-Met ligand (HGF) is secreted by mesenchymal cells. Activation of c-Met coordinates cell proliferation, survival, motility, and differentiation processes, resulting in a complex biological process known as invasive growth (Trusolino and Comoglio, 2002). c-Met activation is essential for the survival of hepatocytes and placental trophoblast cells (Birchmeier and Gherardi, 1998) during normal embryonic development. In adults, the HGF/c-Met pathway is latent, being reactivated under numerous physiological and pathological conditions, such as wound healing (Chmielowiec et al., 2007) and tissue regeneration (Borowiak et al., 2004; Huh et al., 2004). In the case of cancer, however, the pathway is abnormally activated (Birchmeier et al., 2003; Boccaccio and Comoglio 2006; Bussolino et al., 1992; Comoglio and Trusolino 2002). In fact, c-Met is one of the most frequently activated tyrosine kinases in human cancers. Moreover, it is over-expressed in a variety of epithelial tumors, and generates signals that trigger several steps critical to oncogenesis, including the epithelial-to-mesenchymal transition, extracellular matrix degradation, tumor invasion, and metastasis (Birchmeier et al., 2003; Comoglio and Trusolino, 2002; Trusolino and Comoglio, 2002). In addition, c-Met has been shown to be tightly associated with the resistance to cancer therapies by targeting the EGFR and VEGF pathways (Bussolino et al., 1992).

Reflecting the critical roles in cancer, c-Met and its HGF ligand have become leading candidates for targeted cancer therapies (Burgess et al., 2006; Cao et al., 2001; Jo et al., 2011; Kim

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et al., 2006). However, generation and development of inhibitory antibodies targeting c-Met has been difficult because the divalent structure of antibodies often activates c-Met signaling via receptor dimerization and cross-activation (Ohashi et al., 2000; Prat et al., 1998). Therefore, it is necessary to develop a strategy for generation of therapeutic anti-c-Met antibodies that can efficiently block c-Met action (e.g., by preventing HGF binding to c-Met) without activation of its downstream signals. Many human cancers are featured by gene amplifications or activating mutations where c-Met becomes aberrantly activated and over-expressed. Therefore, therapeutic antibodies should preferentially induce c-Met degradation for its removal from the tumor cell surface and consequent down-regulation. To this end, we devised a novel mechanism-based screening method for selecting anti-c-Met antibodies that can down-regulate c-Met without inducing Akt phosphorylation, a major c-Met downstream oncogenic signaling. Using this approach, we successfully identified an antibody, termed F46 that potently inhibits the growth of c-Met-addicted tumor cells. F46 was also capable of attenuating angiogenesis and tumor cell invasion. Furthermore, F46 could block *in vivo* tumor growth, implicating its potential use in cancer therapy.

MATERIALS AND METHODS

Cell lines and cell cultures

The human gastric carcinoma MKN45 (JCRB0254) cell line was purchased from Health Science Research Resource Bank. A549 gastric carcinoma cell lines (CCL-185), Caki-1 renal carcinoma cell lines (HTB-46), and NCI-H441 human lung adenocarcinoma cell lines (HTB-174) were obtained from American Type Culture Collection (ATCC). Human primary hepatocytes (PHH) were purchased from Celsis. MKN45, A549, and NCI-H441 were cultured in RPMI1640 medium (GIBCO) and Caki-1 were cultured in DMEM medium containing 10% (v/v) of fetal bovine serum (FBS, GIBCO) and 1% (v/v) of penicillin/streptomycin (GIBCO). Cells were incubated at 37°C in a humidified atmosphere containing 5% CO₂ and subcultured prior to confluence. Cell counting was performed with the CEDEX Analyzer (Roche Diagnostics).

Tumor xenograft study

To study the effect of F46 on tumor growth *in vivo*, xenograft studies were performed using 5-6 weeks old male BALB/c Nude mice. Mice were acclimated at least for a week before they received tumor inoculation. NCI-H441 or MKN45 cells (5×10^6) were subcutaneously injected into the right flank region of mice under anesthesia by 1-2% isoflurane. After 7 days, when the average tumor size was above 50 mm³, mice were randomized into treatment groups. For NCI-H441 xenograft, mice were treated as follows: 5D5 (10 mg/kg IV, once a week), F46 (10 mg/kg IV, once a week), and vehicle (PBS 0.2 ml IV, once a week). For MKN45 xenograft, mice were treated as follows: F46 (10 mg/kg IP, twice a week), and vehicle (PBS 0.2 ml IP, twice a week). Each treatment groups consisted of 12-15 mice.

CD31 and Ki67 staining

Tumor tissues were fixed in 10% formaldehyde, embedded in paraffin, cut with a microtome in 4 µm sections, mounted on slides, and deparaffinized. The antigen retrieval process involved boiling in 10 mM sodium citrate buffer and immersing in 3% H₂O₂. Sections were then incubated with 100 µl of 1:50 rabbit polyclonal anti-CD31 antibody or 1:100 rabbit monoclonal anti-Ki67 (Abcam) for 60 min at room temperature. Slides were

treated with 100 µl of secondary antibody (polymerized HRP-anti rabbit IgG antibody) for 30 min at 30°C. Color development using DAB solution was performed for 10 min and the slides were counterstained with hematoxylin. CD31 expression area and nuclear Ki67 positive cells were quantified using Image-Pro Plus software (MediaCybernetics).

RESULTS

Identification of F46, an anti-c-Met antibody, by a mechanism-based screening method

Previous attempts to inhibit c-Met using antibody-based approaches have been unsuccessful, as most antibodies have intrinsic agonist activity. For example, 5D5 binds human c-Met with high affinity, but it has high agonist activity that promotes cancer cell proliferation. Thus, we developed a unique mechanism-based screening method for the selection of functional c-Met antibodies that show high affinity to c-Met with low agonist activity. This method comprises two steps: assaying Akt phosphorylation in Caki-1 renal carcinoma cells, a widely used cell line for c-Met signal activation (Nakagawa et al., 2010), and determining the stability of c-Met in MKN45 cells over-expressing c-Met. 5D5, a known agonist c-Met antibody, served as a control. First, we selected antibodies that bind to the extracellular domain (ECD) of human c-Met (data not shown). The selected antibodies were then subjected to a high throughput assays for Akt phosphorylation by using ELISA. Figure 1A shows that several antibodies, such as A9 and A10, have little or no effect on Akt phosphorylation, implying that the antibodies do not exert any agonist activity. However, those antibodies did not induce c-Met degradation (Fig. 1B), suggesting that they show no efficacy for inhibiting c-Met addicted tumor cell proliferation. On the other hand, F46 induced c-Met degradation by about 60% with a marginal Akt phosphorylation in comparison with 5D5. Furthermore, F46 showed a high affinity to c-Met ($K_d = 2.56$ nM), which is comparable to that of 5D5 ($K_d = 1.18$ nM), as determined by surface plasmon resonance (Fig. 1C, top). In addition, F46 showed little or no interaction with other c-Met related receptors, such as RON and Plexin A2, indicating that F46 selectively binds to c-Met (Fig. 1C, bottom).

F46 promotes c-Met degradation with low agonism in c-Met signaling

To clarify whether F46 indeed induces c-Met degradation with low agonism, immunoblot analysis was performed by using anti-c-Met antibody for determining c-Met stability, anti-pTyr antibody specific to Y1234/1235 in c-Met for assaying its activation, and anti-pSer antibody specific to S473 in Akt for detecting the activation of c-Met downstream signaling. F46 strongly down-regulated the expression of c-Met (i.e., induced its degradation) as 5D5 did (Fig. 2A), consistent with the data determined by using ELISA (Fig. 2B). Notably, F46 induced c-Met phosphorylation to an extent much lower than that seen by 5D5 (Fig. 2C). Likewise, the ability of F46 to induce Akt phosphorylation was much lower than that of 5D5, which promoted the Akt activation as well as HGF (Fig. 2E). These results are again consistent with the data obtained by using ELISA (Figs. 2D and 2F), confirming that F46 exerts a significantly lower agonist activity than 5D5.

In addition to Akt, Gab1 serves as a major mediator of the c-Met signaling cascade (Trusolino et al., 2010). Therefore, we compared the agonist activity of F46 with that of 5D5 by measuring the level of Gab1 phosphorylation. F46 induced Gab1 phosphorylation to an extent much lower than that seen with

Fig. 1. Identification of F46, an anti-c-Met antibody, by a mechanism-based screening method. (A) Akt phosphorylation in Caki-1 cells was measured by ELISA using anti-p-Akt antibody as described under “Materials and Methods” in Supplementary Information. Caki-1 cells were incubated with c-Met antibodies (5 μ g/ml) for 30 min. (B) c-Met degradation in MKN45 cells was measured by ELISA. Cells were treated with anti-c-Met antibodies (5 μ g/ml) and incubated for 24 h. The error bars indicate the mean $\pm$ SD of three independent experiments. (C) Binding kinetics of F46 and 5D5 to human c-Met extracellular domain (top), RON and Plexin A2 (bottom) were measured by surface plasmon resonance assay. Representative results from two independent experiments are shown.

Fig. 2. F46 promotes c-Met degradation with low agonism in c-Met signaling. MKN45 cells treated with 5 μ g/ml of F46 or 5D5 were incubated for 24 h. c-Met degradation was measured by Western blot (A) and ELISA (B) as described under “Materials and Methods” in Supplementary Information. Phosphorylation of c-Met and Akt in Caki-1 cells was measured by Western blot (C, E) and ELISA (D, F). Caki-1 cells were incubated for 30 min with 5 μ g/ml of F46 or 5D5 or 100 ng/ml of HGF. The error bars indicate the mean $\pm$ SD of three independent experiments. Phosphorylation of c-Met downstream kinases in Caki-1 (G) and NCI-H441 (I) cells were measured by Western blot. Co-immunoprecipitation experiment showed induced binding between Gab1 and c-Met by 5D5, but not by F46 (H).

Fig. 3. F46 induces c-Met internalization and inhibits HGF-dependent cell invasion and angiogenesis. (A) MKN45 cells were incubated with 5 μ g/ml of F46 for 4 h followed by staining for F46 (red), c-Met (green) and nuclei (blue) as described under “Materials and Methods” in Supplementary Information. (B) MKN45 cells were incubated with increasing amounts of recombinant human HGF. They were added with 0.5 μ g of 5D5 or F46 followed by flow cytometry as described under “Materials and Methods” in Supplementary Information. Δ MFI (y-axis) indicates the decrease in percentage of mean fluorescence intensity (MFI) upon treatment with F46 or 5D5 (top). For the competition ELISA, biotinylated HGF and differing concentrations of IgG, F46, or free HGF were treated on c-Met coated plate (bottom). (C) The Boyden chamber invasion assay was used to measure the anti-invasive ability of F46 in the absence and presence of HGF. The error bars indicate the mean $\pm$ SD of three independent experiments. (* $p < 0.05$, compared with IgG control). (D) HUVECs were incubated with 5 μ g/ml of F46 for 18 h in the absence and presence of HGF. Tube length seen with IgG control was expressed as 100% and the others as its relative values. The error bars indicate the mean $\pm$ SD (* $p < 0.05$, compared with HGF plus IgG control).

5D5 (Fig. 2G). Consistently, the ability of F46 to promote the interaction between c-Met and Gab1 was incomparably lower than that of 5D5 (Fig. 2H). Furthermore, F46 induced the activation of other downstream kinases, such as Shc, Stat1, Erk, Lck, and Akt, much more weakly than 5D5 (Fig. 2I). Collectively, these results indicate that F46, selected by a mechanism-based screening method, could serve as an inhibitory anti-c-Met antibody with low agonist activity.

F46 induces c-Met internalization and inhibits HGF-dependent cell invasion and angiogenesis

Ligand-induced c-Met internalization is a key first step in the regulation of c-Met signaling. Antibody dependent c-Met internalization could promote not only c-Met degradation but also agonist effects. To determine the effect of F46 binding on c-Met trafficking, a direct internalization assay was performed by applying immunocytochemical analysis using MKN45 cell. The results clearly showed the internalization of c-Met upon F46 binding, while F46 unbound c-Met still remained on the cell membrane (Fig. 3A), indicating that F46 efficiently induces c-Met internalization. In addition, co-localization of F46 and c-Met

in this experiment again confirmed the specific binding of F46 on c-Met as discussed in 3.1.

Previously, 5D5 has been shown to compete with HGF for binding to c-Met. To determine whether F46 might also compete with HGF for binding to c-Met, a flow cytometry-based competition assay was performed using MKN45 cells. F46 binding, like that of 5D5, was gradually decreased upon increasing the concentration of HGF that had been pre-treated to cells as assessed by a shift in mean fluorescence intensity (Fig. 3B, top), indicating that F46 and HGF compete with each other for binding to c-Met. A competition ELISA experiment again proved the same conclusion (Fig. 3B, bottom). These results also suggest that F46 and HGF might share the binding site on c-Met.

The HGF/c-Met signaling pathway has been shown to play an important role in cancer cell invasion and angiogenesis (Benvenuti and Comoglio 2007, Hanahan and Weinberg 2011). To determine the effect of F46 on HGF-dependent cell invasion, we carried out a modified Boyden chamber assay (Lamszus et al., 1999; Ma et al., 2005) using A549 cells that do not over-express c-Met protein. HGF treatment led to a marked increase

Fig. 4. F46 inhibits cancer cell proliferation and *in vivo* tumor development. (A) The viability of MKN45 cells was measured by CTG assay after treatment with increasing concentrations of IgG, F46, and 5D5 for 72 h. Growth of the control group (no antibody) was expressed as 100% and the others as its relative values. The error bars indicate the mean $\pm$ SD. (B) After incubation of NCI-H441 lung carcinoma cells with IgG, F46, and 5D5, BrdU incorporation was measured as described under "Materials and Methods" in Supplementary Information. (C) Xenograft studies were performed using 5-6 weeks old male BALB/c nude mice by subcutaneous injection of MKN45 cells (5×10^6) as described under "Materials and Methods". Tumor volumes measured on indicated days were plotted for each F46 treatment group and vehicle group. Asterisks (*) represent p values versus vehicle group according to repeated measures ANOVA (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). (D) Xeno-graft analysis was performed by injecting mice with NCI-H441 cells. (E) Tumor tissues from MKN45 xenografts were subjected to immunohistochemistry using anti-Ki-67 antibody (top) or anti-CD31 antibody (bottom). Arrows indicate the CD31 positive areas. The error bars indicate the mean $\pm$ SD of each group ($n = 12$). Asterisks (*) represent p values versus the vehicle (control) group according to the unpaired *t*-test (*** $p < 0.001$).

in cell invasion, and this increase was significantly reduced by co-treatment with F46 (Fig. 3C). These results indicate that F46 is capable of inhibiting HGF-mediated cell invasion. We next examined whether F46 might also reduce HGF-induced angiogenesis by using HUVECs tube formation assay. HGF strongly induced the angiogenic ability of HUVECs, resulting in an increase of tube length by ~ 3 fold (Fig. 3D). Co-treatment with F46, however, led to a marked inhibition of HGF-mediated increase in the tube length. These results indicate that F46 is also capable of inhibiting HGF-mediated angiogenesis.

F46 inhibits cancer cell proliferation and *in vivo* tumor development

To characterize further the biological consequence of F46-mediated c-Met down-regulation, we first examined the effect of F46 on tumor cell growth. F46 inhibited the growth of MKN45 cells that over-expresses c-Met independently of HGF (Fig. 4A).

In contrast, 5D5 had little or no effect on cell growth, despite the finding that the antibody is capable of inducing c-Met degradation in MKN45 cells (see Figs. 2A and 2B). Thus, it seems possible that the agonist activity of 5D5 leads MKN45 cells to grow even with the reduced c-Met levels. Moreover, 5D5 (at 0.08 μg/ml) increased DNA synthesis by about 2-fold in NCI-H441 lung adenocarcinoma cells, whereas F46 at the same concentration showed little or no effect on DNA synthesis although F46 also increased it $\sim 40\%$ at 2 μg/ml (Fig. 4B). These results indicate that F46 shows an anti-proliferative effect toward cancer cells unlike 5D5.

We finally examined the effect of F46 on *in vivo* tumor development upon heterotopic xenograft analysis. Animals that had been subcutaneous injected with NCI-H441 or MKN45 cells were randomized 7 days after inoculation of F46 or 5D5. The antibodies were treated once or twice a week for 4 weeks as indicated. F46 dramatically reduced MKN45-induced tumor

growth: as compared to a vehicle, it reduced the tumor volume nearly 70% (Fig. 4C). F46 also inhibited the tumor growth induced by NCI-H441, albeit less efficiently than that by MKN45 (Fig. 4D). In contrast, 5D5 rather promoted NCI-H441-induced tumor growth, consistent with its ability increases DNA synthesis. Moreover, immunohistochemical analysis revealed that F46 markedly reduced Ki-67 staining (Fig. 4E, top), indicating its potent ability to inhibit cell proliferation in the tumor tissues. F46 also dramatically reduced CD31 staining (Fig. 4E, bottom), implicating its role in anti-angiogenesis. In addition, we found that F46 could also inhibit U87-MG-induced tumor growth (data not shown), suggesting that F46 can exert its anti-tumor effect also in an HGF-dependent manner (Martens et al., 2006).

DISCUSSION

Over-expression of c-Met is frequently observed in the tumors in correlation with adverse prognosis (Trusolino et al., 2010). In addition, c-Met over-expression has been proposed to be associated with the resistance to EGFR- and VEGF-targeted cancer therapies (Nakagawa et al., 2010). For these reasons, many c-Met targeting anti-cancer therapies are currently under development. While c-Met has attracted much interest as a cancer target, developing effective c-Met therapeutic antibodies has proven difficult since c-Met antibodies can also trigger the downstream signal activation that leads to cell proliferation. Consequently, several strategies have been pursued to target c-Met pathway with minimal agonism. One such approach was the development of OA5D5, a monovalent form of 5D5 that cannot induce receptor dimerization (Jin et al., 2008). Another approach is to target the c-Met ligand, HGF, by developing anti-HGF antibodies (e.g., AMG102) (Burgess et al., 2010). Although these candidates are currently under late-phase clinical trials, these two approaches may still be limited as they may be unable to mitigate the effect of c-Met activation independent of the ligand. Our goal was to identify a c-Met antibody capable of inducing receptor degradation via dimerization/internalization with minimal induction of proliferative signals. We reasoned that a robust screening system for receptor degradation with low agonism would enable us to identify c-Met antibodies whose anti-proliferative signal via receptor degradation is decoupled from the activating downstream signal. To achieve this, we utilized a new mechanism-based dual screening method that measured both c-Met degradation and Akt phosphorylation, which represent sensitive indicators for c-Met neutralization and agonism, respectively.

Using this mechanism-based dual-screening approach, we identified a mouse monoclonal antibody, F46. Lines of evidence presented here suggest that F46 has a potent neutralization activity against tumorigenic c-Met with low agonism. First, F46 showed a very high affinity to c-Met ($K_d = 2.56$ nM). Second, it showed binding specificity to c-Met, but not to its related receptors, such as RON and plexin A2. Third, it exhibited much lower agonist activity than 5D5 (e.g., phosphorylation c-Met, Akt, Gab1, and other c-Met downstream cascade members, such as Shc, Stat1, ERK, Lck, and Alk). Fourth, it was rapidly down-regulated by internalization for degradation. Fifth, it could compete with HGF for binding to c-Met, which should inhibit tumorigenic activity of HGF. Sixth, it could reduce HGF-induced tumor cell invasion and angiogenesis. Seventh, it was capable of inhibiting tumor cell growth as well as DNA synthesis. Finally, F46 could effectively reduce *in vivo* tumor growth as analyzed by tumor xenografts. Thus, we believe that F46 has a potential for the use in cancer therapy.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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REFERENCES

- Benvenuti, S., and Comoglio, P.M. (2007). The MET receptor tyrosine kinase in invasion and metastasis. *J. Cell. Physiol.* 213, 316-325.
- Birchmeier, C., and Gherardi, E. (1998). Developmental roles of HGF/SF and its receptor, the c-Met tyrosine kinase. *Trends Cell Biol.* 8, 404-410.
- Birchmeier, C., Birchmeier, W., Gherardi, E., and Vande Woude, G.F. (2003). Met, metastasis, motility and more. *Nat. Rev. Mol. Cell Biol.* 4, 915-925.
- Boccaccio, C., and Comoglio, P.M. (2006). Invasive growth, a MET-driven genetic programme for cancer and stem cells. *Nat. Rev. Cancer* 6, 637-645.
- Borowiak, M., Garratt, A.N., Wustefeld, T., Strehle, M., Trautwein, C., and Birchmeier, C. (2004). Met provides essential signals for liver regeneration. *Proc. Natl. Acad. Sci. USA* 101, 10608-10613.
- Burgess, T., Coxon, A., Meyer, S., Sun, J., Rex, K., Tsuruda, T., Chen, Q., Ho, S.Y., Li, L., Kaufman, S., et al. (2006). Fully human monoclonal antibodies to hepatocyte growth factor with therapeutic potential against hepatocyte growth factor/c-Met-dependent human tumors. *Cancer Res.* 66, 1721-1729.
- Burgess, T.L., Sun, J., Meyer, S., Tsuruda, T.S., Elliott, G., Chen, Q., Haniu, M., Barron, W.F., Juan, T., Zhang, K., et al. (2010). Biochemical characterization of AMG 102, a neutralizing, fully human monoclonal antibody to human and nonhuman primate hepatocyte growth factor. *Mol. Cancer Ther.* 9, 400-409.
- Bussolino, F., Di Renzo, M.F., Ziche, M., Bocchietto, E., Olivero, M., Naldini, L., Gaudino, G., Tamagnone, L., Coffey, A., and Comoglio, P.M. (1992). Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth. *J. Cell Biol.* 119, 629-641.
- Cao, B., Su, Y., Oskarsson, M., Zhao, P., Kort, E.J., Fisher, R.J., Wang, L.M., and Vande Woude, G.F. (2001). Neutralizing monoclonal antibodies to hepatocyte growth factor/scatter factor (HGF/SF) display antitumor activity in animal models. *Proc. Natl. Acad. Sci. USA* 98, 7443-7448.
- Chmielewicz, J., Borowiak, M., Morkel, M., Stradal, T., Munz, B., Werner, S., Wehland, J., Birchmeier, C., and Birchmeier, W. (2007). c-Met is essential for wound healing in the skin. *J. Cell Biol.* 177, 151-162.
- Comoglio, P.M., and Trusolino, L. (2002). Invasive growth, from development to metastasis. *J. Clin. Invest.* 109, 857-862.
- Cragg, M.S., French, R.R., and Glennie, M.J. (1999). Signaling antibodies in cancer therapy. *Curr. Opin. Immun.* 11, 541-547.
- Ferrara, N., Hillan, K.J., Gerber, H.P., and Novotny, W. (2004). Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nat. Rev. Drug Dis.* 3, 391-400.
- Gschwind, A., Fischer, O.M., and Ullrich, A. (2004). The discovery of receptor tyrosine kinases, targets for cancer therapy. *Nat. Rev. Cancer* 4, 361-370.
- Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer, the next generation. *Cell* 144, 646-674.
- Huh, C.G., Factor, V.M., Sanchez, A., Uchida, K., Conner, E.A., and Thorgerisson, S.S. (2004). Hepatocyte growth factor/c-met signaling pathway is required for efficient liver regeneration and repair. *Proc. Natl. Acad. Sci. USA* 101, 4477-4482.
- Jin, H., Yang, R., Zheng, Z., Romero, M., Ross, J., Bou-Reslan, H., Carano, R.A., Kasman, I., Mai, E., Young, J., et al. (2008). Met-MAB, the one-armed 5D5 anti-c-Met antibody, inhibits orthotopic pancreatic tumor growth and improves survival. *Cancer Res.* 68, 4360-4368.
- Jo, S., Hong, S.W., Yoo, J.W., Lee, C.H., Kim, S., Kim, S., and Lee, D. (2011). Selection and optimization of asymmetric siRNA targeting the human c-MET gene. *Mol. Cells* 32, 543-548.

- Kim, K.J., Wang, L., Su, Y.C., Gillespie, G.Y., Salhotra, A., Lal, B., and Lateral, J. (2006). Systemic anti-hepatocyte growth factor monoclonal antibody therapy induces the regression of intracranial glioma xenografts. *Clin. Cancer Res.* 12, 1292-1298.
- Lamszus, K., Lateral, J., Westphal, M., and Rosen, E.M. (1999). Scatter factor/hepatocyte growth factor (SF/HGF) content and function in human gliomas. *Int. J. Dev. Neurosci.* 17, 517-530.
- Li, S., Schmitz, K.R., Jeffrey, P.D., Wiltzius, J.J., Kussie, P., and Ferguson, K.M. (2005). Structural basis for inhibition of the epidermal growth factor receptor by cetuximab. *Cancer Cell* 7, 301-311.
- Ma, P.C., Jagadeeswaran, R., Jagadeesh, S., Tretiakova, M.S., Nallasura, V., Fox, E.A., Hansen, M., Schaefer, E., Naoki, K., Lader, A., et al. (2005). Functional expression and mutations of c-Met and its therapeutic inhibition with SU11274 and small interfering RNA in non-small cell lung cancer. *Cancer Res.* 65, 1479-1488.
- Martens, T., Schmidt, N.O., Eckerich, C., Fillbrandt, R., Merchant, M., Schwall, R., Westphal, M., and Lamszus, K. (2006). A novel one-armed anti-c-Met antibody inhibits glioblastoma growth *in vivo*. *Clin. Cancer Res.* 12, 6144-6152.
- Nakagawa, T., Tohyama, O., Yamaguchi, A., Matsushima, T., Takahashi, K., Funasaka, S., Shirotori, S., Asada, M., and Obaishi, H. (2010). E7050, a dual c-Met and VEGFR-2 tyrosine kinase inhibitor promotes tumor regression and prolongs survival in mouse xenograft models. *Cancer Sci.* 101, 210-215.
- Ohashi, K., Marion, P.L., Nakai, H., Meuse, L., Cullen, J.M., Bordier, B.B., Schwall, R., Greenberg, H.B., Glenn, J.S., and Kay, M.A. (2000). Sustained survival of human hepatocytes in mice, A model for *in vivo* infection with human hepatitis B and hepatitis delta viruses. *Nat. Med.* 6, 327-331.
- Prat, M., Crepaldi, T., Pennacchietti, S., Bussolino, F., and Comoglio, P.M. (1998). Agonistic monoclonal antibodies against the Met receptor dissect the biological responses to HGF. *J. Cell Sci.* 111 (Pt 2), 237-247.
- Trusolino, L., and Comoglio, P.M. (2002). Scatter-factor and semaphorin receptors, cell signalling for invasive growth. *Nat. Rev. Cancer* 2, 289-300.
- Trusolino, L., Bertotti, A., and Comoglio, P.M. (2010). MET signalling, principles and functions in development, organ regeneration and cancer. *Nat. Rev. Mol. Cell Biol.* 11, 834-848.