p85β regulates autophagic degradation of AXL to activate oncogenic signaling

Ling Rao1,7, Victor C. Y. Mak1,7, Yuan Zhou1, Dong Zhang2, Xinran Li1, Chloe C. Y. Fung1, Rakesh Sharma3, Chao Gu4, Yiling Lu5, George L. Tiope1, Annie N. Y. Cheung6, Gordon B. Mills2 & Lydia W. T. Cheung1

PIK3R2 encodes the p85β regulatory subunit of phosphatidylinositol 3-kinase and is frequently amplified in cancers. The signaling mechanism and therapeutic implication of p85β are poorly understood. Here we report that p85β upregulates the protein level of the receptor tyrosine kinase AXL to induce oncogenic signaling in ovarian cancer. p85β activates p110 activity and AKT-independent PDK1/SGK3 signaling to promote tumorigenic phenotypes, which are all abolished upon inhibition of AXL. At the molecular level, p85β alters the phosphorylation of TRIM2 (an E3 ligase) and optineurin (an autophagy receptor), which mediate the selective regulation of AXL by p85β, thereby disrupting the autophagic degradation of the AXL protein. Therapeutically, p85β expression renders ovarian cancer cells vulnerable to inhibitors of AXL, p110, or PDK1. Conversely, p85β-depleted cells are less sensitive to these inhibitors. Together, our findings provide a rationale for pharmacological blockade of the AXL signaling axis in PIK3R2-amplified ovarian cancer.
The p85 regulatory subunit of class 1A phosphatidylinositol 3-kinase (PI3K) binds to the p110 catalytic subunit, initiating signaling downstream of activated receptor tyrosine kinases (RTKs). Although there is functional specificity among p110 isoforms, the roles of p85 isoforms in cancers are only beginning to be understood. PIK3R1 (encodes p85α) has been suggested to act as a tumor suppressor through functions such as inhibiting p110 kinase activity and stabilizing phosphatase and tensin homolog (PTEN). Depletion of p85α can thus lead to enhanced p110 activity and PTEN destabilization, as well as cell context-dependent activation of oncogenic signaling. Indeed, loss-of-function disruptions in PIK3R1 are frequent in cancers, including breast cancer cell lines in vitro and hampers colon carcinogenesis in Pk3r2−/− mice. p85α also promotes invadopodium formation and melanoma invasion, possibly through increased activity of the GTPases Cdc42 and Rac1. Further, ectopic expression of PIK3R2 in breast cancer cell lines in vitro and hampers colon carcinogenesis elucidates.

Results
p85β upregulates the activity but not the expression of p110. An increased PIK3R2 copy number was detected in 49% of The Cancer Genome Atlas (TCGA) serous ovarian tumor samples (n = 579). PIK3R2 copy numbers positively correlated with corresponding mRNA levels measured by RNA-Sequencing (r = 0.7, P < 0.0001) or microarray (r = 0.6, P < 0.0001) (Supplementary Fig. 1a). PIK3R2 mRNA expression was higher in PIK3R2-amplified tumors than in diploid tumors (P < 0.0001) (Supplementary Fig. 1b). Intriguingly, high PIK3R2 mRNA levels were significantly associated with relatively poor overall survival and progression-free survival in ovarian cancer patients (Fig. 1a).

The functional consequences of silencing PIK3R2 were evaluated in three serous ovarian cancer cell lines with high p85β protein levels (OVCA4, OVCA8, and SKOV3) using two independent small interfering RNA (siRNA). Knockdown efficiency is shown in Supplementary Fig. 1c. PIK3R2 depletion impaired cell proliferation, long-term clonogenic survival, and cell invasion (Fig. 1b–d). Stable short hairpin RNA (shRNA)-mediated PIK3R2 knockdown induced similar phenotypic changes in vitro and decreased intraperitoneal growth in vivo (Supplementary Fig. 1c–g). To evaluate the functional consequences of increased p85β levels, p85β was stably expressed in serous ovarian cancer cell lines with low endogenous p85β protein levels (DOV13 and EFO21). This p85β overexpression led to enhancements in tumorigenic phenotypes (Fig. 1e–g and Supplementary Fig. 2a–c). These increases were markedly abolished by pan-p110 inhibitors (GDC-0941; PIK-90), p110α-specific inhibitors (A66; BYL719), or a p110β-specific inhibitor (TGX-221), indicating the contribution of p110 to the activity of p85β (Fig. 1e–g and Supplementary Fig. 2a–c). Remarkably, two AKT inhibitors (MK-2206; GDC-0068) did not alter the induced phenotypes, indicating that the effects of p85β are independent of AKT signaling. This is further supported by the observation that knocking down AKT1/2/3 expression with siRNA had minimal impacts on the p85β-induced phenotypes (Supplementary Fig. 2d).

p85α binds to p110 to stabilize p110 proteins and inhibit p110 kinase activity. Strikingly, we found that p85β promoted p110 kinase activity, which was reflected by the production of phosphatidylinositol 3,4,5-trisphosphate (PIP3) from phosphatidylinositol 4,5-bisphosphate (PIP2) in p110α- or p110β-immunoprecipitated lysates (Fig. 1h). p110 activity associated with either p85α or p85β was enhanced (Supplementary Fig. 2e); therefore, the effect is not limited to p85β-bound p110. Total cellular PIP3 levels in p85β-overexpressing cells were also increased (Supplementary Fig. 2f). Consistently, p110 activities and PIP3 levels were decreased by PIK3R2 siRNA (Supplementary Fig. 2g, h). However, the protein levels of p110α and p110β were not altered in p85β-overexpressing (Fig. 1i) or p85β-depleted cells (Supplementary Fig. 2i).

p85β increases the protein level of AXL to activate p110. To understand the effects of p85β, we analyzed TCGA serous ovarian cancer protein data generated by reverse-phase protein array (RPPA). The samples were divided according to PIK3R2 copy number. Intriguingly, the levels of AXL (an RTK in the TAM family) were significantly elevated in the tumors with PIK3R2 amplification (P = 0.01). We also demonstrated a positive correlation between p85β and AXL levels in an independent serous ovarian tumor set (n = 49) by immunohistochemistry (Pearson’s correlation coefficient r = 0.6, P < 0.0001; Fig. 2a, b). Western blotting confirmed that both the AXL protein level (Fig. 2c) and tyrosine phosphorylation (Fig. 2d) were increased in p85β-overexpressing DOV13 and EFO21 cells. Conversely, PIK3R2 siRNA treatment or stable PIK3R2 knockdown led to decreases in the AXL protein level and phosphorylation (Fig. 2e, f and Supplementary Fig. 3a). Remarkably, depletion of p85α (PIK3R1) had no appreciable effect on the AXL protein level (Supplementary Fig. 3b), indicating the specific effect of PIK3R2. Further, this regulation was specific to AXL, because the protein levels of other TAM members (MERTK and Tyro3) were not changed (Supplementary Fig. 3c).

As AXL is an upstream regulator of PI3K, we investigated the involvement of AXL in p85β-induced phenotypes and p110 activity. Cell proliferation, colony formation, and cell invasion induced by p85β were all abrogated upon inhibition of AXL by dominant-negative (DN) AXL or an AXL inhibitor (TP-0903) (Fig. 2g–i and Supplementary Fig. 3d–f). The induced catalytic activities of p110α and p110β were also abolished by AXL inhibition (Fig. 2j and Supplementary Fig. 3g). Together, these data indicate that p85β increases the AXL protein level and activation to promote p110 activities, which may in turn initiate downstream oncogenic signaling.

The regulation of AXL by p85β was, however, independent of p110 activity. Treatment with the pan-110 inhibitor (PIK-90), p110α-specific inhibitor (BYL719), or p110β-specific inhibitor (TGX-221) did not abolish the increased AXL levels by p85β in two cell lines (Fig. 3a and Supplementary Fig. 4a). Phosphorylation of AKT and S6 was inhibited reflecting the effectiveness of the inhibitors. Crosstalk between AXL and EGFR family members, for example, via AXL-EGFR heterodimerization, has previously been demonstrated. Neither lapatinib (targets EGFR and HER2) nor erlotinib (targets EGFR) had any influence...
on the increased phosphorylation and total protein level of AXL induced by p85β (Supplementary Fig. 4b), suggesting that the activation of AXL is unlikely to occur through an interaction with EGFR or HER2. Gas6 is a ligand that activates AXL but may also decrease the AXL level by promoting AXL protein degradation12. Our data showed that the level of Gas6 was unaffected by PIK3R2 depletion (Supplementary Fig. 4c), and that Gas6 had minimal interference with the effect of p85β on AXL. First, Gas6 caused...
transient activation of AXL in vector control-expressing or p85β-overexpressing cells (Fig. 3b). Importantly, the induced phosphorylation of AXL by p85β and Gas6 was additive. Second, total AXL protein levels remained high in p85β-overexpressing cells compared with vector control-expressing cells after Gas6 stimulation (Fig. 3b). Third, we examined the potential effect of Gas6 on PIK3R2-knockdown cells. The decreases in AXL phosphorylation and protein level upon PIK3R2 depletion were not affected by Gas6 (Fig. 3c). Last, the protein interaction between p85β and AXL remained essentially unchanged in Gas6-stimulated cells, as revealed by an in situ proximity ligation assay (PLA) (Fig. 3d).
Fig. 2 p85β increases the protein level of AXL, which in turn activates p110. a Serous ovarian tumor tissue samples were subjected to immunohistochemical staining for p85β and AXL. Scale bars, 200 µm. The boxes depict magnified areas. b Scatter plot illustrating the correlation of the immunostaining scores corresponding to p85β and AXL. Pearson's correlation coefficient r with P-value are shown. c Protein levels of AXL, p85β, and Erk2 (loading control) in cells stably expressing PIK3R2 (R2OX) or vector control. d AXL was immunoprecipitated (IP) from whole cell lysates and western blotting (IB) was performed with anti-p-Tyr and anti-AXL antibodies (upper). IP with mouse IgG was negative control. AXL levels in the input lysates are shown (lower). Protein levels of AXL and p85β in cells with or without PIK3R2 silencing. NS, nonspecific siRNA. f AXL was immunoprecipitated from cell lysates and western blotting was performed (upper). AXL levels in input lysates is shown (lower).

g–j EFO21 cells with or without stable PIK3R2 overexpression were either treated with AXL inhibitor TP-0903 (0.5 µM) or transfected with dominant-negative (DN) AXL or its vector control. These cells were subjected to (g) BrdU cell proliferation assay, (h) colony formation assay, (i) cell invasion assay, and (j) immunoprecipitation-based p110α or p110β PI3-kinase activity assay. The western blotting experiments (c–f) were repeated three times with independent lysates and results were reproducible. Assays in g–j were done in triplicate and representative data of three independent experiments are presented as mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001 using two-tailed t-test. Source data are provided as a Source Data file.

Fig. 3 The regulation of AXL by p85β does not involve p110 activity and is not affected by Gas6. a EFO21 cells with or without stable PIK3R2 overexpression (R2OX) were treated with 10 µM PIK-90 (pan-p110 inhibitor) for 24 h. Protein was then collected and analyzed using the indicated antibodies. b, c DOV13 or EFO21 cells stably expressing PIK3R2 (R2OX) or vector control or (c) OVCAR8 cells with or without PIK3R2 silencing (R2 siRNA) were serum-starved for 24 h. Cells were then treated with human recombinant Gas6 (500 ng/mL) for the indicated times. The western blotting experiments were repeated three times with independent lysates and results were reproducible. d Parental OVCAR8 cells were serum-starved for 24 h prior to stimulation with Gas6 (500 ng/mL) for 6 h. Cells were subjected to proximity ligation assay with anti-p85β and anti-AXL antibodies. Negative controls with one of the antibodies replaced by IgG were included. The assay was repeated three times and reproducible results were obtained. Representative images are shown (left). The number of signals per nucleus was counted in ≥8 fields and data represent mean ± SD (right). Scale bars, 20 µm. ns, not statistically significant by two-tailed t-test. Source data are provided as a Source Data file.
p85β regulates the autophagic degradation of AXL. Although p85α has been implicated in the recycling of activated platelet-derived growth factor receptor in NIH3T3 cells, the regulation of any RTK by p85β has not been reported. The AXL mRNA level and promoter activity were not changed in PIK3R2-depleted cells (Supplementary Fig. 5a–b). Cycloheximide-chase experiments revealed that PIK3R2 depletion reduced the half-life of the endogenous AXL protein (Fig. 4a and Supplementary Fig. 5c). Proteins are mainly degraded by the proteasomal or lysosomal pathway. As shown in Fig. 4b and Supplementary Fig. 5d, PIK3R2 depletion failed to decrease the AXL protein level upon treatment with chloroquine (lysosome inhibitor), but not with MG132 or lactacystin (proteasome inhibitors), suggesting that AXL is regulated by p85β via the lysosomal pathway. Ubiquitination was analyzed by immunoblotting with two different ubiquitin-specific antibodies prior to stripping the membranes for reprobing with anti-AXL antibody. The anti-ubiquitin clone FK2 recognizes both conjugated monoubiquitin and polyubiquitin chains, whereas the clone P4D1 recognizes all forms of ubiquitin including conjugated or free ubiquitin. Notably, our data revealed an increase in ubiquitinated AXL in the presence of PIK3R2 siRNA (Fig. 4c).
exposures (Supplementary Fig. 5e). Precise characterization of the types of ubiquitination is warranted.

We next attempted to identify the proteins that mediate the entry of AXL into the degradation pathway. Phosphorylation modulates the activity and substrate selectivity of proteins within the degradation machinery. Quantitative mass spectrometry (MS)-based phosphoproteomic analysis was therefore performed to reveal proteins with an altered phosphorylation status upon PIK3R2 depletion. This profiling yielded 90 unique altered phosphopeptides, mapping to 29 and 45 proteins whose levels were downregulated or upregulated at P < 0.05 by PIK3R2-specific siRNA, respectively (Supplementary Table 1). These proteins were analyzed by Gene Ontology (GO) enrichment analysis. Strikingly, we observed significant enrichment in ubiquitin-mediated proteolysis (log10 P-value = −5.36) and autophagy regulation (log10 P-value = −2.58) (Fig. 5a). Selective autophagy represents a major lysosome-mediated degradation mechanism in which ubiquitinated proteins are recognized by autophagic receptors forming autophagosomes prior to fusion with the lysosomes.

We performed siRNA experiments targeting the 12 proteins with phosphorylation changes within these two GO categories (Supplementary Fig. 6a). In addition, we included siRNAs targeting the E3 ubiquitin ligases Cbl-b and c-Cbl, because these ligases have been demonstrated to mediate the degradation of RTKs including AXL. The screen revealed that knocking down the expression of two genes, OPTN and TRIM2, could reverse the decrease in the AXL protein level induced by PIK3R2 depletion. In contrast, the inhibition of the other proteins, including Cbl-b or c-Cbl, had no effect (Supplementary Fig. 6a). The involvement of TRIM2 and OPTN in AXL degradation was further confirmed by multiple independent siRNAs (Fig. 5b). Nedd4, which has been shown to be an RTK E3 ligase, was chosen as a negative control, because it had no effect on AXL levels in the screen. Consistently, TRIM2 and OPTN knockdown but not Nedd4 knockdown reversed PIK3R2 loss-induced AXL degradation (Fig. 5b). Remarkably, TRIM2- and OPTN-specific siRNAs had no effect on Tyro3 or MERTK, indicating the specificity of their activities (Supplementary Fig. 6b).

TRIM2 is a tripartite motif (TRIM) RING finger E3 ligase that ubiquitinates the neurofilament light subunit and the pro-apoptotic protein Bim22. Our data suggested TRIM2 as an E3 ligase of AXL. Depletion of TRIM2 by siRNA reduced the amount of ubiquitinated AXL (Fig. 5c), whereas TRIM2 overexpression reversed the upregulation of the AXL level mediated by p85β (Fig. 5d). Importantly, phosphorylation-defective mutation of S443 in TRIM2 (TRIM2S443A), a residue whose phosphorylation was increased by p85β knockdown, rendered TRIM2 incapable of downregulating AXL level. S443 is located within the filamin domain in TRIM2, but the function of this domain is unknown. Binding between AXL and TRIM2 was promoted by PIK3R2 knockdown, as revealed by immunoprecipitation (Fig. 5e) and a PLA (Supplementary Fig. 6c). Importantly, Gas6 stimulation at a condition that effectively induced AXL phosphorylation did not disrupt the binding (Supplementary Fig. 6c).

OPTN encodes optineurin, which is a selective autophagy receptor that mediates the interactions between ubiquitinated proteins and the autophagy machinery. Immunoprecipitation and PLA consistently demonstrated that PIK3R2 knockdown enhanced the binding between AXL and optineurin (Fig. 5e), which was again unaffected by Gas6 (Supplementary Fig. 6d). PIK3R2 knockdown increased the phosphorylation of optineurin at S526, which is a highly conserved residue close to the ubiquitin-binding domain that has not been previously reported to be phosphorylated. We confirmed the involvement of optineurin in AXL degradation, because the increased AXL level induced by p85β was abrogated upon optineurin overexpression (Fig. 5f). The phosphorylation-defective optineurin mutant S526A failed to decrease the AXL level (Fig. 5f), suggesting the importance of optineurin phosphorylation in the degradation of AXL. We also investigated the involvement of the other previously characterized amino acids in optineurin, including S177, S473, and S513, which may alter ubiquitin binding or other functions of optineurin in mitophagy23–27. S473A but not S177A or S513A reversed the decrease in the AXL level induced by p85β (Supplementary Fig. 6e). Double mutants composed of S526A with S177A or S473A had effects similar to those of single S526A mutant. Western blotting with a commercial antibody against S177 showed that its phosphorylation was unaffected by p85β (Supplementary Fig. 6f).

Autophagic flux, which reflects autophagic degradation activity, was induced by PIK3R2 depletion. The protein level of the autophagosome marker LC3B-II was increased by PIK3R2 depletion and treatment with chloroquine, which inhibits autophagosome–lysosome fusion, resulted in further accumulation of LC3B-II (Fig. 5g). This increase in LC3B-II level was accompanied by an increase in LC3B puncta formation in PIK3R2-depleted cells, as revealed by confocal double-labeling immunofluorescence (Fig. 5h). Further, we observed pronounced colocalization between AXL and LC3B, ATG5 and ATG12 (autophagy markers)29,30, or Rab7 (a Rab GTPase that regulates late endosomal trafficking and the autolysosomal pathway)31 in PIK3R2-depleted cells (Fig. 5h). The presence of AXL in these vesicles is consistent with degradation through the autophagy–lysosomal pathway. In contrast, the associations between AXL and Rab4 or Rab11 (which regulated receptor recycling to the plasma membrane)31 were not altered by PIK3R2 depletion (Supplementary Fig. 6g).

p85β signals through SGK3 instead of AKT. To elucidate the downstream signaling events that mediate the effects of p85β, five ovarian cancer cell lines transfected with PIK3R2-specific siRNA were subjected to RPPA analysis. AXL level was downregulated in four of the five cell lines (Fig. 6a). Consistent with the observation above (Fig. 5g), we observed increased LC3 protein levels in PIK3R2-knockdown cells. Intriguingly, PIK3R2-specific siRNA also led to decreased amounts of p-PDK1 (S241), an upstream kinase of AKT and SGK32, and p-NDRG1 (pT346), a marker of SGK activity33,34 (Fig. 6a). Consistent with the observations that the effects of PIK3R2 were insensitive to AKT inhibitors, an RPPA and subsequent western blotting validation showed no significant differences in the levels of phosphorylated AKT, the AKT substrate PRAS40, mammalian target of rapamycin (mTOR), p70S6K, and S6 in PIK3R2-depleted cells (Fig. 6b). Indeed, AKT phosphorylation at S473 was modestly reduced in p85β-overexpressing ovarian cancer cells (Supplementary Fig. 7a). Phosphorylation of PRAS40, mTOR, and p70S6K was not changed in these PIK3R2-overexpressing cells (Supplementary Fig. 7a). SGK has been proposed as to be a downstream effector of AKT-independent PI3K oncogenic signaling35,36. SGK antibodies were not included in the RPPA due to inability to validate on that platform. We therefore evaluated the phosphorylation of SGK1 and SGK3 using western blotting. Although the p-SGK1 (S78) level was not altered, the p-SGK3 (T320) level was decreased upon PIK3R2 silencing (Fig. 6b). The decreases in the p-PDK1 and p-NDRG1 levels were reproducible across the cell lines (Fig. 6b). Importantly, p85β-overexpressing ovarian cancer cells demonstrated coordinated enhanced phosphorylation of PDK1, SGK3, and NDRG1 (Fig. 6c).

Next, we sought to assess the contribution of each of these molecules to the effects of p85β on SGK3 signaling by inhibiting...
AXL (TP-0903), p110 (GDC-0941), or PDK1 (GSK2334470). On-target inhibition of AXL phosphorylation and p110 activity by TP-0903 or GDC-0941 is shown in Fig. 6c and Supplementary Fig. 7b, respectively. Inhibition of PDK1 phosphorylation could also be observed upon GSK2334470 treatment (Fig. 6c). All the inhibitors suppressed the induction of PDK1, SGK3, and NDRG1 phosphorylation by p85β expression (Fig. 6c). These results collectively indicate activation of the AXL/p110/PDK1/SGK3/NDRG1 signaling cascade by p85β in ovarian cancer. Notably, these inhibitions had no effect on the induction of AXL protein.
levels by p85β (Fig. 6c), Concordantly, with the notion that the regulation of AXL by p85β is independent of p110 activity. To further investigate whether this signaling axis in turn activates p110 activity, we assessed PI3P production in p110α or p110β immunoprecipitates from PIK3R2-overexpressing cells treated with PDK1 inhibitors (GSK2334470 and OSU-03012). The inhibition of PDK1 did not abolish p85β-induced p110α or p110β activity, consistent with PDK1 being downstream of p110 (Supplementary Fig. 7c).

p85β sensitizes cells to inhibition of AXL signaling. p85β could confer sensitivity to inhibitors targeting molecules in the AXL/p110/ PDK1/SK3/NDRG1 signaling cascade. p85β-overexpressing 3D spheroids were treated with serial dilutions of inhibitors of AXL and P3K/PDK1 signaling to obtain IC50 values in the presence and absence of p85β expression. Strikingly, p85β-overexpressing 3D spheroids displayed significantly higher sensitivity to AXL (BGB324 and TP-0903), pan-P3K (GDC-0941), p110α-specific (A66), p110β-specific (TGX-221), and PDK1 (OSU-03012 and GSK2334470) inhibitors than control spheroids (Fig. 7a, c). Drug sensitivity was also assessed in spheroids of PIK3R2-depleted cells. In contrast to the results for the overexpression spheroids, PIK3R2 siRNA-mediated knockdown led to reduced sensitivity to all of these inhibitors (Supplementary Fig. 8a, b). Consistent with our earlier data suggesting that AKT and SGK1 activation was not required for the effects of p85β, p85β-overexpressing cells were not more sensitive to an AKT (MK-2206) or SGK1/2 inhibitor (Supplementary Fig. 8b). However, p85β increased the sensitivity of cells to PIK3R2 siRNA for 72 h. Protein was extracted from the tumors and western blotting demonstrated decreased AXL phosphorylation in all TP-0903-treated mice (Supplementary Fig. 9a). The tumors developed from p85α overexpressing cells preserved the signaling features observed in vitro, including stronger phosphorylation of PDK1 and SGK3, but not AKT or mTOR than that in the vector control-expressing tumors (Supplementary Fig. 9b). Moreover, TP-0903 effectively decreased p-PDK1 and p-SGK3. Together, these findings suggest that therapeutic blockade of AXL signaling may be an effective strategy for ovarian tumors with PIK3R2 amplification.

Discussion

AXL is overexpressed in multiple cancer types, including ovarian cancer, causing malignant phenotypes and a poor prognosis39–41. Genomic aberrations in AXL are relatively rare in cancers. It appears that AXL is primarily regulated at the levels of the promoter42–44 and protein stability12,45–47. In this study, we showed that p85β but not p85α regulated the autophagy-lysosomal machinery to promote AXL protein stabilization and downstream signaling (Fig. 7e). Previous studies have suggested that the E3 ligase chromatin immunoprecipitation promotes the ubiquitination-mediated proteasomal degradation of AXL47, whereas cbl-b targets all three members of TAM (Tyro3, AXL, and MERTK) for ubiquitination46. Monoubiquitination of AXL has been reported, but the ligase involved has yet to be identified12. We demonstrated that AXL was ubiquitinated by TRIM2, which had not been demonstrated to target any RTK. Importantly, AXL was a selective TRIM2 target among TAM family members, contributing to selective regulation of AXL by p85β. A second layer of regulation of AXL lies in the phosphorylation of optineurin. Several optineurin phosphorylation sites have been previously characterized. Data so far have indicated that these sites may relate to distinct functions. For example, S177 facilitates autophagosome formation at least partly by enhancing LC3 binding23–25, whereas S443 promotes the binding of optineurin to ubiquitin24,25. Unphosphorylated optineurin preferentially binds polyubiquitin chains, not monoubiquitin25. Intriguingly, phosphorylated optineurin at S473 displays enhanced binding to monoubiquitin with an affinity comparable to that for polyubiquitin chains25, therefore widening the spectrum of ubiquitinated targets that can be recognized by optineurin. Our data demonstrated that mutation of S473 caused an effect on AXL level similar to that of mutating S526. Further, combined muta-
tion of the two residues was not additive. It remains to be examined whether S526 also alters the binding capacity of optineurin to ubiquitin. Moreover, whether p85β regulates optineurin phosphorylation at S473 is not known, because phosphorylation of this residue could not be detected in our samples. Accordingly, a low endogenous level of S473 phosphorylation has been previously reported24,27. The other optineurin phosphorylation site we investigated is S513, which did not alter the effect of p85β on AXL. Studies have shown that S513 alone does not appear to regulate ubiquitin binding24,27 but that S513 together with S473 may mediate mitophagy by promoting the recruitment and retention of optineurin on damaged mitochondria24,27.

p85β was not previously linked to regulation of the autophagy-lysosomal pathway. How p85β regulates the phosphorylation of TRIM2 and optineurin remains to be elucidated. The effect of p85β on AXL is likely mediated by the interaction of p85β with a set of proteins that do not interact coordinately with p85a, which may indeed also account for the differential activities of p85a and p85β. Nevertheless, p110 activity and SGK3/PDK1 signaling are not involved, because inhibition of these molecules had no effect on AXL regulation. The previously identified kinases of optineurin include TBK1, IKKβ, and Plik27,48,49. The potential roles of these kinases in mediating the regulatory effect of p85β on AXL warrant...
**Fig. 6** p85β activates the AXL/PDK1/SGK3 signaling axis. 

**a** Heatmap illustrating proteins downregulated (<0.8) or upregulated (>1.2) in at least three ovarian cancer cell lines transfected with PIK3R2 siRNA, compared with the mock control by RPPA analysis. **b** Lysates from cells transfected with PIK3R2 siRNA for 72 h were immunoblotted using the indicated antibodies. NS, nonspecific siRNA. **c** Stable PIK3R2-overexpressing (R2OX) or vector control-expressing cells were treated with pan-p110 inhibitor GDC-0941 (10 µM), AXL inhibitor TP-0903 (0.5 µM), or PDK1 inhibitor GSK2334470 (2 µM) for 48 h. Lysates were subjected to western blotting. The western blotting experiments were repeated three times with independent lysates and results were reproducible. Source data are provided as a Source Data file.
further investigation. Our data showed that the induction of AXL protein stability and phosphorylation by p85β was sustained even in the presence of Gas6. Further, the phosphorylation of AXL induced by p85β and Gas6 appeared to be additive, suggesting p85β and Gas6 as two independent activators of AXL. The level of Gas6 was shown to be elevated in ovarian cancer patients\textsuperscript{50,51}. It would be interesting to determine the co-occurrence of Gas6 elevation and PIK3R2 amplification, as well as the corresponding AXL activation status in cancer patients. In ovarian cancer, an increased PIK3CA copy number is common (79%; n = 579), but PIK3CA mutation is rare (2 cases out of 316)\textsuperscript{52}. Remarkably, PIK3R2 amplification has been detected in 81% of non-PIK3CA-
amplified tumors compared with 52% of PIK3CA-amplified tumors (P < 0.0001) by McNemar’s test. However, whether PIK3CA amplification leads to the same downstream effects as PIK3R2 amplification remains to be fully characterized.

In summary, we describe herein that p85β upregulates the level of the upstream kinase AXL. The rationale for AXL inhibition in cancer therapy so far has been the frequent overexpression of AXL and the associated poor prognosis in cancers. Our findings that AXL mediates the oncogenic signaling of p85β provide further support for targeting AXL as a cancer treatment and suggest that PIK3R2 amplification could serve as a biomarker for the efficacy of AXL inhibitors.

Methods

Cell lines and inhibitors. OVCA48, OVCA49, OVCA83, EFO21, and DOV13 were obtained from National Cancer Institute. SKOV3 was from American Type Culture Collection. The cell lines were maintained in RPMI-1640 media (Gibco, Carlsbad, CA) supplemented with 5% fetal bovine serum (FBS) (Gibco), and 1% penicillin–streptomycin at 37 °C and 5% CO2. Cell lines were authenticated using negative mycoplasma contamination. PI3K inhibitors (GDC-0941, PIK-90, A66, BYL719, and TGX-221), Akt inhibitors (MK-2206 and GDC-0068), and AXL inhibitor (GSK2334470) and SGK1/2 inhibitor (GS650394) were purchased from Santa Cruz Biotechnology (Dallas, TX). The used doses of the inhibitors in vitro are as follows: GDC-0941, 10 μM; PIK-90, 10 μM; A66, 2 μM; BYL719, 2 μM; TGX-221, 10 μM; MK-2206, 5 μM; GDC-0068, 5 μM; GSK2334470, 1 μM; TGX-221, 0.5 μM; A66, 2 μM; GDC-0941, 10 μM; 5% dimethylsulfoxide (DMSO), 0.2 μM.

siRNA, shRNA, and plasmids. ON-TARGETplus siRNA targeting human PIK3R2, SMARTpool siRNA targeting the 14 proteins in degradative pathways, and nonspecific siRNA were obtained from Dharmaco (Lafayette, CO). Additional individual siRNA against TRIM2, OPTN, and NEDD4 was purchased from Integrated DNA Technologies (Coraville, IA). shRNA transfection was performed using Lipofectamine RNAiMAX (Life Technologies, Carlsbad, CA). OVCA48 and SKOV3 with stable PIK3R2 knockdown and the corresponding vector control were established by infection of lentivirus with pLKO.1-PIK3R2 shRNA or pLKO.1 vector prior to puromycin selection. DOV13 and EFO21 cells with stable PIK3R2 overexpression or vector control were constructed by infection of lentivirus with p Lent6–PIK3R2 or empty pLent6 vector followed by blastidcin selection. AXL and DN-AXL plasmids were kind gifts from Professor Axel Ullrich (Max Planck Institute for Biochemistry, Germany). Expression plasmids of human TRIM2 in pHBV1 lentiviral vector was purchased from Genemedi (Shanghai, China) and human OPTN in pEFP-N1 was from Addgene (Watertown, MA). Mutants of OPTN or TRIM2 were generated using QuickChange Lightning Site-Directed Mutagenesis kit (Agilent Technologies, Santa Clara, CA). Plasmids were introduced to cells using Lipofectamine 3000 reagent according to the manufacturer’s protocol. All constructs were validated by DNA sequencing. Sequence information of siRNA and shRNA was shown in Supplementary Table 2.

Proliferation assay. Cells, with or without treatment, were seeded at the density of 1000 cells/well in triplicate in 96-well plates for 72 h. Cell proliferation was detected using sulforhodamine B (SRB) cell proliferation assay kit (Cell Signaling) according to the manufacturer’s instruction. Experiments were performed three times independently.

Coloncy formation assay. DOV13 and EFO21 PIK3R2-stably expressing cells were either treated with inhibitors or transfected with DN-AXL-vector and were seeded into 6-well plates at a density of 200 cells/well in triplicate. Likewise, OVCA48, OVCA49, and SKOV3 cells were transfected with PIK3R2 siRNA or nonspecific siRNA for 24 h prior to cell seeding. After 14 days, colonies were washed twice with phosphate-buffered saline (PBS), fixed with methanol for 10 min, and stained with crystal violet for 15 min at room temperature. Experiments were performed three times independently.

Invasion assay. Stable cells or siRNA-transfected cells were suspended in serum-free medium and seeded into insert with pore size of 8.0 μm (Millipore, Billerica, MA) precoated with 1 mg/mL Matrigel (Corning, NY). Cell culture medium containing 10% FBS was added to the lower chambers and cells were incubated for 24 h. Invasive cells were fixed by methanol and stained with crystal violet. The number of invasive cells was counted in five randomly captured fields using light microscope (×100). All assays were performed in triplicate.

Cell viability assay. Cells were seeded at a density of 1000 cells/well in triplicate in 96-well plates. Culture medium was replaced at day 3. Afterwards, cells were incubated with 10 μL Resazurin solution (0.2 mg/mL; Cell Signaling, Danvers, MA) for 3 h prior to measurement of absorbance 600 nm. Experiments were performed three times independently.

In vivo tumorigenicity assay. The protocols were approved by the Committee on the Use of Live Animals in Teaching and Research of The University of Hong Kong and all mouse experiments were conducted according to ethical regulations. OVCA48 or SKOV3 cells (5 × 10⁶) with stable PIK3R2 knockdown or vector control in sterile PBS were injected intraperitoneally into 6-week-old female athymic BALB/c mice (n = 5 per group) (Charles River Lab, USA). Six weeks after injection, mice were killed and the disseminated tumor nodules in the peritoneal cavity were quantified and weighed. To evaluate the antitumor effect of TP-0903 (AXL inhibitor), 1.5 × 10⁷ DOV13 cells stably expressing PIK3R2 or vector control were mixed with Matrigel (2:1) and implanted subcutaneously into the right flank of each mouse. Four weeks after inoculation, mice were randomly divided into two subgroups and orally administered with 50 mg/kg TP-0903 (n = 5) or vehicle (5% dimethylsulfoxide (DMSO), n = 5) daily for 10 days. Tumor volume was calculated as length × width × 0.5 (length is the major tumor axis, whereas width is the minor axis). One mouse injected with PIK3R2-expressing cells treated with DMSO died during the treatment period and was excluded from the analysis. Tumor burden based on tumor volumes and weight was compared among subgroups. Protein collected from the tumor samples was used for western blotting. Some tumor nodules were fixed and were subjected to hemotoxylin and eosin staining for histopathology.

3D spheroid-based drug sensitivity assay. PIK3R2 siRNA-transfected or PIK3R2-overexpressing cells were suspended in 2% Matrigel-containing medium and seeded at a density of 1000 cells/well in triplicate in Matrigel-precoated 96-well plates. Cells were cultured for 7 days for 3D spheroids formation, followed by treatment of inhibitors at serial concentrations for 72 h. Luminescent readout as viability indicator was detected by CellTiter-Glo® 3D cell viability assay according to the manufacturer’s instruction (Promega, Madison, WI). The relative percentage of cell viability after inhibitor treatment was shown in the dose–response curves with the untreated cells as 100%. IC50 values of tested drugs were calculated by nonlinear regression analysis using GraphPad Prism 7 (La Jolla, CA). Assays were performed in triplicate.

Real-time PCR. Five micrograms of total RNA isolated using TRIzol reagent (Life Technologies) were reverse transcribed using SuperScript IV and oligo (dT) primers (Life Technologies), followed by cDNA amplification using Power SYBR
Green PCR Master Mix system according to the manufacturer’s instructions (Applied Biosystems, Foster City, CA). GAPDH was used as an internal control to calculate the relative mRNA levels. Primer sequences are listed in Supplementary Table 3. All assays were performed in triplicate.

Luciferase assay. pGL3 luciferase plasmid with human AXL gene promoter (2376 bp) was kindly provided by Professor Heike Allgayer at the Mannheim Medical Faculty of University of Heidelberg, Germany. Cells transfected with siRNA for 24 h were transfected again with pGL3 basic reporter vector or pGL3-AXL vector with Renilla luciferase plasmid (pRL-TK). After 48 h, firefly and Renilla luciferase activities (for normalization) were measured by Dual-Luciferase Reporter Assay System (Promega). Assays were performed in triplicate.

Protein extraction, immunoprecipitation, and immunoblotting. Total protein for western blotting was prepared using RIPA lysis buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.1% SDS, 1% NP-40, 1% sodium deoxycholate (Sigma-Aldrich, St. Louis, MO). Prior to incubation with two primary antibodies overnight (Supplementary Table 4), cells were fixed by 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. After blocking with 3% bovine serum albumin, cells were incubated with primary antibody overnight (Supplementary Table 4). After washing with TBST buffer, cells were incubated with secondary antibody for 1 h. Images were captured with Carl Zeiss LSM 700 (Zeiss, Jena, Germany).

Immunofluorescent staining. OVCA8R cells (3 x 10^6) seeded onto sterilized cover slips were transfected with PIK3R2 siRNA for 48 h. Cells were then fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. After blocking with 3% bovine serum albumin, cells were incubated with primary antibody overnight (Supplementary Table 4). After washing with TBST buffer, cells were incubated with secondary antibody for 1 h. Images were captured with Zeiss LSM 700 (Zeiss, Jena, Germany).

Proximity ligation assay. Cells seeded onto sterilized cover slips were subjected to Duolink PLA fluorescence according to the manufacturer’s instructions (Sigma-Aldrich, St. Louis, MO). Prior to incubation with two primary antibodies overnight (Supplementary Table 4), cells were fixed by 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Negative control was performed by replacing one of the antibodies with IgG. The cells were then incubated with PLA probe (PLUS and MINUS) for 1 h. Probe ligation were performed, followed by polymerase-mediated amplification of PLA signals. Fluorescence signals were observed at 461 nm (4’-diamidino-2-phenylindole) and 594 nm (Texas Red) by Carl Zeiss LSM 700 (Zeiss). At least eight fields of each slide were captured and the number of spots per nucleus was counted. Experiments were performed three times independently.

PI3K activity assay. PI3K catalytic subunit p110α or p110β was isolated by immunoprecipitation and then kinase activities were assessed by measurement of P(3,4,5)P3 generated from P(4,5)P2 substrate solution using the competitive in vitro PI3-Kinase Activity ELISA Pico (Echelon Biosciences, Salt Lake City, UT) according to the manufacturer’s indication. PI3 protein production was calculated by the standard curve using sigmoidal dose–response (variable slope) correlation and PI3-kinase activities were indicated by relative protein levels normalized with corresponding control. All assays were performed in triplicate.

P(3,4,5)P3/P(4,5)P2 quantification. After treatment, cells were collected in ice-cold 0.5 M trichloroacetic acid. The organic phases containing acidic lipids were sequentially extracted with water and chloroform containing acidic lipids. The organic phases containing acidic lipids were sequentially extracted with water and chloroform containing acidic lipids. The organic phases containing acidic lipids were sequentially extracted with water and chloroform containing acidic lipids. The organic phases containing acidic lipids were sequentially extracted with water and chloroform containing acidic lipids.

Phosphoproteome sample preparation and MS analyses. Sample processing and liquid chromatography tandem mass spectrometry (LC-MS/MS) were performed at LKS Faculty of Medicine, Proteomics, and Metabolomics Core Facility, Faculty of Medicine, University of Hong Kong. A total of 1.5 mg of protein lysate per sample was digested by the FASP method.13 For total proteome analysis, 50 μg of the protein was used and the remaining were subjected to phosphoprotein enrichment using TiO2 beads.54 The peptides were desalted using C18 ziptips. Total and enriched peptides were subjected to LC-MS/MS analyses on Orbitrap Fusion Lumos ETD mass spectrometer interfaced with Dionex 3000 RSLC nanoLC. The high-resolution, high-mass accuracy MS data obtained were processed using Maxquant version 1.6.0.1. MS data analyzed in triplicate for each condition were searched using inbuilt Andromeda algorithm against Uniprot human protein database (95,000 entries). Search parameters--Enzyme specificity was set to trypsin, up to two missed cleavages allowed for digestion, cysteine carboxamidomethylation as a fixed modification, and phosphorylation of Ser, Thr, and Tyr, and oxidation of methionine as variable modifications. Phosphorylated site quantification was calculated using the label free quantification intensities. Data visualization and bioinformatic analysis was performed in the Perseus software version 1.6.2.1. For statistical analysis, replicates were grouped and analysis of variance test was carried out with permutation-based 1% false discovery rate. Hierarchical clustering of phosphoproteins was performed on logarithmized intensities.

Reverse-phase protein array. RPPA was carried out by our group55,56. Cells were lysed in lysis buffer containing 1% Triton X-100, 50 mM HEPES (pH 7.4), 150 mM NaCl, 1.5 mM MgCl2, and 1 mM EGTA. Protein concentration was adjusted to 1.5 μg/μl and denatured protein was printed onto nitrocellulose-coated slides. The slides were incubated with primary antibodies. Signals were visualized by HRP and DAB colorimetric reaction. Slides were scanned for the quantification of signal intensities, which were determined by “Supercurve Fitting” with a logistic regression model. All data points were normalized for protein loading.

Immunohistochemistry. Human ovarian tumor tissue array (OVCl021) was obtained from Pantonics (Richmond, CA). The slides were deparaffinized and rehydrated in graded ethanol prior to antigen retrieval using citrate buffer pH 6.0. They were then incubated with 3% H2O2 to quench endogenous peroxide activity and goat serum for blocking. Incubation with primary antibody (1:25 for pS6 and 1:40 for AXL) was performed at 4 °C overnight and subsequently with biotin-conjugated secondary antibody (Dako, Carpinteria, CA) for 1 h at room temperature. HRP was detected by DAB (Amresco, Solon, OH). Protein levels were represented by histoscore scores on an arbitrary scale: 0, no immunoreactivity; 1, weak; 2, moderate; 3, intense; and 4, very intense.

Statistical analyses. All experiments were repeated three times. Experiment data were presented as mean ± SD and P-values were calculated using Student’s unpaired two-tailed t-test (unless indicated otherwise) by Prism software. P-values < 0.05 were considered significant.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. Source Data file contains the raw data underlying all reported averages in graphs and uncropped versions of blots presented in the figures. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE57 partner repository with the dataset identifier PXD018449. Data pertaining to TCGA ovarian cancer samples were obtained from the eBioPortal (https://www.ebiportal.org). Additional information is available from the authors upon reasonable request.

Received: 29 July 2019; Accepted: 9 April 2020; Published online: 08 May 2020.

References
1. Utormak, T. et al. The p110α and p110β isoforms of PI3K play divergent roles in mammary gland development and tumorigenesis. Genome Dev. 26, 1573–1586 (2012).
2. Guillermet-Guibert, J. et al. The p110β isoform of phosphoinositide 3-kinase signals downstream of G protein–coupled receptors and is functionally redundant with p110γ. Proc. Natl Acad. Sci. USA 105, 8292–8297 (2008).
3. Thorpe, L. M. et al. PI3K alpha is essential for female fertility and regulates the development of the female reproductive tract by mediating the oncogenic activity induced by loss of the novel tumor suppressor PI3K-p85α. Proc. Natl Acad. Sci. USA 114, 7095–7100 (2017).
4. Li, X. et al. Deregulated Gab2 phosphorylation mediates aberrant AKT and STAT3 signaling upon PIK3R1 loss in ovarian cancer. Nat. Commun. 10, 716 (2019).
5. Lin, Y. et al. PIK3R1 negatively regulates the epithelial–mesenchymal transition and stem-like phenotype of renal cancer cells through the AKT/ GSK3β/CTNNB1 signaling pathway. Sci. Rep. 5, 8997 (2015).
6. Cortés, I. et al. p85β phosphoinositide 3-kinase subunit regulates tumor progression. Proc. Natl Acad. Sci. USA 109, 11318–11323 (2012).
7. Cariglia-Martinez, A. E. et al. Phosphoinositide 3-phosphate regulates invadopodium formation. Biol. Open 3, 924–936 (2014).
8. Cheung, L. W. et al. High frequency of PIK3R1 and PIK3R2 mutations in endometrial cancer elucidates a novel mechanism for regulation of PTEN protein stability. Cancer Discov. 1, 170–185 (2011).
9. Yu, J. et al. Regulation of the p85/p110 phosphatidylinositol 3-kinase: stabilization and inhibition of the p110α catalytic subunit by the p85 regulatory subunit. Mol. Cell. Biol. 18, 2577–2587 (1998).

10. Vougiouka, M. et al. Axl-EGFR receptor tyrosine kinase hetero-interaction provides EGFR with access to pro-invasive signalling in cancer cells. Oncogenesis 5, e266 (2016).

11. Revach, O. Y. et al. Cross-talk between receptor tyrosine kinases AXL and ERBB3 regulates invadopodia formation in melanoma cells. Cancer Res. 79, 2634–2648 (2019).

12. Valverde, P. Effects of Gαs and hydrogen peroxide in Axl ubiquitination and downregulation. Biochem. Biophys. Res. Commun. 333, 180–185 (2005).

13. Chamberlain, M. D. et al. Deregulation of Rab5 and Rab4 proteins in p53R274A-expressing cells alters PGDF trafficking. Cell. Signal. 22, 1562–1575 (2010).

14. Shaid, S., Brandts, C., Serve, H. & Dikic, I. Ubiquitination and selective autophagy. Cell Death Differ. 20, 21 (2013).

15. Vittal, V., Stewart, M. D., Brzovic, P. S. & Klevit, R. E. Regulating the regulators: recent revelations in the control of E3 ubiquitin ligases. J. Biol. Chem. 290, 21344–21551 (2015).

16. Kirkin, V., McEwan, D. G., Novak, I. & Dikic, I. A role for ubiquitin in monitoring autophagy (3rd edition) Mol. Cell. Biol. 34, 1137–13378 (2014).

17. Giosa, R. et al. CBL controls a tyrosine kinase network involving AXL, SYK and LYN in nulitobin-resistant chronic myeloid leukaemia. J. Pathol. 237, 14–24 (2015).

18. Paolino, M. et al. The E3 ligase Cbl-b and TAM receptors regulate cancer metastasis via natural killer cells. Nature 507, 508 (2014).

19. Krishnamoorthy, G. P. et al. Molecular mechanism of 17-allylamino-17-deoxygeldanamycin (17-AAG)-induced AXL receptor tyrosine kinase degradation. J. Biol. Chem. 288, 17481–17494 (2013).

20. Gleason, C. E. et al. Polyubiquitin binding to optineurin is required for optimal activation of TANK-binding kinase 1 and production of interferon β. J. Biol. Chem. 286, 35663–35674 (2011).

21. Kachaner, D. et al. PI3K-dependent phosphorylation of optineurin provides a negative feedback mechanism for mitotic progression. Mol. Cell. 45, 553–566 (2012).

22. Sun, W. et al. Coexpression of Gαs/Ad in human ovarian cancers. Oncology 66, 450–457 (2004).

23. Roux, C. et al. Meta-analysis of microarray data identifies Gαs expression as an independent predictor of poor survival in ovarian cancer. Biomed. Res. Int. 2013, 238284 (2013).

24. Cerami, E. et al. The cBio Cancer Genomics Portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2, 401–404 (2012).

25. Wilkiewicz, J. R., Zougman, A., Nagaraj, N. & Mann, M. Universal sample preparation method for proteome analysis. Nat. Methods 6, 359–362 (2009).

26. Sharma, K. et al. Ultradeep human phosphoproteome reveals a distinct regulatory nature of Tyr and Ser/Thr-based signaling. Cell Rep. 8, 1583–1594 (2014).

27. Cheung, L. W. et al. Naturally occurring neomorphic PIK3R1 mutations activate the MAPK pathway, dictating therapeutic response to MAPK pathway inhibitors. Cancer Cell 26, 479–494 (2014).

28. Shibata, R. et al. Reverse phase protein array: validation of a novel proteomic technology and utility for analysis of primary leukemia specimens and hematopoietic stem cells. Mol. Cancer Ther. 5, 2512–2521 (2006).

29. Perez-Riverol, Y. et al. The PRIDE database and related tools and resources in 2019: improving support for quantification data. Nucleic Acids Res. 47, D442–D450 (2019).

Acknowledgements

This study was supported by Hong Kong Research Grants Council (17116118) to L.W.C. We thank the RPPA Core Facility (funded by NCI #CA016672) of the MD Anderson Cancer Center (Houston, TX) and the Faculty Core Facility of the LKS Faculty of Medicine HKU for the help with confocal microscopy. G.B.M. is supported by a kind gift from the Adelson Medical Research Foundation, the Ovarian Cancer Research Foundation, The Breast Cancer Research Foundation, The Komen Foundation SAC110052, and NCI grants CA217685, CA217842, and CA098258. We also thank Professor Axel Ullrich (Max Planck Institute of Biochemistry, Germany) for generously providing the AXL plasmids and Professor Heike Allgayer (University of Heidelberg, Germany) for the human AXL gene promoter.

Author contributions

L.W.C. conceived and coordinated the project. L.R., V.C.M., and L.W.C. designed the experiments. L.R., V.C.M., Y.Z., D.Z., X.L., C.C.F., C.G., Y.L., and L.W.C. performed experiments. L.R., V.C.M., and L.W.C. wrote the manuscript. L.W.C. thank the RPPA Core Facility (funded by NCI #CA016672) of the MD Anderson Cancer Center (Houston, TX) and the Faculty Core Facility of the LKS Faculty of Medicine HKU for the help with confocal microscopy. G.B.M. is supported by a kind gift from the Adelson Medical Research Foundation, the Ovarian Cancer Research Foundation, The Breast Cancer Research Foundation, The Komen Foundation SAC110052, and NCI grants CA217685, CA217842, and CA098258. We also thank Professor Axel Ullrich (Max Planck Institute of Biochemistry, Germany) for generously providing the AXL plasmids and Professor Heike Allgayer (University of Heidelberg, Germany) for the human AXL gene promoter.

Competing interests

G.B.M. consults with AstraZeneca, ImmunoMET, Ionis, Nuevodata, PDIx bio, Signalchem, Symphogen, and Tarveda; has stock options with Catena Pharmaceuticals, ImmunoMet, SignalChem, Spindle Top Ventures, and Tarveda; sponsored research from AstraZeneca, ImmuneNet, Pfizer, Nanostrin, and Tesaro; travel support from Chrysalis Bio; and has licensed technology to Nanostrin and Myriad Genetics. The other authors declare no competing interests.
