

Dsh Homolog DVL3 Mediates Resistance to IGFIR Inhibition by Regulating IGF-RAS Signaling

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Abstract

Drugs that inhibit insulin-like growth factor 1 (IGFI) receptor IGFIR were encouraging in early trials, but predictive biomarkers were lacking and the drugs provided insufficient benefit in unselected patients. In this study, we used genetic screening and downstream validation to identify the WNT pathway element DVL3 as a mediator of resistance to IGFIR inhibition. Sensitivity to IGFIR inhibition was enhanced specifically *in vitro* and *in vivo* by genetic or pharmacologic blockade of DVL3. In breast and prostate cancer cells, sensitization tracked with enhanced MEK–ERK activation and relied upon MEK activity and DVL3 expression. Mechanistic investigations showed that DVL3 is present in an adaptor complex that links IGFIR to RAS, which includes Shc, growth factor receptor–bound-2 (Grb2), son-of-sevenless (SOS), and the tumor suppressor DAB2. Dual DVL and DAB2 blockade synergized in activating ERKs and sensitizing cells to IGFIR inhibition, suggesting a nonredundant role for DVL3 in the Shc–Grb2–SOS complex. Clinically, tumors that responded to IGFIR inhibition contained relatively lower levels of DVL3 protein than resistant tumors, and DVL3 levels in tumors correlated inversely with progression-free survival in patients treated with IGFIR antibodies. Because IGFIR does not contain activating mutations analogous to EGFR variants associated with response to EGFR inhibitors, we suggest that IGF signaling achieves an equivalent integration at the postreceptor level through adaptor protein complexes, influencing cellular dependence on the IGF axis and identifying a patient population with potential to benefit from IGFIR inhibition. *Cancer Res*; 74(20); 5866–77. ©2014 AACR.

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Introduction

Type 1 insulin-like growth factor 1 receptor (IGFIR) signals via recruitment of adaptors, including insulin receptor substrate-1 (IRS-1) and Shc, to drive proliferation, invasion, and cell survival (1). In the clinical and experimental setting, low IGF bioactivity protects from tumor development and metastasis, suggesting that IGFs provide a potent protumorigenic signal (2, 3). In early clinical trials, IGFIR inhibitory drugs induced objective regressions, some dramatic and durable, as monotherapy in Ewing sarcomas and other uncommon tumors, and with chemotherapy or targeted agents in common cancers (4–9). However, these encouraging early reports have not translated to phase III benefit in unselected patients (10). Clearly, a better understanding of IGFIR biology is required if this therapeutic approach is to be successful. Key priorities are to understand what makes tumors resistant to IGFIR inhibition, use this information to target these drugs to potentially responsive patients, and select rational treatment combinations for clinical testing. To address these issues, we used genetic screening and downstream validation to identify novel determinants of resistance to an IGFIR antagonist.

Materials and Methods

Cell lines and reagents

DUI145, PC3, LNCaP, and LNCaP-LN3 prostate cancer and MCF-7, MDA-MB-231, and MDA-MB-468 breast cancer cells were from Cancer Research UK Cell Services (Clare Hall Laboratories), 22Rv1 prostate cancer cells from the ATCC, and BT20 and BT549 from Dr. Anthony Kong, University of Oxford, Oxford, United Kingdom. All cell lines were mycoplasma-free when tested with MycoAlert (Lonza Rockland Inc.). AZ12253801 and gefitinib were provided by AstraZeneca, DVL-PDZ inhibitor II (DVLi) purchased from Calbiochem, and BMS-754807, XAV939, and AZD6244 from Selleck Chemicals. DVL3 cDNA (Addgene) was amplified using primers 5'-GGATCCATGGACTACAAGGACGACGACGA-3' and 5'-CTCGAGTCACATCACATCCACAAAGAACT-3' incorporating BamHI and XhoI sites, respectively (underlined), digested with BamHI and XhoI (New England Biolabs), cloned into BamHI-XhoI-digested pHRISIN-CSGW HIV vector, and after verification of the insert by DNA sequencing, used for virus production as described (11). Western blotting, immunoprecipitation, and pulldown assays were performed using reagents described in ref. 12, Supplementary Methods, and Supplementary Table S1.

siRNA screens

Screens were performed as described previously (13 and 14), using kinase siRNA library (siARRAY, targeting 779 known and putative human protein kinases; Dharmacon) containing SMARTpools of four siRNAs targeting each transcript, and human DNA Repair siRNA Set V1.0 siRNA library (Qiagen), together with siPLK1 and Allstars siRNA as positive and negative controls, respectively. Cells were reverse-transfected with 50 nmol/L siRNAs using Dharmafect 1 reagent for DUI145 and Dharmafect 3 for MCF-7. Two days later, cells were exposed to vehicle (0.01% DMSO) or AZ12253801 at the GI_{50} for 5 days, and viability was assessed by CellTiter Glo (CTG) assay (Promega). Duplicate primary screens were analyzed to derive Z' factors (dynamic range) and Z -scores (effect of siRNA on viability, corrected for within/between plate variation), as described (14–16). Triplicate second-round screens used four individual siRNAs and the four siRNAs pooled; these and additional siRNAs are listed in Supplementary Table S2. Data were analyzed to generate log2 surviving fractions as the final score for the effect of IGFIR inhibition on viability; scores <-0.2 were regarded as significant, as described (13, 14). Hits were validated by siRNA transfection as above, analyzing after 48 hours by Western blot or quantitative reverse-transcription PCR (qRT-PCR, with primers listed in Supplementary Table S3), or treating with solvent or AZ12253801 and assaying for viability after 5 days or clonogenic survival after 10 to 20 days. Pooled data from ≥ 3 independent experiments were curve-fitted using GraphPad Prism v5 to interpolate GI_{50} and SF_{50} (concentrations inhibiting 50% of growth or survival).

Immunofluorescence and IHC. Cells underwent immunofluorescent staining using ERK antibody (#4695; Cell Signaling Technology). IHC used DVL3 antibody 4D3 (Santa Cruz Biotechnology; see Supplementary Methods).

Xenografts. As detailed in Supplementary Methods, mice bearing DUI145 xenografts were randomly allocated to 14 days treatment with 0.05-mL solvent (DMSO), 25 mg/kg AZ12253801 twice daily, 50 mg/kg DVLi once daily, or combination treatment.

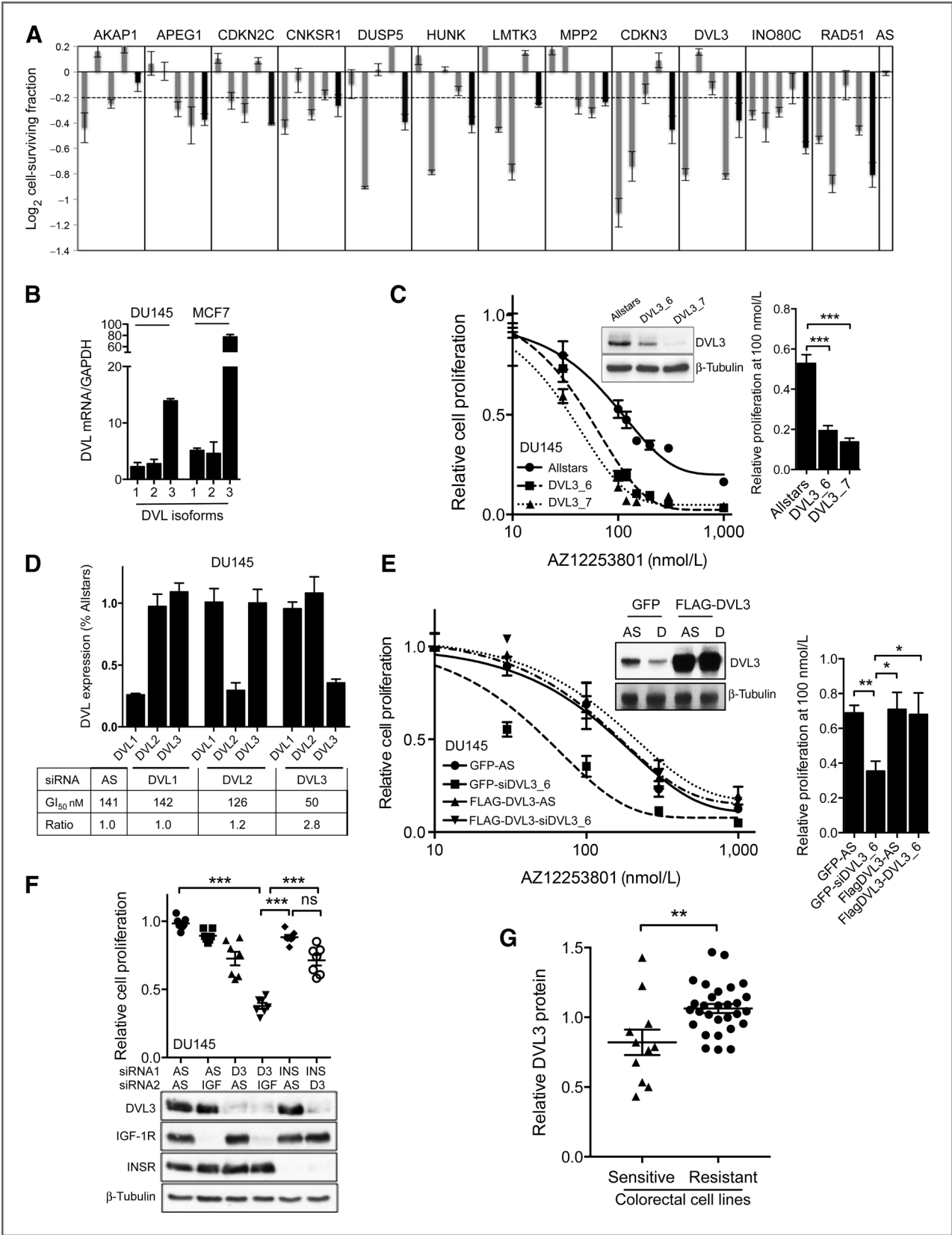
Results and Discussion

siRNA screens for sensitization to IGFIR inhibition

IGFIR overexpression occurs frequently in common cancers, but is a poor predictor of clinical sensitivity to IGFIR inhibition (10). Consistent with this, IGFIR overexpression in PC3 prostate cancer cells did not influence response to IGFIR tyrosine kinase inhibitor (TKI) AZ12253801 that has approximately 10-fold selectivity over the insulin receptor (INSR; Supplementary Fig. S1A; ref. 17). To identify proteins that influence response to AZ12253801, we performed siRNA screens in DUI145 prostate cancer and MCF7 breast cancer cells, confirming that AZ12253801 inhibited IGFIR phosphorylation and cell viability (Supplementary Fig. S1B). Primary screens were performed to deplete approximately 1,000 targets; given our interest in the involvement of IGFIR kinase in the DNA damage response (12, 18), we selected siRNA libraries targeting kinase-related and DNA repair-associated proteins, with positive (siPLK1) and negative (Allstars) control siRNAs, as described (13, 14). Forty-eight hours after siRNA transfection to allow target depletion, cells were treated with solvent or AZ12253801 at the GI_{50} , and viability was assayed 5 days later. Duplicate DUI145 screens were highly reproducible (R^2 values ≥ 0.8) and sensitive, with Z' -factors of 0.23 to 0.6 (Supplementary Table S4) indicating good discrimination between positive and negative controls (15). We calculated drug sensitization Z -scores (13) for each siRNA, rank-ordered siRNAs by Z -score, and selected 54 genes for validation (Supplementary Table S5). Triplicate second-round screens identified 12 putative resistance mediators, including regulators of the cell cycle and DNA damage response, and proteins with poorly characterized functions (Fig. 1A). Seven hits in DUI145 screens (CDKN2C, CNKSR1, DUSP5, HUNK, LMTK3, MPP2, and DVL3) were also candidate hits in MCF7 cells. Validation in low-throughput format confirmed that depletion of each hit enhanced AZ12253801 sensitivity (Supplementary Table S6).

DVL3 mediates resistance to IGFIR inhibition

Known cross-talk between the IGF and WNT axes (19–21) prompted us to investigate one of the most robust hits: Dishevelled homolog 3 (DVL3), a poorly characterized WNT component. DVL3 is one of three mammalian homologs of *Drosophila Dsh*, a cytoplasmic protein that is phosphorylated on binding of Wnts to Frizzled (Fz) receptors, blocking the β -catenin destruction complex (22). DVL3 was the dominant isoform in both DUI145 and MCF7 cells (Fig. 1B), and depletion of DVL3 but not DVL 1 or 2 sensitized to AZ12253801 (Fig. 1C and D). Expression of siRNA-resistant FLAG-DVL3 was able to rescue from AZ12253801 sensitization induced by DVL3 siRNA targeting the 3'-UTR of endogenous *DVL3* mRNA (Fig. 1E), suggesting that sensitization by DVL3 depletion was unlikely to be an off-target effect of DVL3 siRNA. As noted above, AZ12253801 can also inhibit the closely related INSR. Although



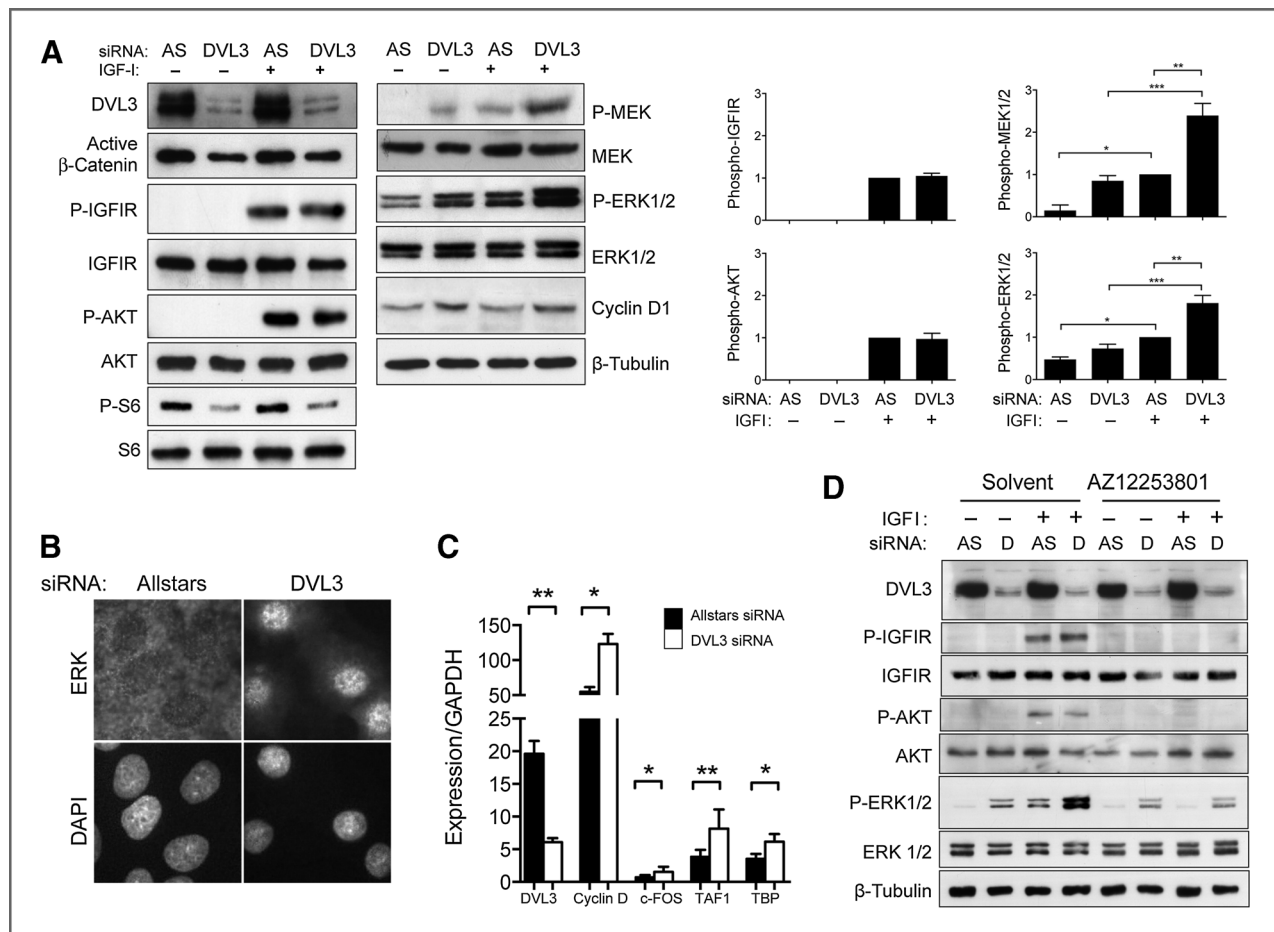


Figure 2. DVL3 depletion enhances IGF signaling via MEK-ERK. **A**, DU145 cells were Allstars (AS) or DVL3 siRNA-transfected, and after 48 hours, treated with 10 nmol/L IGF-I for 10 minutes. Right, graphs, mean $\pm$ SEM phospho-IGFIR, phospho-AKT, phospho-MEK, and phospho-ERKs corrected for equivalent total proteins, expressed as % IGF-treated Allstars-transfectants from three independent experiments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, by one-way ANOVA). **B**, Allstars or DVL3 siRNA-transfected cells were serum-starved for 12 hours and stained for total ERKs, with DAPI nuclear staining. **C**, qPCR for ELK1 target genes in Allstars-transfected and DVL3-depleted cells, mean $\pm$ SEM of triplicate independent analyses (*, $P < 0.05$; **, $P < 0.01$). **D**, Allstars or DVL3 (D) siRNA-transfected DU145 cells were treated with 120 nmol/L AZ12253801 for one hour and in the final 10 minutes with 10 nmol/L IGF-I. Whole-cell extracts were analyzed by Western blot. DVL3 depletion enhanced IGF-induced ERK activation, and this effect was suppressed in AZ12253801-treated cells.

DVL3-depleted DU145 and MCF7 cells were sensitized to IGFIR depletion, INSR-depleted cells showed no significant reduction in viability upon DVL3 depletion (Fig. 1F and Sup-

plementary Fig. S1C), supporting the contention that functional interaction between DVL3 and AZ12253801 is related to the ability of AZ12253801 to block IGFIR. We next tested

Figure 1. DVL3 mediates resistance to IGFIR inhibition. **A**, hits from second-round screens in DU145 cells, showing inhibition of cell viability (mean $\pm$ SEM log₂ cell-surviving fraction), induced by single siRNAs (gray bars), siRNA pools (black), with Allstars (AS) control. Dashed line, -0.2 threshold for significant growth inhibition in AZ12253801-treated cells versus controls. **B**, isoform-specific qPCR showing mean $\pm$ SEM expression of DVLs 1–3 relative to GAPDH. Data from three independent experiments, each with three technical replicates. **C**, DU145 cells were transfected with 50 nmol/L Allstars siRNA or DVL3 siRNAs, 48 hours later treated with AZ12253801, and after 5 days assayed for viability. Graph, mean $\pm$ SEM viability expressed relative to solvent-treated controls; pooled data from three independent assays. Inset, Western blot to check DVL3 depletion. Graph to right, viability at 100 nmol/L AZ12253801 (***, $P < 0.001$, by one-way ANOVA). **D**, DU145 cells were transfected with isoform-specific DVL siRNAs, after 48 hours DVLs were measured by qPCR, normalized to GAPDH. Graph, mean $\pm$ SEM DVL3 expression relative to Allstars transfectants ($n = 3$). Table, AZ12253801 GI₅₀ values in cells depleted of each DVL, fold sensitization calculated as ratio to GI₅₀ in Allstars transfectants. **E**, DU145 cells infected with vectors encoding GFP or FLAG-DVL3 were transfected with 3' UTR siRNA DVL3_6 and viability assayed after 5 days. Inset, Western blotting of parallel cultures. Right, graph, viability at 100 nmol/L AZ12253801 (*, $P < 0.05$; **, $P < 0.01$, by one-way ANOVA). **F**, DU145 cells were transfected with Allstars, DVL3, and/or IGFIR or INSR siRNA (100 nmol/L total siRNA), and viability assayed after 5 days. Parallel cultures were collected after 60 hours to check target depletion. Compared with controls, depletion of IGFIR or DVL3 suppressed viability to 89% $\pm$ 2% and 73% $\pm$ 5%, respectively, and combined depletion to 38% $\pm$ 2% (***, $P < 0.001$, by one-way ANOVA), suggesting supraadditive growth inhibition. Viability in INSR-depleted cells was 88% $\pm$ 2% of control levels, without significant reduction (71% $\pm$ 4%) upon DVL3 depletion. **G**, DVL3 protein levels in 40 colorectal cancer cell lines previously assayed for figitumumab sensitivity, divided by growth inhibition induced by 10 μ g/mL figitumumab into sensitive (>67% growth inhibition, $n = 11$) and resistant cells (moderately, 33%–67% inhibition, $n = 8$ or highly resistant, 0%–33% inhibition, $n = 21$). DVL3 protein levels were significantly lower in the figitumumab-sensitive cells (*, $P = 0.003$, by t test).

whether intrinsic sensitivity to IGFIR inhibition is related to endogenous DVL3 expression. In prostate ($n = 5$) and breast cancer ($n = 5$) cell lines, there was no apparent relationship between endogenous DVL3 protein and AZ12253801 GI_{50} (Supplementary Fig. S1D), perhaps reflecting genotypic differences in these small panels. In a larger sample ($n = 40$) of well-characterized colorectal cancer cell lines (23, 24), mean DVL3 protein levels were significantly lower in cell lines that were sensitive to IGFIR antibody figitumumab ($n = 11$), compared with cell lines that were moderately ($n = 8$) or highly ($n = 21$) figitumumab resistant (Fig. 1G), supporting the hypothesis that DVL3 protein is associated with resistance to IGFIR inhibition. There was no correlation with IGFIR expression (Supplementary Fig. S1E and S1F), consistent with the unchanged response to AZ12253801 in IGFIR-overexpressing PC3 prostate cancer cells (Supplementary Fig. S1A). We also noted lack of correlation between DVL3 protein and mRNA in the colorectal cancer cell lines, likely due to posttranscriptional regulation of DVL3 expression (25), and *DVL3* mRNA did not associate with response to figitumumab (Supplementary Fig. S1G).

DVL3-depleted cells show enhanced ERK activation

We speculated that the association between DVL3 and resistance to IGFIR inhibition may reflect cross-talk between the WNT and IGF pathways. Previously, we and others showed that the adaptor protein IRS-1 undergoes IGF-induced interaction with β -catenin, promoting β -catenin stabilization and transcriptional activity (19, 20). However, in DU145 cells, there was no evidence that IGF1 affected levels of active (nonphosphorylated) β -catenin or phosphorylated DVL3 (Fig. 2A). DVL3 depletion suppressed active β -catenin and phosphorylation of mTOR effector S6, consistent with known roles for DVL3 in canonical WNT signaling and mTOR activation (22, 26). DVL3-depleted cells showed no change in IGFIR expression or activation, but unexpectedly showed increased MEK-ERK phosphorylation (Fig. 2A). Quantification of these data indicates that DVL3 depletion had a detectable (although not statistically significant) effect on basal (ligand-unstimulated) ERK activation, and significantly enhanced IGF-induced ERK activation. This effect was accompanied by ERK nuclear translocation and upregulation of ERK-E twenty-six (ETS)-like transcription factor (ELK) target genes (Fig. 2B and C; ref. 27).

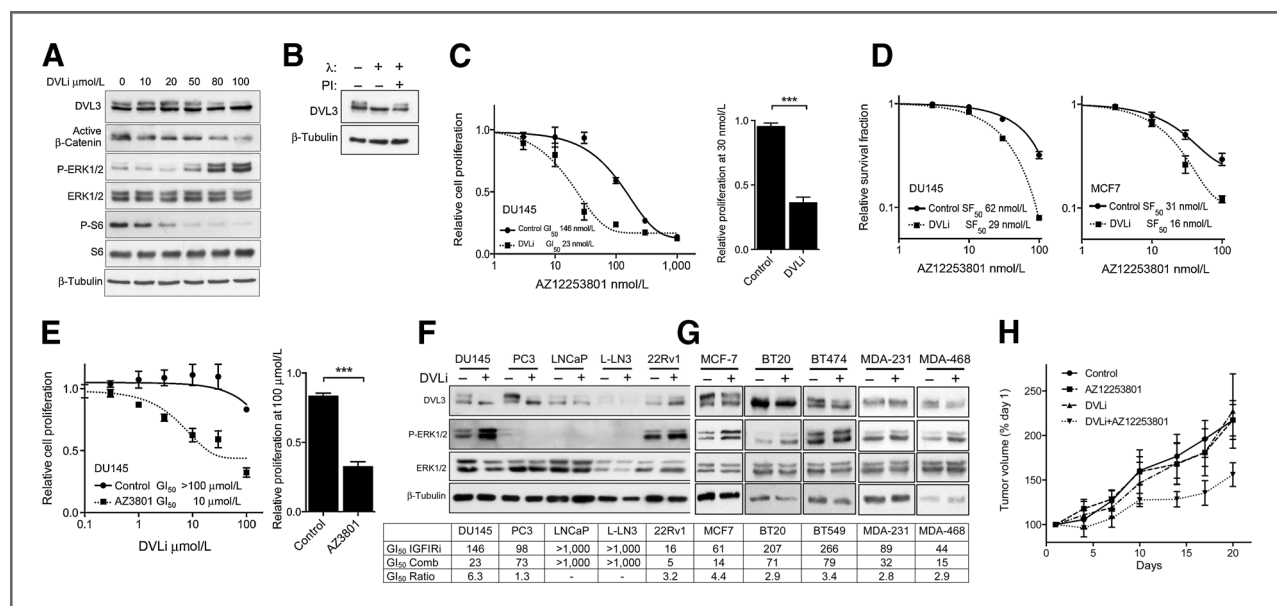


Figure 3. DVL inhibition recapitulates effects of DVL3 depletion and sensitizes to AZ12253801 *in vitro* and *in vivo*. A, serum-starved DU145 cells were DVLi-treated for 16 hours, and whole-cell extracts were analyzed by Western blot using the indicated antibodies. DVLi inhibited DVL3 phosphorylation, reduced active β -catenin and phospho-S6, and increased levels of phospho-ERKs. B, DU145 whole-cell extract was treated with lambda phosphatase (λ) in the absence or presence of phosphatase inhibitors (PI). The upper band of DVL3 immunoreactivity was abolished by λ phosphatase and restored by PI, consistent with DVL phosphorylation, as reported (22). C, DU145 cells were treated with AZ12253801 alone or with 100 μ M DVLi. Legend shows GI_{50} values derived from pooled data in three independent experiments. Right, graph, viability at 30 nmol/L AZ12253801 (***, $P < 0.001$, by *t* test). D, DU145 cells (left, 3,000 cells/well) and MCF-7 cells (right, 5,000 cells/well) were treated with AZ12253801 alone or with AZ12253801 and 50 μ M DVLi for 10 days, and surviving colonies were fixed, stained, and counted. Graphs, mean $\pm$ SEM cell survival, expressed as survival relative to solvent-treated controls. Legends show SF_{50} from pooled data in two independent experiments (6 data points). E, DU145 cells were treated with DVLi alone or with 120 nmol/L AZ12253801. Graph, pooled data from three independent experiments. Right, graph, viability at 100 μ M DVLi (***, $P < 0.001$, by the *t* test). F and G, prostate cancer (F) and breast cancer (G) cells were serum-starved, treated with 100 μ M DVLi for 16 hours, and analyzed by Western blot for phospho-ERK. Bottom, parallel cultures were treated with AZ12253801 alone or with 100 μ M DVLi. Table, pooled data from at least three independent CTG assays, showing GI_{50} (nmol/L) for AZ12253801 alone (IGFIRi) or with DVLi (Comb); fold sensitization calculated as GI_{50} ratio (GI_{50} IGFIRi/ GI_{50} Comb). H, male mice bearing DU145 xenografts were treated for 14 days with intraperitoneal solvent (0.05 mL DMSO), 25 mg/kg AZ12253801 twice daily, 50 mg/kg DVLi once daily, or the combination (DVLi + AZ12253801). Tumor growth in groups treated with AZ12253801 or DVLi was not significantly different from controls. The combination treatment group showed significant tumor growth retardation compared with groups treated with solvent (control, $P < 0.001$), AZ12253801 alone ($P < 0.001$), and DVLi alone ($P < 0.01$) by repeated measures ANOVA.

The IGF-induced component of ERK activation in DVL3-depleted cells was effectively suppressed by AZ12253801 (Fig. 2D). These data suggest that DVL3 depletion enhances both basal and IGF-stimulated ERK activation, analogous to effects of PTEN loss on basal and ligand-induced AKT activation (28).

Of 12 candidate screen hits (Fig. 1A), five have known or putative roles as signaling regulators. In addition to DVL3, these include dual-specificity phosphatase 5 (DUSP5), connector enhancer of kinase suppressor of RAS (CNKSR1), lemur tyrosine kinase 3 (LMTK3), and hormonally upregulated neu-associated kinase (HUNK), reported to regulate ERKs, RAS, AKT, and EGFR, respectively (29–32). Indeed, in DU145 cells, HUNK depletion enhanced IGF signaling to AKT (Supplementary Fig. S2A–S2C), supporting the concept that response to IGFIR inhibition is regulated by factors downstream of IGFIR.

Proximal WNT inhibition mimics effects of DVL3 depletion

Aiming to find a drug that recapitulates effects of DVL3 depletion, we tested compounds that block WNT signaling at different levels. Consistent with a role in Axin stabilization (33), the toolbox tankyrase inhibitor XAV939 upregulated Axin1 and inhibited mTOR, but did not activate ERKs or sensitize to

AZ12253801 (Supplementary Fig. S3A–S3C). We then tested effects of WNT inhibition at a more proximal step, blocking DVL function directly. The DVL-PDZ domain has a peptide-binding cleft, involved in transducing signals from the membrane receptor Fz to downstream canonical and noncanonical pathways (22). The small-molecule inhibitor DVLi competitively binds to the cleft of the DVL-PDZ domain and blocks WNT signaling in *Xenopus* embryo and PC3 prostate cancer cells (34). This agent inhibited DVL3 phosphorylation and phenocopied DVL3 depletion, reducing active β -catenin and phospho-S6, activating ERKs in the absence of ligand, and sensitizing to AZ12253801 in viability and clonogenic assays (Fig. 3A–D). DVLi also sensitized to IGFIR TKI BMS-754807 that is being evaluated clinically (35; Supplementary Fig. S3D). We noted evidence for reciprocal sensitization: despite suppressing active β -catenin and mTOR, DVLi alone caused negligible growth inhibition, perhaps related at least in part to ERK activation, but with AZ12253801 caused significant loss of viability (Fig. 3E).

Although the DVLi we used has relatively low potency, the WNT pathway is an intense focus for drug development (34, 36), offering the prospect of more potent inhibitors in future. Therefore, we used this DVLi to perform proof-of-

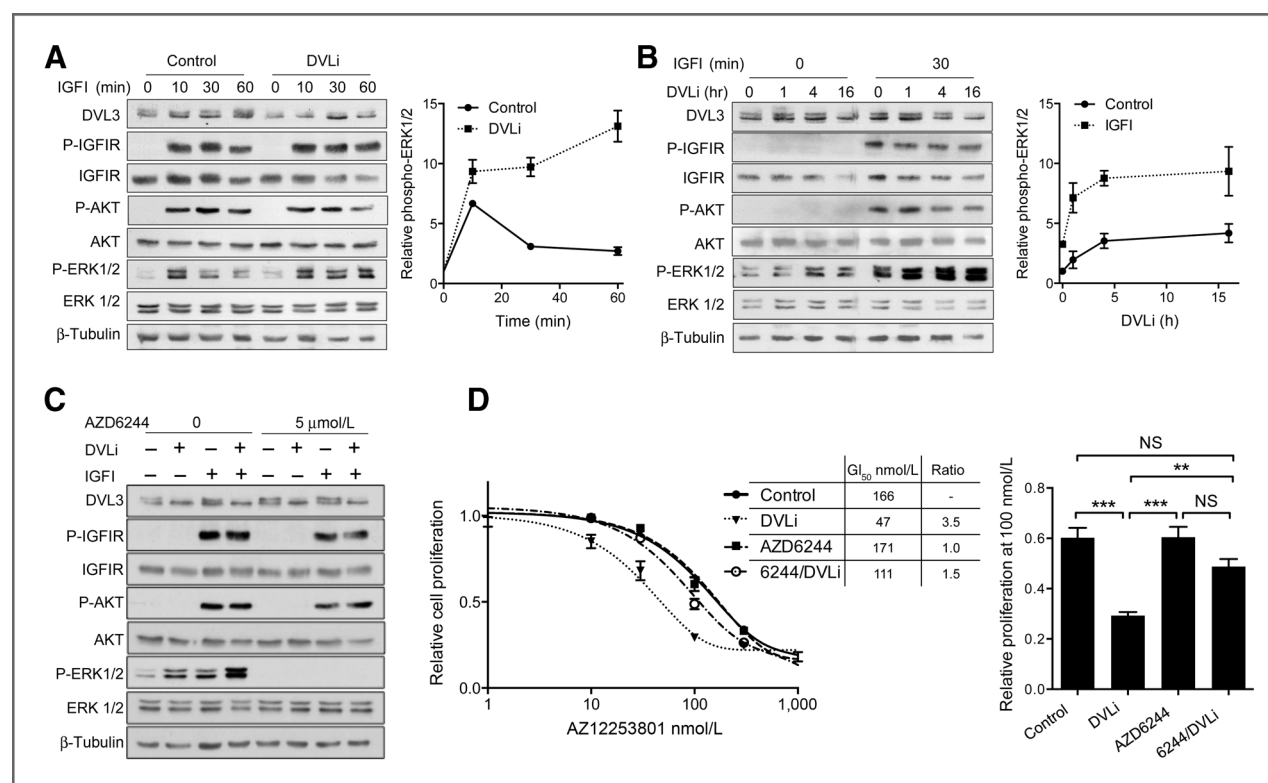


Figure 4. The ability of DVL3 to influence sensitivity to IGFIR inhibition is linked to its capacity to regulate ERK activation. **A**, serum-starved DU145 cells were treated with 100 μ mol/L DVLi for 16 hours and in the final 0 to 60 minutes with 10 nmol/L IGF1. **B**, serum-starved DU145 cells were treated with 100 μ mol/L DVLi for 1 to 16 hours and in the final 30 minutes with 10 nmol/L IGF1. Graphs (A and B) show phospho-ERK (mean $\pm$ range), corrected for total ERK, from two independent experiments. **C**, serum-starved DU145 cells were treated with 100 μ mol/L DVLi and/or 5 μ mol/L AZD6244 for 16 hours and in the final 30 minutes with 10 nmol/L IGF1. **D**, DU145 cells were treated with AZ12253801 alone or 100 μ mol/L DVLi alone or with 5 μ mol/L MEK inhibitor AZD6244, and cell viability was assayed after 5 days. Graph shows pooled data from four independent experiments; legend shows GI_{50} values and fold sensitization (GI_{50} ratio). Right, graph, relative proliferation at 100 nmol/L AZ12253801 (**, $P < 0.01$; ***, $P < 0.001$, by one-way ANOVA).

principle experiments, testing its ability to influence sensitivity to IGFIR inhibition in the prostate and breast cancer cell lines we characterized previously (Supplementary Fig. S1D). Two prostate cancer cell lines (DU145 and 22Rv1) were sensitized to AZ12253801 by DVL inhibition; both had detectable basal ERK phosphorylation that was enhanced by DVLi (Fig. 3F). The other three (PC3, LNCaP, and LNCaP-LN3) did not manifest detectable basal or DVLi-induced ERK phosphorylation and were not sensitized to AZ12253801 by DVLi. The five breast cancer cell lines all showed increased ERK activation and enhanced response to AZ12253801 upon DVL inhibition (Fig. 3G). Thus, 7 of 10 cell lines showed DVLi-induced ERK activation, and in each, DVLi enhanced response to IGFIR inhibition. Supporting the existence of functional cross-talk between the IGF axis and proximal WNT components, upregulation of IGF-binding protein 5 was shown to mediate growth inhibitory effects of a soluble Wnt inhibitor in murine MMTV-Wnt1-driven tumors (36). Therefore, to test the potential clinical relevance of our findings, we evaluated IGFIR and DVL inhibition in mice bearing DU145 prostate cancer xenografts. AZ12253801 or DVLi alone had no significant effects on tumor growth, but growth in the combination treatment group was significantly retarded compared with control-treated ($P < 0.001$),

AZ12253801-treated ($P < 0.001$), and DVLi-treated animals ($P < 0.01$; Fig. 3H).

DVL3 regulates IGF1-induced ERK activation

These data indicate that blockade of proximal WNT signaling enhances sensitivity to IGFIR inhibition, and suggest that this property tracks with regulation of MEK-ERK signaling (Figs. 2A–C and 3F and G). To characterize this effect further, we performed time-course experiments in DVL-inhibited DU145 cells. IGF1 induced rapid activation of IGFIR and AKT that persisted in control cells for at least 60 minutes, whereas ERK activation peaked at 10 minutes and resolved to basal levels by 60 minutes (Fig. 4A). In contrast, there was clear persistence of IGF-induced ERK phosphorylation at 30 to 60 minutes in cells where DVL3 was inhibited (Fig. 4A) or depleted (Supplementary Fig. S4A). Persistent ERK activation in DVL-inhibited cells was apparent within 4 hours of DVLi treatment, and was strikingly enhanced upon IGF treatment (Fig. 4B). An increase in IGF-induced ERK activation was also observed in MCF7 cells (Supplementary Fig. S4B). These results support the existence of a link between the ability of DVL3 to influence sensitivity to IGFIR inhibition and to attenuate the response of the IGF axis to a mitogenic stimulus via MEK-ERK. Consequently, DVL3 depletion or inhibition creates an environment

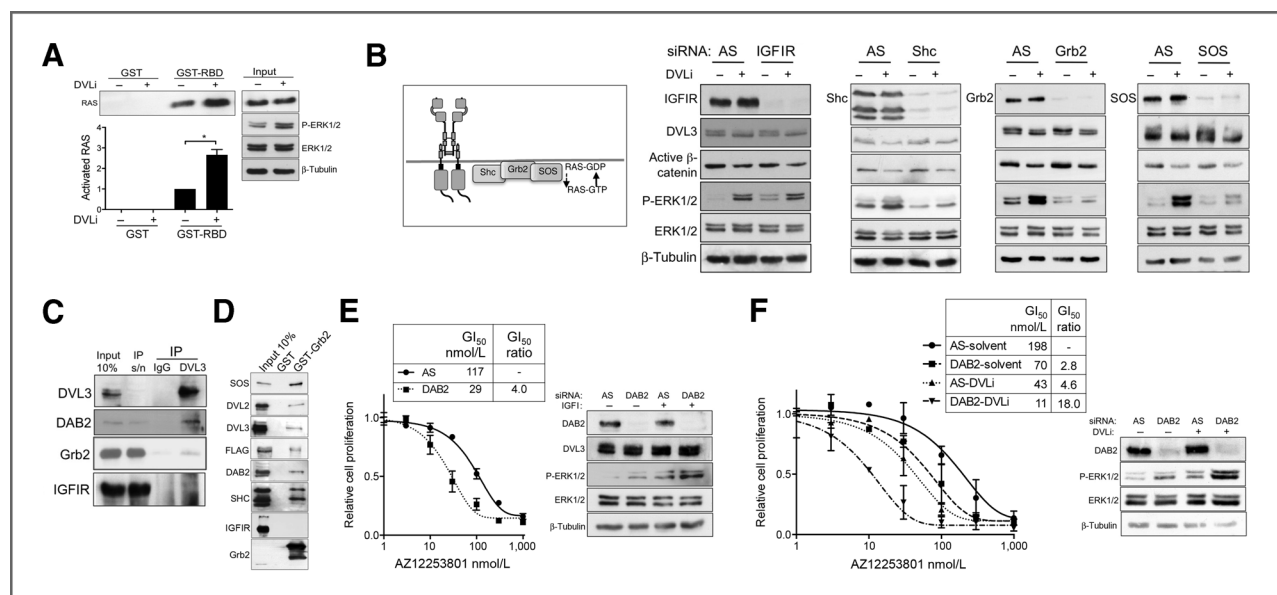


Figure 5. DVL3 regulates signaling from IGFIR to RAS. **A**, DU145 cells were treated with 100 μ M DVLi for 16 hours, lysates were incubated with GST or GST-RAS-binding domain (RBD) of RAF, and pull-downs analyzed by Western blotting for activated (RBD-bound) RAS. Right, blot confirms ERK activation in whole-cell extracts. Bottom, graph, RAS activity in three independent assays, expressed relative to control (DVLi-untreated) cells, *, $P < 0.05$, by t test. **B**, cartoon, IGFIR activates RAS via a protein complex that includes Shc, Grb2, and SOS. DU145 cells were transfected with Allstars (AS) siRNA or siRNAs to deplete IGFIR, Shc, Grb2, or SOS. After 48 hours, cells were treated with 100 μ M DVLi for 16 hours and analyzed by Western blotting. DVLi-induced ERK activation was abolished by depletion of Shc, SOS, or Grb2 but not IGFIR. **C**, DU145 whole-cell extracts were immunoprecipitated with control (IgG) or DVL3 antibodies and analyzed by Western blot, in parallel with DVL3 IP supernatant (s/n) to confirm DVL3 immunodepletion. DVL3 IPs contained DAB2 and Grb2 but not IGFIR. The same result was obtained in two further independent experiments. **D**, DU145 whole-cell extracts incubated with GST or GST-Grb2, and precipitated proteins analyzed by Western blot, revealing the presence of DVL2, DVL3, Shc, and DAB2 in Grb2 pull-downs. Representative of three independent experiments. **E**, DU145 cells were transfected with Allstars or DAB2 siRNA and after 48 hours, treated with AZ12253801 (left), and viability assayed after 5 days, showing GI₅₀ values from three independent assays; right, serum-starved overnight and treated with 10 nmol/L IGF1 for 10 minutes. DAB2 knockdown sensitized to AZ12253801 and also activated ERKs. **F**, Allstars-transfected or DAB2-depleted cells were treated with 100 μ M DVLi and/or AZ12253801, and cell viability assayed after 5 days. Graph, mean $\pm$ SEM viability, pooled data from three independent assays. Legend shows GI₅₀ values and fold sensitization to AZ12253801. Parallel cultures were analyzed by Western blot, shown to right.

that is permissive for signaling, recently characterized as "signalability" (37). In contrast, DVL inhibition did not influence the response to IGF1 in PC3 prostate cancer cells that were not sensitized to AZ12253801 by DVLi (Supplementary Fig. S4C). To test whether ERK activation is required for DVL3 to modify the response to IGFIR inhibition, we abolished ERK activity using MEK inhibitor AZD6244 (Fig. 4C). MEK-inhibited DU145 cells showed almost complete rescue from DVLi-induced sensitization to IGFIR inhibition (Fig. 4D), suggesting that the ability of DVL3 to regulate MEK-ERK contributes to the sensitization effect.

DVL3 complexes with SHC, Grb2, and SOS to regulate RAS activation

To investigate how DVL3 regulates ERKs, we performed RAS activation assays and found that RAS was activated in DVL-inhibited cells (Fig. 5A). This contrasts with previously reported WNT:ERK cross-talk, occurring at more distal WNT signaling nodes, that generates positive feedback between the two pathways (38). Given that DVL3 depletion did not activate IGFIR itself (Fig. 2A and Supplementary Fig. S4B), and DVL3-depleted or DVL-inhibited cells showed no change in EGF-induced ERK activation or sensitivity to EGFR inhibitor gefitinib (Supplementary Fig. S5A and S5B), we reasoned that RAS activation in DVL-depleted or DVL-inhibited cells was unlikely to be initiated at the level of these RTKs.

It is increasingly recognized that mitogenic signals generated by RTKs are integrated by complexes of adaptor and scaffolding proteins, including IRS-1, a well-recognized focus for feedback signaling via ERKs and mTOR-S6 kinase (39). However, IRS-1 knockdown did not influence AZ12253801 sensitivity or ERK activation induced by DVL inhibition (Supplementary Fig. S5C and S5D). In contrast, ERK activation in DVL-inhibited cells was suppressed by depletion of the adaptor protein Shc, exchange factor son-of-sevenless (SOS), or growth factor receptor-bound-2 (Grb2; Fig. 5B). ERK activation was not abolished by depletion of IGFIR (Fig. 5B), suggesting that the ability of DVL3 depletion or DVL inhibition to activate basal (ligand-unstimulated) MEK-ERK signaling was IGFIR independent, perhaps induced by another RTK or signaling complex.

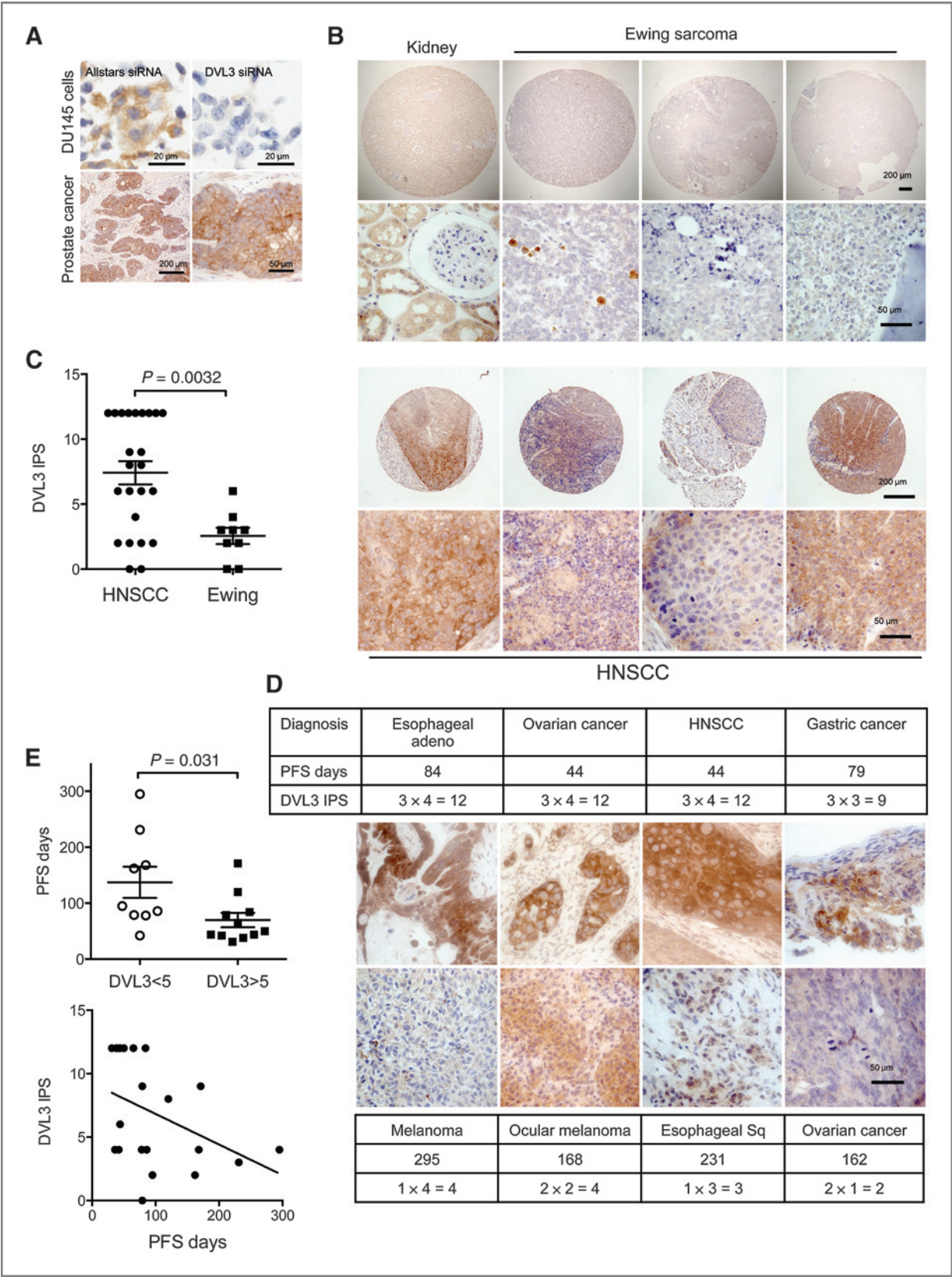
These data suggest that DVL3 suppresses signal transduction at the level of the Shc-Grb2-SOS complex. DVLs were not identified as Grb2 interactors in HEK293 cells (40), but DVL3 does contain the atypical proline-rich region shown in DVL2 to bind Grb2 and promote canonical WNT signaling (41). Indeed, complexes containing Grb2 and DVL3 were detectable in DU145 cells by immunoprecipitation and Grb2 pulldown (Fig. 5C and D). These complexes also contained the putative tumor suppressor Disabled 2 (DAB2), reported to limit RAS activation by competing with SOS for Grb2 binding (42-44). Consistent with this role, and with the association between ERK activation and sensitization to IGFIR inhibition (Fig. 3F and G and Fig. 4D), DAB2 depletion mimicked DVL3 depletion in sensitizing to AZ12253801 and enhancing IGF-induced ERK activation (Fig. 5E). DVL3 and DAB2 showed similar binding patterns to individual Grb2

domains (Supplementary Fig. S5E), interacting principally with the amino-terminal SH3 domain and central SH2 domain, whereas as reported, SOS bound to Grb2 amino and carboxy-terminal SH3 domains (45). We speculated that DVL3 interacts with Grb2 via DAB2, but found that DAB2-depleted cells still contained DVL3:Grb2 complexes (Supplementary Fig. S5F), and could be further sensitized to IGFIR inhibition by DVLi, with 18-fold reduction in AZ12253801 GI_{50} in DAB2-deleted, DVL-inhibited cells, compared with 2.8- and 4.6-fold sensitization induced separately by DAB2 depletion or DVL inhibition (Fig. 5F). These data suggest that DVL3 has a different role from DAB2 in regulating signal transduction to RAS.

DVL3 expression in clinical cancers

To investigate the clinical relevance of these findings, we evaluated DVL3 expression in patient tumors. Given the lack of correlation shown previously between DVL3 mRNA and protein (Supplementary Fig. S1G), we developed an IHC protocol, with controls including DVL3-depleted DU145 cells, to assess DVL3 expression by intensity $\times$ percentage score (IPS; Supplementary Methods; Fig. 6A and Supplementary Fig. S6A and S6B). Approximately 50% of breast and prostate cancers contained moderate or heavy cytoplasmic DVL3 (Supplementary Fig. S6C) that did not correlate with stage, grade, or patient survival (Supplementary Table S7). As a first approach to testing for the correlation between DVL3 protein and sensitivity to IGFIR inhibition, we assessed DVL3 in Ewing sarcomas, reported to be responsive to IGFIR inhibitor monotherapy (4, 5, 46, 47), and head and neck squamous cell cancers (HNSCC) that are resistant (48). The results are shown in Fig. 6B and C; all but one of the Ewing sarcomas contained low or no detectable DVL3, and the mean DVL3 staining score was significantly higher in HNSCCs ($P = 0.0032$). We then assessed DVL3 protein expression in archival tumors from patients recruited to early-phase clinical trials of IGFIR antibodies figitumumab or AVE164 (Supplementary Table S8; refs. 9, 48). Figure 6D shows examples of DVL3 staining in eight of the trial cases, and Fig. 6E shows analysis with respect to progression-free survival (PFS). Although there was overlap in DVL3 expression between patients experiencing early progression versus prolonged control, it was notable that of eight patients achieving prolonged disease control (>84 days, including one partial remission), six of these tumors had low DVL3 expression (IPS <5 ; Supplementary Table S8). PFS was longer in patients whose tumors showed no/low DVL3 ($n = 9$; PFS, 137 ± 28 days) compared with patients whose tumors had moderate or strong DVL3 ($n = 11$; PFS, 70 ± 13 days; $P = 0.031$), and DVL3 staining was negatively correlated with PFS (Spearman $r = -0.55$; $P = 0.0125$; Fig. 6E). Included here were melanomas, ovarian, gastrointestinal, and HNSCCs (Supplementary Table S8), suggesting that DVL3 expression may have predictive value for response to IGFIR inhibition in a range of tumor types.

These data define new roles for DVL3 in suppressing signal transduction from IGFIR to RAS, and regulating response to IGFIR blockade. The association between RAS



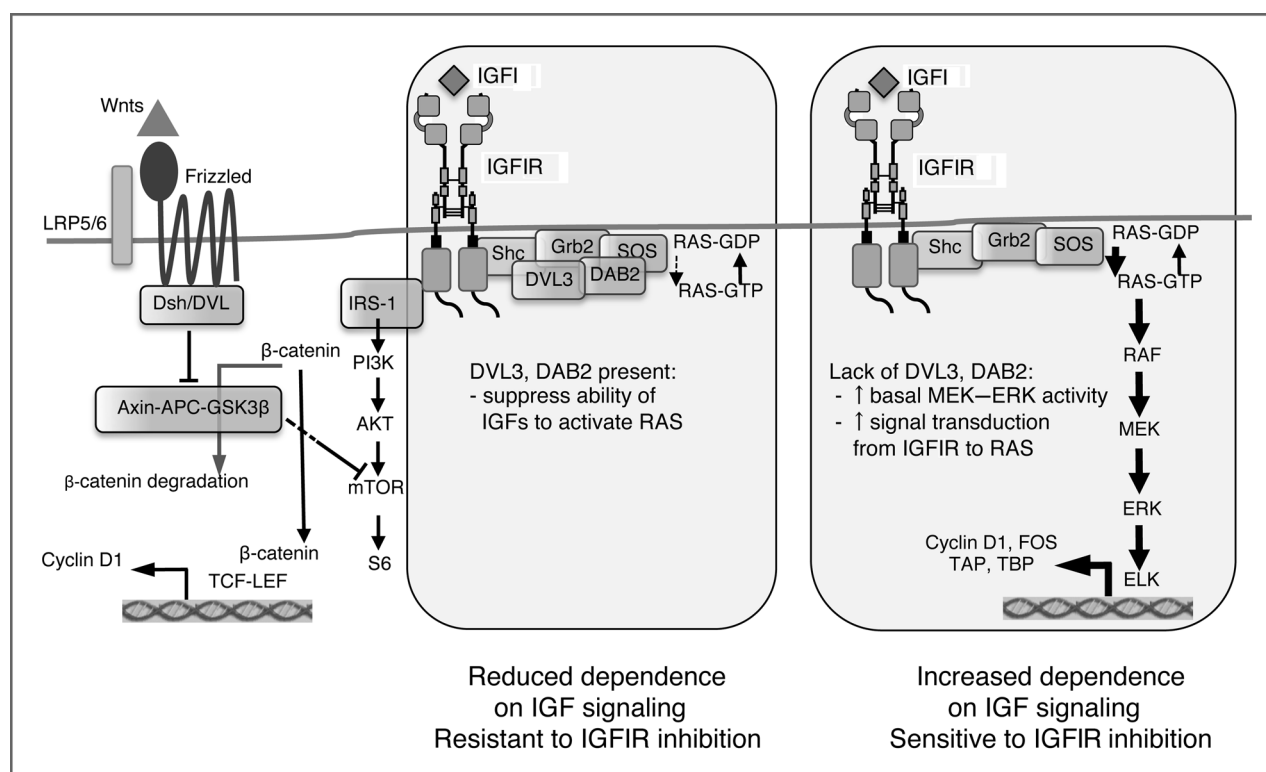


Figure 7. Model for DVL3 as a regulator of RAS activation and mediator of resistance to IGFIR inhibition. DVL3 exists in a complex of adaptor proteins that includes Shc, Grb2, and the tumor suppressor DAB2. Both DVL3 and DAB2 suppress signal transduction from IGFIR to RAS. When DVL3 and/or DAB2 are depleted, or DVLs are inhibited, there is enhanced basal (ligand-unstimulated) RAS–MEK–ERK activation, and in the presence of ligand, selective amplification of IGF signaling via the RAS–MEK–ERK pathway. This state is associated with increased dependency on IGF signaling and enhanced sensitivity to IGFIR inhibition.

activation and sensitization to IGFIR inhibition is counter-intuitive, but is consistent with a report that KRAS-mutated non-small cell lung cancer cells show IGF-dependent PI3K activity and are sensitive to IGFIR inhibition (49). The finding that responses to IGFIR inhibition are modified by manipulating DVL3 expression or function suggests that DVL3 contributes to the resistance phenotype. Thus, DVL3 depletion or inhibition leads to amplification of IGF-induced RAS–MEK–ERK activation, and this property is associated with enhanced sensitivity to IGFIR inhibition (Fig. 7). Similar AZ12253801 sensitization and ERK activation were induced by depletion of DAB2 (Fig. 5E), reported to interact with Grb2 and suppress RAS activation (42–44). DAB2-depleted cells were capable of responding to DVL inhibition with enhanced ERK activation and AZ12253801 sensitization (Fig.

5F), suggesting that DVL3 has a nonredundant function in the Shc–Grb2–SOS complex.

Our screen identified additional candidate resistance mediators (DVL3, DUSP5, CNKSR1, LMTK3, and HUNK) with roles in regulating receptor or postreceptor signaling. The ability to influence IGFIR sensitivity to both ligand and receptor inhibition is reminiscent of the functional effects of EGFR kinase mutations, which favor the active conformation of EGFR kinase and render EGFR sensitive to EGFR TKIs (50). Comparable mutations have been sought but not reported in IGFIR; it is plausible that similar tuning of IGF signaling is achieved by changes in signal transduction complexes downstream of IGFIR. In summary, our data identify a role for DVL3 in suppressing signal transduction from IGFIR to RAS and attenuating response to IGFIR blockade. We propose that the

Figure 6. DVL3 protein expression is inversely correlated with response to IGFIR antibody. A, DU145 cells were transfected with Allstars or DVL3 siRNA, after 48 hours were formalin-fixed, paraffin-embedded and used as controls for immunostaining, in parallel with sections of a transurethral resection of prostate, in which 100% of chippings were involved by Gleason grade 4+5 prostate cancer from a previously untreated patient. These cell and tissue controls were included in every staining run for quality control purposes. B, TMAs of Ewing sarcoma and HNSCC underwent IHC staining in the same staining run. Representative images of Ewing sarcoma (top), a sample of normal kidney included as a control on the same TMA slide (left), and HNSCC (bottom). Most of the Ewing sarcomas had light or no DVL3 signal; intense focal signal in one tumor may be due to staining of macrophages. C, DVL3 IPS scores for nine cases of Ewing sarcoma and 24 cases of HNSCCs, showing significantly higher mean score in HNSCCs. D, examples of DVL3 IHC on tumors from patients on IGFIR antibody trials, showing diagnosis, PFS (days), and DVL3 IPS. E, graph of PFS versus DVL3 IPS in 20 patients treated on IGFIR antibody trials.

proportion of potentially responsive patients in IGF1R trials could be significantly increased by selecting cases with low-DVL3 tumors.

Disclosure of Potential Conflicts of Interest

T.M. Jones received a commercial research grant from GSK. V.M. Macaulay is a consultant/advisory board member for Consultancy. No potential conflicts of interest were disclosed by the other authors.

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References

- Chitnis MM, Yuen JS, Protheroe AS, Pollak M, Macaulay VM. The type 1 insulin-like growth factor receptor pathway. *Clin Cancer Res* 2008; 14:6364–70.
- Pollak M. The insulin and insulin-like growth factor receptor family in neoplasia: an update. *Nat Rev Cancer* 2012;12:159–69.
- Guevara-Aguirre J, Balasubramanian P, Guevara-Aguirre M, Wei M, Madia F, Cheng CW, et al. Growth hormone receptor deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans. *Sci Transl Med* 2011;3:70ra13.
- Olmos D, Postel-Vinay S, Molife LR, Okuno SH, Schuetze SM, Paccagnella ML, et al. Safety, pharmacokinetics, and preliminary activity of the anti-IGF-1R antibody figitumumab (CP-751,871) in patients with sarcoma and Ewing's sarcoma: a phase 1 expansion cohort study. *Lancet Oncol* 2010;11:129–35.
- Tolcher AW, Sarantopoulos J, Patnaik A, Papadopoulos K, Lin CC, Rodon J, et al. Phase I, pharmacokinetic, and pharmacodynamic study of AMG 479, a fully human monoclonal antibody to insulin-like growth factor receptor 1. *J Clin Oncol* 2009;27:5800–7.
- Javle MM, Varadhachary GR, Fogelman DR, Shroff RT, Overman MJ, Ukegbu L, et al. Randomized phase II study of gemcitabine (G) plus anti-IGF-1R antibody MK-0646, G plus erlotinib (E) plus MK-0646 and G plus E for advanced pancreatic cancer. *J Clin Oncol* 2011;29 (suppl: abstr 4026).
- Molife LR, Fong PC, Paccagnella L, Reid AH, Shaw HM, Vidal L, et al. The insulin-like growth factor-I receptor inhibitor figitumumab (CP-751,871) in combination with docetaxel in patients with advanced solid tumours: results of a phase Ib dose-escalation, open-label study. *Br J Cancer* 2010;103:332–9.
- Macaulay VM, Middleton MR, Eckhardt SG, Juergens RA, Rudin CA, Manukyan A, et al. Phase I study of OSI-906, dual tyrosine kinase inhibitor of insulin-like growth factor-1 receptor (IGF-1R) and insulin receptor (IR) in combination with erlotinib (E) in patients with advanced solid tumors. *J Clin Oncol* 2011;29 (suppl: abstr 3098).
- Macaulay VM, Middleton MR, Protheroe AS, Tolcher A, Dieras V, Sessa C, et al. Phase I study of humanized monoclonal antibody AVE1642 directed against the type 1 insulin-like growth factor receptor (IGF-1R), administered in combination with anticancer therapies to patients with advanced solid tumors. *Ann Oncol* 2013;24:784–91.
- Yee D. Insulin-like growth factor receptor inhibitors: baby or the bath-water? *J Natl Cancer Inst* 2012;104:975–81.
- Demaion C, Parsley K, Brouns G, Scherr M, Battmer K, Kinnon C, et al. High-level transduction and gene expression in hematopoietic repopulating cells using a human immunodeficiency [correction of immunodeficiency] virus type 1-based lentiviral vector containing an internal spleen focus forming virus promoter. *Hum Gene Ther* 2002; 13:803–13.
- Chitnis MM, Lodhia KA, Aleksic T, Gao S, Protheroe AS, Macaulay VM. IGF-1R inhibition enhances radiosensitivity and delays double-strand break repair by both non-homologous end-joining and homologous recombination. *Oncogene* 2013 Nov 4. [Epub ahead of print].
- Iorns E, Turner NC, Elliott R, Syed N, Garrone O, Gasco M, et al. Identification of CDK10 as an important determinant of resistance to endocrine therapy for breast cancer. *Cancer Cell* 2008;13:91–104.
- Lord CJ, McDonald S, Swift S, Turner NC, Ashworth A. A high-throughput RNA interference screen for DNA repair determinants of PARP inhibitor sensitivity. *DNA Repair (Amst)* 2008;7:2010–9.
- Boutros M, Ahringer J. The art and design of genetic screens: RNA interference. *Nat Rev Genet* 2008;9:554–66.
- Martin SA, Hewish M, Sims D, Lord CJ, Ashworth A. Parallel high-throughput RNA interference screens identify PINK1 as a potential therapeutic target for the treatment of DNA mismatch repair-deficient cancers. *Cancer Res* 2011;71:1836–48.
- Aleksic T, Chitnis MM, Perestenko OV, Gao S, Thomas PH, Turner GD, et al. Type 1 insulin-like growth factor receptor translocates to the nucleus of human tumor cells. *Cancer Res* 2010;70:6412–9.
- Turney BW, Kerr M, Chitnis MM, Lodhia K, Wang Y, Riedemann J, et al. Depletion of the type 1 IGF receptor delays repair of radiation-induced DNA double strand breaks. *Radiother Oncol* 2012;103: 402–9.
- Playford MP, Bicknell D, Bodmer WF, Macaulay VM. Insulin-like growth factor 1 regulates the location, stability, and transcriptional activity of beta-catenin. *Proc Natl Acad Sci U S A* 2000;97:12103–8.

20. Chen J, Wu A, Sun H, Drakas R, Garofalo C, Cascio S, et al. Functional significance of type 1 insulin-like growth factor-mediated nuclear translocation of the insulin receptor substrate-1 and beta-catenin. *J Biol Chem* 2005;280:29912–20.
21. McManus EJ, Sakamoto K, Armit LJ, Ronaldson L, Shpiro N, Marquez R, et al. Role that phosphorylation of GSK3 plays in insulin and Wnt signalling defined by knockin analysis. *EMBO J* 2005;24:1571–83.
22. Gao C, Chen YG. Dishevelled: the hub of Wnt signaling. *Cell Signal* 2010;22:717–27.
23. Liu Y, Bodmer WF. Analysis of P53 mutations and their expression in 56 colorectal cancer cell lines. *Proc Natl Acad Sci USA* 2006;103:976–81.
24. Ashraf SQ, Nicholls AM, Wilding JL, Ntourogi TG, Mortensen NJ, Bodmer WF. Direct and immune mediated antibody targeting of ERBB receptors in a colorectal cancer cell-line panel. *Proc Natl Acad Sci USA* 2012;109:21046–51.
25. Chan DW, Chan CY, Yam JW, Ching YP, Ng IO. Prickle-1 negatively regulates Wnt/beta-catenin pathway by promoting dishevelled ubiquitination/degradation in liver cancer. *Gastroenterology* 2006;131:1218–27.
26. Inoki K, Ouyang H, Zhu T, Lindvall C, Wang Y, Zhang X, et al. TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. *Cell* 2006;126:955–68.
27. Boros J, O'Donnell A, Donaldson IJ, Kasza A, Zeef L, Sharrocks AD. Overlapping promoter targeting by Elk-1 and other divergent ETS-domain transcription factor family members. *Nucleic Acids Res* 2009;37:7368–80.
28. Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, et al. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell* 1998;95:29–39.
29. Caunt CJ, Keyse SM. Dual-specificity MAP kinase phosphatases (MKPs): shaping the outcome of MAP kinase signalling. *FEBS J* 2013;280:489–504.
30. Therrien M, Wong AM, Rubin GM. CNK, a RAF-binding multidomain protein required for RAS signaling. *Cell* 1998;95:343–53.
31. Giamas G, Filipovic A, Jacob J, Messier W, Zhang H, Yang D, et al. Kinome screening for regulators of the estrogen receptor identifies LMTK3 as a new therapeutic target in breast cancer. *Nat Med* 2011;17:715–9.
32. Komurov K, Padron D, Cheng T, Roth M, Rosenblatt KP, White MA. Comprehensive mapping of the human kinome to epidermal growth factor receptor signaling. *J Biol Chem* 2010;285:21134–42.
33. Huang SM, Mishina YM, Liu S, Cheung A, Stegmeier F, Michaud GA, et al. Tankyrase inhibition stabilizes axin and antagonizes Wnt signaling. *Nature* 2009;461:614–20.
34. Grandy D, Shan J, Zhang X, Rao S, Akunuru S, Li H, et al. Discovery and characterization of a small molecule inhibitor of the PDZ domain of dishevelled. *J Biol Chem* 2009;284:16256–63.
35. Haluska P, Dhar A, Hou X, Huang F, Nuyten DSA, Park J, et al. Phase II trial of the dual IGF-1R/IR inhibitor BMS-754807 with or without letrozole in aromatase inhibitor-resistant breast cancer. *J Clin Oncol* 2011;29 (suppl; abstr TPS111).
36. Liu BY, Soloviev I, Huang X, Chang P, Ernst JA, Polakis P, et al. Mammary tumor regression elicited by Wnt signaling inhibitor requires IGF1R. *Cancer Res* 2012;72:1568–78.
37. Lito P, Pratilas CA, Joseph EW, Tadi M, Halilovic E, Zubrowski M, et al. Relief of profound feedback inhibition of mitogenic signaling by RAF inhibitors attenuates their activity in BRAFV600E melanomas. *Cancer Cell* 2012;22:668–82.
38. Bikkavilli RK, Malbon CC. Mitogen-activated protein kinases and Wnt/beta-catenin signaling: molecular conversations among signaling pathways. *Commun Integr Biol* 2009;2:46–9.
39. Buck E, Eyzaguirre A, Rosenfeld-Franklin M, Thomson S, Mulvihill M, Barr S, et al. Feedback mechanisms promote cooperativity for small molecule inhibitors of epidermal and insulin-like growth factor receptors. *Cancer Res* 2008;68:8322–32.
40. Bisson N, James DA, Ivosev G, Tate SA, Bonner R, Taylor L, et al. Selected reaction monitoring mass spectrometry reveals the dynamics of signaling through the GRB2 adaptor. *Nat Biotechnol* 2011;29:653–8.
41. Crampton SP, Wu B, Park EJ, Kim JH, Solomon C, Waterman ML, et al. Integration of the beta-catenin-dependent Wnt pathway with integrin signaling through the adaptor molecule Grb2. *PLoS ONE* 2009;4:e7841.
42. Xu XX, Yi T, Tang B, Lambeth JD. Disabled-2 (Dab2) is an SH3 domain-binding partner of Grb2. *Oncogene* 1998;16:1561–9.
43. Zhou J, Hsieh JT. The inhibitory role of DOC-2/DAB2 in growth factor receptor-mediated signal cascade. DOC-2/DAB2-mediated inhibition of ERK phosphorylation via binding to Grb2. *J Biol Chem* 2001;276:27793–8.
44. Hocevar BA, Mou F, Rennolds JL, Morris SM, Cooper JA, Howe PH. Regulation of the Wnt signaling pathway by disabled-2 (Dab2). *EMBO J* 2003;22:3084–94.
45. Egan SE, Giddings BW, Brooks MW, Buday L, Sizeland AM, Weinberg RA. Association of Sos Ras exchange protein with Grb2 is implicated in tyrosine kinase signal transduction and transformation. *Nature* 1993;363:45–51.
46. Tap WD, Demetri G, Barnette P, Desai J, Kavan P, Tozer R, et al. Phase II study of ganitumab, a fully human anti-type-1 insulin-like growth factor receptor antibody, in patients with metastatic Ewing family tumors or desmoplastic small round cell tumors. *J Clin Oncol* 2012;30:1849–56.
47. Malempati S, Weigel B, Ingle AM, Ahern CH, Carroll JM, Roberts CT, et al. Phase I/II trial and pharmacokinetic study of cixutumumab in pediatric patients with refractory solid tumors and Ewing sarcoma: a report from the Children's Oncology Group. *J Clin Oncol* 2012;30:256–62.
48. Schmitz S, Kaminsky-Forrett MC, Henry S, Zanetta S, Geoffrois L, Bompas E, et al. Phase II study of figitumumab in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck: clinical activity and molecular response (GORTEC 2008-02). *Ann Oncol* 2012;23:2153–61.
49. Molina-Arcas M, Hancock DC, Sheridan C, Kumar MS, Downward J. Coordinate direct input of both KRAS and IGF1 receptor to activation of PI3 kinase in KRAS-mutant lung cancer. *Cancer Discov* 2013;3:548–63.
50. Pao W, Chmielecki J. Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer. *Nat Rev Cancer* 2010;10:760–74.

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Dsh Homolog DVL3 Mediates Resistance to IGF1R Inhibition by Regulating IGF-RAS Signaling

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