PRL3-zumab as an immunotherapy to inhibit tumors expressing PRL3 oncoprotein

Min Thura, Abdul Qader Al-Aidaroos, Abhishek Gupta, Cheng Ean Chee, Soo Chin Lee, Kam Man Hui, Jie Li, Yeoh Khay Guan, Wei Peng Yong, Jimmy So, Wee Joo Chng, Chin Hin Ng, Jianbiao Zhou, Ling Zhi Wang, John Shyi Peng Yuen, Henry Sun Sien Ho, Sim Mei Yi, Edmund Chiong, Su Pin Choo, Joanne Ngeow, Matthew Chau Hsien Ng, Clarinda Chua, Eugene Shen Ann Yeo, Iain Bee Huat Tan, Joel Xuan En Sng, Nicholas Yan Zhi Tan, Jean Paul Thiery, Boon Cher Goh & Qi Zeng

Tumor-specific antibody drugs can serve as cancer therapy with minimal side effects. A humanized antibody, PRL3-zumab, specifically binds to an intracellular oncogenic phosphatase PRL3, which is frequently expressed in several cancers. Here we show that PRL3-zumab specifically inhibits PRL3+ cancer cells in vivo, but not in vitro. PRL3 antigens are detected on the cell surface and outer exosomal membranes, implying an ‘inside-out’ externalization of PRL3. PRL3-zumab binds to surface PRL3 in a manner consistent with that in classical antibody-dependent cell-mediated cytotoxicity or antibody-dependent cellular phagocytosis tumor elimination pathways, as PRL3-zumab requires an intact Fc region and host FcγII/III receptor engagement to recruit B cells, NK cells and macrophages to PRL3+ tumor microenvironments. PRL3 is overexpressed in 80.6% of 151 fresh-frozen tumor samples across 11 common cancers examined, but not in patient-matched normal tissues, thereby implicating PRL3 as a tumor-associated antigen. Targeting externalized PRL3 antigens with PRL3-zumab may represent a feasible approach for anti-tumor immunotherapy.
A major challenge in cancer therapy is the lack of drug specificity. Many cancer-associated targets of currently approved drugs are also often expressed in normal tissues, inadvertently causing off-target tissue damage. Consequently, the “holy grail” of cancer research has been the exploration of more efficacious therapies against druggable, tumor-specific oncotargets. Antibody-based therapies have proven superior to standard chemotherapy in precisely targeting malignant cells with reduced side effects, acting like magic bullets. Therefore, for a breakthrough in tumor-specific cancer therapy, safety and efficacy, there is an urgent need to continuously identify additional tumor-specific antigens targetable by precise antibody-based drugs.

Phosphatase of regenerating liver 3 (PRL3 or PRL-3) belongs to a unique family of C-terminal prenylated phosphatases within the protein tyrosine phosphatase superfamily, consisting of 3 closely-related members—PRL1, PRL2, and PRL3. In 2001, the Vogelstein group showed that PRL3 was overexpressed in metastatic liver lesions compared to corresponding primary colorectal tumors or normal colon epithelium. PRL3 upregulation has subsequently been reviewed to show a ubiquitous correlation with advanced cancers and poorer prognosis. Traditionally, to target intracellular oncoproteins such as PRL3, small chemical inhibitors (rather than antibodies) are screened in vitro systems as the first-line assay for anti-cancer cell activity, primarily because intracellular compartments are presumed to be inaccessible to large antibody molecules. Since antibodies are more specific and cause fewer side effects than small chemical compounds, our group has actively worked for more than a decade at the forefront of unconventional immunotherapy using antibodies (rather than small chemical inhibitors) to block tumors expressing PRL3, as well as other intracellular oncoproteins, in various animal models. Challenging the dogma, we first proved that intravenously administered PRL3 or PRL1 antibodies could block metastatic lung tumors expressing intracellular PRL3 or PRL1 oncoproteins. In follow-up studies, we established a general concept of targeting multiple intracellular oncoproteins with antibodies from different species or vaccination in several animal models. Today, this concept has been recognized as an emerging field of cancer immunotherapy. In 2016, we proved that a humanized PRL3 antibody (PRL3-zumab; IgG1) could suppress PRL3+ gastric tumors in a clinically relevant orthotopic model for evaluating drug efficacy. Collectively, these reproducible findings cement the targetability of intracellular oncoproteins using antibodies.

Hepatocellular carcinoma (HCC), the most common type of liver cancer, is the second main cause of cancer-related mortalities and fifth most common cancer worldwide. Herein, we explored the utility of PRL3-zumab in treating HCC, a disease with frequent PRL3 overexpression and with an unmet need for efficacious and well-tolerated targeted drugs.

Using clinically relevant orthotopic liver “seed and soil” tumor models, we show that PRL3-zumab specifically inhibits PRL3+ liver tumors. In freshly dissociated tumor tissues, we demonstrate that PRL3 can be detected on the surface of live tumor cells, a phenomenon that can be partially recapitulated on serum-starved cancer cells in vitro where PRL-3 also localizes to the outer surface of secreted exosomes. The presence of surface PRL3 antigens suggests that PRL3-zumab recognizes and targets PRL3+ tumors for elimination in a similar manner as antibodies against classical extracellular targets, as implicated by the requirement of intact Fc region in PRL3-zumab to interact with host FcγRs for recruitment of immune effectors and effective elimination of PRL3+ tumors. Finally, we showcase the clinical relevance of PRL3 as a frequently expressed tumor antigen across 11 major cancer types globally, warranting the exploration of PRL3-zumab as a potential drug against these common malignancies.

Results

In mice, PRL3-zumab blocks PRL3+ liver tumors. Orthotopic tumor models, wherein human cancer cells (“seeds”) are implanted into the organs (“soil”) from which the cancer originated, replicate human disease with high fidelity and more accurately recapitulate clinically relevant therapeutic responses. To dissect the mechanism of how PRL3-zumab could target tumors that express intracellular PRL3, in this study, we established an orthotopic liver model to test the ability for PRL3-zumab to block liver tumors within their natural niche. In a panel of six human (Fig. 1a, lanes 1–6) and two murine (Fig. 1a, lanes 7–8) liver cancer cell lines screened for PRL3 protein expression status, we identified three human liver cancer cell lines—MHCC-LM3, Huh-7, and Hep3b.1.1 (Fig. 1a, lanes 1, 3, and 5)—which expressed endogenous PRL3 (20 kDa). However, only MHCC-LM3 (Fig. 1a, lane 1) could robustly form sizeable orthotopic liver tumors within our manageable short timeframe (≤5 weeks). The MHCC-LM3 cell line was used as the most suitable model for studying PRL3-zumab therapeutic response in view of two prominent factors: (1) PRL3 positivity and (2) rapid orthotopic liver tumor formation. Figure 1b illustrates our orthotopic liver model wherein liver cancer cells were implanted into mouse liver to generate orthotopic liver tumors, followed by treatment with PRL3-zumab for 5 weeks (2 doses per week). Compared to untreated mice, MHCC-LM3 liver tumor formation in PRL3-zumab -treated mice was visibly reduced and measurement of tumor volumes revealed a significant, 7-fold reduction in mean tumor burden between treated mice and untreated mice (Fig. 1c).

To study if PRL3-zumab could extend mice survival beyond the 5-week treatment duration, independent groups of treated and untreated mice were monitored post treatment until the appearance of morbid characteristics (classified as a death event). Clearly, treated mice had a longer median survival time of 12 weeks compared to 8 weeks for untreated mice (Supplementary Fig. 1). Since both PRL3− HepG2 and SNU499 human liver cancer cell lines (Fig. 1a, lanes 2 and 4, respectively) could not form tumors within 5 weeks, we next utilized the highly tumorigenic Hep53.4 murine PRL3− liver cancer cell line as a negative control for PRL3-zumab therapy (Fig. 1a, lane 7), as it robustly forms sizeable liver tumors within 5 weeks. In addition, we engineered a Hep53.4 cell line overexpressing an enhanced green fluorescence protein-tagged PRL3 fusion protein (EGFP-PRL3; 45 kDa) with forced PRL3 expression (Hep53.4-PRL3; Fig. 1a, lane 8) and established Hep53.4 (PRL3−) or Hep53.4-PRL3 (PRL3+) orthotopic liver tumors in mice for PRL3-zumab treatment. As expected, PRL3-zumab failed to inhibit Hep53.4 tumors that lacked PRL3 expression, with no differences observed between treated and untreated mice (Fig. 1d). In contrast, PRL3-zumab strongly inhibited Hep53.4-PRL3 tumors, with significant differences between treated and untreated mice (Fig. 1e).

Collectively, these findings from orthotopic liver models reinforce the fundamental principle that PRL3-zumab therapy specifically blocks PRL3+ (but not PRL3−) tumors.

In culture, PRL3-zumab does not inhibit PRL3+ cancer cells. Because PRL3-zumab blocked PRL3+ tumors in vivo, we then investigated if PRL3-zumab could inhibit the growth of PRL3+ cancer cells in vitro: MHCC-LM3 (PRL3+; Fig. 1c) or Hep53.4-PRL3 cells (ectopic PRL3+; Fig. 1e) by adding high doses (up to 50 μg mL−1) of PRL3-zumab directly to cultured cells. Regardless of dose or PRL3 expression status in this simplified in vitro system, we found that PRL3-zumab had no inhibitory effects (Fig. 1f, red boxes) on the growth of MHCC-LM3 cells (PRL3+; Fig. 1f), Hep53.4 cells (PRL3+; Fig. 1g), or Hep53.4-PRL3 cells (PRL3+; Fig. 1h). In contrast, cisplatin, a well-known chemotherapeutic...
**Fig. 1** PRL3-zumab inhibits PRL3⁺ liver tumors in vivo but not cancer cells in vitro. a Representative western blot (WB) of PRL3 protein expression in human (lanes 1-6) and murine (lanes 7 and 8) liver cancer cells. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as a loading control. Asterisks indicate cell lines that rapidly generate orthotopic liver tumors within 5 weeks. b Outline of orthotopic “seed and soil” liver tumor model for treatments. c-e Mean volumes at the end of the experiment in treated (filled squares) and untreated (filled triangles) groups of mice bearing PRL3⁺ MHCC-LM3 tumors (n=10 mice per group; c), PRL3⁻ Hep53.4 tumors (n=3 mice per group; d), and Hep53.4-PRL3 tumors (n=6 mice per group; e). The mean value was calculated by the Student’s t test (mean ± s.e.m.). P values between treatment pairs as indicated. Lower panels, representative liver tumors at the end of experiment. Scale bar, 10 mm. f-h The viabilities of MHCC-LM3 cells (f), Hep53.4 cells (g), and Hep53.4-PRL3 cells (h) cultured for 48 h with PBS control (filled squares), 5 µg mL⁻¹ PRL3-zumab (filled upright triangles), 50 µg mL⁻¹ PRL3-zumab (filled inverted triangles), 2 µg mL⁻¹ cisplatin (filled diamonds), or 10 µg mL⁻¹ cisplatin (filled circles) were evaluated by an MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. The mean value was calculated by the Student’s t test (mean ± s.e.m., n = 3 biologically independent samples each). *P < 0.05, **P < 0.01, NS, not significant, as compared between treatment and control group for each cell line. Source data are provided as a Source Data file.

drug, resulted in a nonspecific dose-dependent growth inhibition in all three cell lines (Fig. 1f-h, red arrows). These findings indicated that PRL3-zumab only works in vivo, reflecting the requirement of host immune system for its therapeutic effect.

**PRL3-zumab binds to externalized surface PRL3 antigens.** As PRL3-zumab could inhibit tumors expressing PRL3 in mice, we investigated if intracellular PRL3 antigen could be externalized in vivo as an extracellular target for PRL3-zumab binding. Orthotopic PRL3⁺ MHCC-LM3 liver tumors were freshly harvested and dissected into live, single-cell suspensions to compare the percentages of surface PRL3 expression on these ex vivo tumor cells vs. parental MHCC-LM3 cultured cells using PRL3-zumab or cetuximab, a well-known chimeric antibody against the epidermal growth factor receptor (EGFR) as a positive surface protein control (Fig. 2a). Live and dead cells were also...
distinguished using the nonspecific uptake of fluorescent dyes, which freely passes through the compromised membranes of non-viable (dead) cells and thus labels them brightly. As dead cells had pronounced nonspecific uptake of control human IgG (hIgG) antibodies (Fig. 2b; top right quadrants), we only considered surface antigen expression on live cells (Fig. 2b–d; top left quadrants) for the rest of this study. The percentage of surface positive (surface+) cells was subsequently calculated based on the number of surface+ live cells (upper left quadrants) divided by the total live cells (sum of upper and lower left quadrants; Fig. 2e).
Unlike EGFR, which was abundantly expressed on both cultured and tumor cells (Fig. 2c–e), surface PRL3 was poorly expressed on cultured cells, yet expressed on most tumor cells (1.1% vs. 64.9% respectively; Fig. 2d–e). After correcting for nonspecific binding signals, EGFR surface+ cell populations between tumor and cultured cells were comparable (Fig. 2f), while a 57-fold increase in mean PRL3 surface+ cell population was noted in tumors compared to cultured cells (Fig. 2f). Consistent with MHCC liver tumors, we observed a similar increase in PRL3 surface+ cells in metastatic lung tumors formed by tail vein injection of B16F0 melanoma cells (Supplementary Fig. 2a), which express endogenous PRL3 and respond well to PRL3 antibody therapy. The PRL3 surface+ cell population was upregulated 14-fold on tumor isolates from these metastatic PRL3+ B16F0 melanoma lung tumors compared to the cognate cultured cells (Supplementary Fig. 2b). These results show that upregulation of the PRL3 surface+ cell population could be a common feature of PRL3+ solid tumors in vivo. Since total expression levels of PRL3 are only slightly higher in tumor isolates compared to cultured cells (Supplementary Fig. 3a), our findings suggest that the increase in PRL3 surface+ cell populations could be attributed primarily to enhanced PRL3 relocalization rather than an increase in absolute PRL3 expression per se.

Since mechanical and enzymatic tumor dissociation ex vivo might induce cell death or membrane damage (liver tumors, in particular, are considered as “tough” tissues based on their histological composition and require extended tissue processing), we next considered whether the increase in PRL3 surface+ cell populations observed might be related to apoptotic induction. Although early apoptotic cells may still have intact cellular membranes and could thus appear “live” in our Live/Dead analysis, they can be readily identified using Annexin-V, which specifically binds phosphatidylserine, a phospholipid extensively “flipped” onto the outer plasma membranes of early apoptotic cells. Using EGFR as a positive surface protein control, we found that 15–25% of both EGFR surface+ and PRL3 surface+ “live” tumor cells were viable (Annexin-V−), whereas the remaining population were in early stages of apoptosis (Annexin-V+; Supplementary Fig. 3b, c). These results validate that, like EGFR, surface PRL3 is naturally expressed on viable tumor cells, and its externalization does not depend on apoptosis.

The microenvironment of solid tumors is characterized by numerous stressors, including nutrient deprivation, low pH, hypoxia, and oxidative stress. We hypothesized that the difference in PRL3 surface+ cell populations between cultured and tumor cells might be due to a limitation of standard, empirically defined culture conditions to faithfully recapitulate such stresses present within the tumor microenvironment. To investigate the possible influence of microenvironmental stress conditions on surface PRL3 expression in vitro, we serum-starved MHCC-LM3-cultured cells as a simplified model of an in vivo stress faced by solid tumors and assayed for expression of both EGFR and PRL3 on live cells (Supplementary Fig. 3d, e). Prolonged serum starvation of MHCC-LM3 cells for 72 h did not induce significant changes in EGFR surface+ cell population (Fig. 2g), whereas PRL3 surface+ cell population increased 8.4-fold upon serum starvation (Fig. 2g). Interestingly, at the molecular level, we detected antagonistic activation of pro-survival vs. pro-apoptosis and autophagy pathways upon serum starvation (Supplementary Fig. 4), resulting in a complex milieu that might enhance PRL3 externalization in starved cells. Likewise, we reasoned that the upregulation of PRL3 surface+ population was greater in tumor cells (57-fold; Fig. 2f) compared to serum-starved cultured cells (8.4-fold; Fig. 2g) likely due to the additional stresses faced within the tumor microenvironment, such as hypoxia or pH stress, which might further exacerbate PRL3 surface relocalization. Taken together, we provide evidence for stress-inducible cell surface relocalization of intracellular PRL3 antigens to demonstrate mechanistic support for PRL3-zumab’s ability to recognize and target PRL3+ tumor cells in vivo.

PRL3 may be externalized via the exosomal secretion pathway. Since PRL3 lacks a signal sequence that could direct it across the classical endoplasmic reticulum—Golgi secretory pathway, a key question was how PRL3 could be recruited from the cytoplasmic leaflets of the plasma membrane and/or early endosomes to the outer leaflet of the plasma membrane to be localized on the tumor cell surface. Numerous intracellular proteins, including heat-shock protein 70 (HSP70), heat-shock protein 90 (HSP90), and glucose-regulated protein 78 (GRP78), have been reported to be specifically relocalized to the cell surface only in tumor cells, but not in normal cells. In addition, while apoptosis and necrosis could result in leakage and relocalization of intracellular antigens, antibodies against intracellular gp75 can reject tumors where there is no necrosis, suggesting alternative specific pathway(s) enabling antigen externalization for antibody binding. This could include unconventional protein transport pathways, such as exosome secretion, which occurs independently of the endoplasmic reticulum and Golgi to enhance protein translocation to the tumor cell surface. As exosomes have been characterized to stem from endosomes, a compartment where PRL3 protein accumulates, we investigated if metastasis-associated PRL3 might also be externalized via the exosomal pathway. To avoid contamination from exosomes abundantly present in fetal bovine serum used for cell culture, we assayed for endogenous PRL3 expression in exosomes harvested from MHCC-LM3 (PRL3+) and Hep53.4 (PRL3−) cells cultured under serum-free conditions for 24 h. Endogenous PRL3 readily accumulated in exosomes secreted specifically by MHCC-LM3 cells but not by Hep53.4 cells (Fig. 3a; lanes 1–4). Exosomal enrichment was validated using the focal adhesion protein, paxillin (exosome-negative), and the ESCRT-1 complex protein component, TSG101 (exosome-positive; Fig. 3a). To test if PRL3 might possess specific localization signal(s) for packaging into exosomes, we transiently transfected MHCC-LM3 cells with GFP (control) or GFP-tagged PRL3 to generate MHCC-GFP and
MHCC-PRL3 cells, the latter clearly demonstrating plasma membrane localization of PRL3 (Fig. 3b). We found that unlike free GFP, which could not be detected in MHCC-GFP-derived exosomes (Fig. 3c, lane 3), GFP-tagged PRL3 robustly accumulated in exosomes secreted by MHCC-PRL3 cells (Fig. 3c, lane 4). Exosome enrichment was validated here using the endoplasmic reticulum-anchored protein calnexin (exosome-negative) and TSG101 (Fig. 3c, lane 4). We repeated these studies in Hep53.4 and Hep53.4-PRL3 cells and reproducibly demonstrated that PRL3 was not detected in exosomes derived from Hep53.4 parental cells (Fig. 3d, lane 3), but present in exosomes derived from Hep53.4-PRL3 cells (Fig. 3d, lane 4). To test the specificity of PRL3 localization to exosomes, we compared several proteins with varying subcellular localizations, including endosomes (TSG101, Alix), ER (calnexin), focal adhesions (paxillin), nucleus (fibrillarin), nuclear membrane (nucleoporin), and cytosol (actin; Supplementary Fig. 5). Like PRL3, the endosomal proteins tested (TSG101, Alix) robustly accumulated in exosomes (Supplementary Fig. 5). In contrast, except for actin (Supplementary Fig. 5), none of the other proteins tested with varying subcellular localizations accumulated in exosomal fractions. It should be noted that the presence of actin in exosomes was not due to its cytosolic localization, as we observed that cytosolic GFP (Fig. 3b) did not localize to exosomes at all (Fig. 3c, lane 3). Thus, PRL3 possesses specific localization signal(s) driving its exosomal localization.

Recently, a proteomic study found that ~33% of 410 exosomal membrane-bound proteins assays possessed an unconventional flipped “inside-out” protein topology, with typically intracytoplasmic-facing regions of these membrane-bound cellular proteins somehow exposed on the outside of secreted exosomes28. To study if membrane-anchored PRL3 might also share such an unconventional inside-out topology to present PRL3 on the outer exosome surface, we employed a protease-protection assay using proteinase K, a broad-acting protease impermeable to lipid bilayers29,30. We found that GFP-PRL3 was readily digested by proteinase K (Fig. 3e, lanes 2 and 5), implicating a predominantly extracellular, membrane-associated PRL3 localization on exosomes. In contrast, the classical exosome marker proteins TSG101 and Alix were relatively resistant to proteinase K (Fig. 3e, lanes 2 and 5) and required disruption of the exosomal double membrane with 1% Triton X-100 detergent for comparable sensitivity to proteolytic digestion (Fig. 3e, lanes 3 and 6). These results suggest an intravesicular localization for...
these proteins, consistent with previous reports28. Based on these findings, we propose a model for the localization of PRL3 to the surface of exosomes vs. TSG101 and Alix, which are mainly localized within the exosome lumen (Fig. 3f). Collectively, these observations demonstrate that (1) PRL3 possesses specific localization signal(s) for unconventional externalization via exosomes, and that (2) exosomal PRL3 could be a key transport route for “inside-out” PRL3 to the cell surface via membrane fusion.

**PRL3-zumab therapeutic efficacy requires its Fc domain.** Since intracellular PRL3 could be externalized via several unconventional protein-releasing pathways (including exosome secretion), we reasoned that PRL3-zumab might mechanistically exploit tumor clearance pathways similar to conventional antibody therapy targeting other extracellular or surface oncoproteins. To evaluate this, we first investigated the requirement of the Fc region of PRL3-zumab for therapeutic efficacy. Fc receptors (FcRs) on immune cells bind to the constant region (Fc) of IgGs in antigen–antibody complexes and bridge the antibody–antigen complex to host effector cells, such as B lymphocytes, natural killer cells, and macrophages. This results in their recruitment and activation of effector pathways for target antigen/cell clearance via antibody-dependent cell-mediated cytotoxicity (ADCC) or phagocytosis (ADCP)31. To investigate the involvement of host FcRs in PRL3-zumab’s mechanism of action (MOA), two complementary experiments were designed. First, CH1 and CH2 domains were deleted from the original PRL3 antibody to create a (scFv-CH3)2 PRL3-minibody with a truncated Fc portion (Fig. 4a, upper cartoon), which still retained PRL3-zumab’s variable regions for specific binding to PRL3, but not to its two homologs, PRL1 and PRL2 (Supplementary Fig. 6a, b). Second, we co-treated mice with anti-CD16/32 antibodies (Fig. 4a, lower cartoon), which bind to FcγII and FcγIII receptors and inhibit FcR-mediated immune clearance (“FcR blocker”)32.

As the Fc portion of IgG is essential for binding to FcRs33,34, we evaluated the therapeutic efficacies of full-length PRL3-zumab vs. partial Fc-deleted PRL3-minibody. We generated mice bearing PRL3+ MHCC-LM3 orthotopic liver tumors and divided them into untreated (group I), nonspecific human IgG treatment (group II), PRL3-zumab treatment (group III), PRL3-minibody treatment (group IV), PRL3-zumab co-treatment with FcR blocker (group V), and FcR blocker alone (group VI). Our results showed that compared to untreated tumors (group I), only PRL3-zumab treatment (group III) resulted in significant tumor suppression, whereas the other treatments (groups II, IV, V, and VI) lacked anti-tumor efficacy (Fig. 4b). We further reproduced the lack of PRL3-minibody therapeutic efficacy in a different orthotopic gastric tumor model using the PRL3+ SNU-484 human gastric cell line (Supplementary Fig. 6c), illustrating that this lack of therapeutic efficacy was not an organ-specific defect. Since deletion of PRL3-zumab’s CH1 and CH2 domains did not affect the resulting PRL3-minibody’s binding to PRL3 (Supplementary Fig. 6a and b), we reasoned that the loss of therapeutic effect was not due to potential antigen-binding defects, but rather due to the lack of FcR binding ability. Taken together with the abolishment of PRL3-zumab therapeutic efficacy upon blockage of host FcγII/III receptors, our results establish that the interaction between the Fc domain of PRL3-zumab and host FcγII/III receptors is essential for anti-tumor therapeutic effects of PRL3-zumab.

**PRL3-zumab recruits immune cells to PRL3+ tumor niches.** The finding that PRL3-zumab required Fc-FcR interaction for in vivo anti-tumor activity indicated the involvement of the classical ADCC pathway of immune-mediated tumor clearance. We thus performed in situ immunofluorescence analysis with antibodies specific to B cells (B220/CD45R) and NK cells (CD335) on PRL3+ MHCC-LM3 orthotopic liver tumor sections derived from untreated mice (group I), PRL3-zumab monotherapy (group II), FcR blocker monotherapy (group III), or

---

**Fig. 4** PRL3-zumab eliminates tumors in an Fc- and FcR-dependent manner. **a** Cartoon depicting domain architecture of PRL3-zumab (intact Fc) vs. PRL3-minibody (truncated Fc lacking CH1 and CH2 domains) and their ability to engage Fc receptors (FcR) on host immune cells. The anti-CD16/32 FcR blocker antibody prevents IgG from binding murine FcγII/III receptors. **b** Both Fc region of PRL3-zumab and FcR binding are required for anti-tumor effects of PRL3-zumab. Upper panel, representative images of livers from each treatment group at day 35 (5-week endpoint). Tumor areas are framed with black lines. Scale bar, 10 mm. Lower panel, tumor volumes in each group. The mean tumor volumes were calculated using one-way analysis of variance (ANOVA) (mean ± s.e.m.) for independent groups of untreated (n = 12), human IgG-treated (n = 3), PRL3-zumab-treated (n = 6), PRL3-minibody-treated (n = 5), PRL3-zumab + FcR-blocked-treated (n = 7), and FcR blocker-treated (n = 4) mice. Source data are provided as a Source Data file.
In PRL3-zumab-treated tumors (group II), we found a significant enrichment of both B cells (Fig. 5a) and NK cells (Fig. 5b), a phenomenon not observed in untreated and FcR blocker mono-therapy groups (Fig. 5a, b, groups I and III, respectively). Importantly, FcR blocker co-treatment abolished PRL3-zumab-induced accumulation of both B cells and NK cells (Fig. 5a, b, group IV). Whereas NK cells are the major ADCC effectors, macrophage-dependent ADCP is increasingly recognized as the MOA behind many antibodies previously approved to treat cancer. Previous studies have demonstrated that CD45+Lin−CD11b+Ly-6C+ tumor-associated myeloid cells, which include macrophages, can express F4/80 antigen. Interestingly, significantly higher F4/80+ cell recruitment was evident specifically in PRL3-zumab-treated tumors (Fig. 5c). Using a myeloid immunoprofiling panel with a sequential gating strategy (Supplementary Fig. 7a), we validated the accumulation of tumor-infiltrating CD45+Lin−CD11b+Ly-6G−Ly-6C+F4/80+ macrophages in PRL3-zumab-treated tumors (Supplementary Fig. 7b, c). In these tumors, we also noted an enrichment of Ly-6C+F4/80low myeloid cells (Supplementary Fig. 7d), a population that typically comprises of phenotypically similar, but functionally distinct, monocytes and/or monocytic myeloid derived suppressor cells (M-MDSCs). Unlike monocytes, which can function...
PRL3 is frequently overexpressed in multiple human cancers. We previously demonstrated the value of PRL3 as a gastric cancer oncotarget, where PRL3 expression was detected in approximately 85% of fresh-frozen gastric tumor tissues, but not in patient-matched normal gastric tissues. Since elevated PRL3 is frequently overexpressed in multiple human cancers, its overexpression may contribute to immune evasion pathways.

Discussion
In this study, we present five key findings: (1) generation of an orthotopic “seed and soil” liver tumor model using the human PRL3+ HCC cell line, MHCC-LM3, as a clinically relevant liver tumor model for PRL3-zumab therapeutic evaluation, (2) externalization of PRL3 to the cell surface of tumor isolates and serum-starved cultured cells as a direct surface target for PRL3-zumab, (3) exosomal secretion of PRL3 as a possible pathway for intracellular PRL3 to become externalized as surface PRL3, (4) identification of the essential role for the Fc region of PRL3-zumab to bind FcRs and recruit host immune cells for anti-tumor efficacy, akin to classical mechanisms for antibody therapy, and (5) the clinical value of PRL3 as a frequently expressed oncotarget in 11 common cancer types.

Our finding that PRL3-zumab effectively suppressed PRL3+ orthotopic liver tumors presents a solution to a long-standing challenge in medical treatment of HCC, whose pathophysiologic complexity is often exacerbated by underlying functional liver insufficiency. For instance, sorafenib, the first targeted therapy for advanced HCC that demonstrated a slight improvement in mortality, actually resulted in worse survival outcomes in patients with both advanced HCC and liver dysfunction (Child-Pugh B patients) due to toxicity. Given that at least five major phase 3 trials of molecular-targeted agents against advanced liver cancer have failed in the past decade, the frequent overexpression of PRL3 in liver cancer patients (80%; Fig. 6a) highlights PRL3-zumab as a potentially valuable therapeutic option for HCC by fulfilling the unmet need for efficacious and well-tolerated targeted drugs to treat this morbid disease.

Using the MHCC-LM3 orthotopic liver tumor model, we consolidated the MOA for PRL3-zumab by providing key evidence demonstrating how intracellular PRL3 oncoprotein can be externalized as surface PRL3 for PRL3-zumab to trigger the host immune system’s canonical pathways of antibody-mediated tumor clearance within tumour microenvironment. It should be highlighted that PRL3 is not the only intracellular protein to be externalized by malignant cells. HSP70, HSP90, GRP78, actin, cytokeratins, vimentin, and fetu-acinar pancreatic protein are all related examples of intracellular proteins in normal cells, which are externalized by cancerous cells. Early studies have also documented the generally higher permeability of tumor cell membranes as compared to normal cells. Although surface PRL3 could arise by several possible pathways, we found that intracellular, lipid-anchored PRL3 could be externalized on the surface of exosomes with an “inside-out” topology. Notably, most lipid-anchored Rab family proteins have also been predicted as having such a topology on exosomes, an observation experimentally validated for Rab5. Regardless of the route employed, we propose that the externalization of PRL3 in tumors makes it possible to selectively target this oncoprotein with antibody therapy in the same manner as conventional targeting of classical cell surface or secreted targets, ultimately resulting in feedforward “kill-and-leak” cascades facilitating tumor elimination (Fig. 5d).
**Fig. 6** PRL3 is frequently overexpressed in multiple human cancers. 

- **a-k** Representative full western blot (WB) of PRL3 protein levels in tumor (“T”) tissues and, where available, patient-matched normal (“n”) tissues from **a** liver, **b** lung, **c** colon, **d** breast, **e** stomach, **f** thyroid, **g** pancreas, **h** kidney, **i** acute myeloid leukemia (bone marrow aspirates), **j** bladder, and **k** prostate tissues. Relative molecular masses (in kDa) are indicated on the right of each immunoblot. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as a loading control. Source data are provided as a Source Data file.
Unable to be faithfully recaptured using in vitro systems. This is coupled with the preclinical efficacy of PRL3 in vitro. In PRL3 patient-derived xenografts (PDX), we found that serum starvation, which mimics the deprivation of host factors within the tumor microenvironment. True to this, we demonstrate that the in vivo environment plays a crucial role in the MOA together with well-established cell surface-targeting drugs such as antibody–drug conjugates (ADCs). Despite potent anti-tumor activity in vivo, PRL3-zumab did not result in any killing or suppression of PRL3+ cancer cell growth in vitro. Our findings in vitro support several possible explanations for this discrepancy. First, the proportion of PRL3 surface+ cells is much lower in cultured cells compared to tumor cells, likely failing to reach sufficient threshold levels to trigger PRL3-zumab-mediated inhibitory effects in vitro. Second, in vitro artificial culture conditions fail to recapitulate complex host factors within the tumor microenvironment. True to this, we found that serum starvation, which mimics the deprivation of growth-promoting factors common in poorly vascularized tumors, enhanced the presence of surface PRL3+ surface on cancer cells. Third, complex tumor–host immunity interactions are unable to be faithfully recapitulated using in vitro systems. This is a major limitation, as we found that in vivo Fc-FcyR binding is essential for anti-tumor effects of PRL3-zumab by recruiting immune cells into PRL3+ tumor microenvironments. In tumor sections, PRL3-zumab promoted tumoral infiltration of B cells, NK cells, and macrophages, which are important mediators of ADCC and ADCP. Intriguingly, this sets PRL3-zumab apart from other intracellular-targeting antibodies, which typically rely on cell penetration and target inhibition.46,47, instead and places its MOA together with well-established cell surface-targeting drugs such as trastuzumab and rituximab. Our findings herein demonstrate that the in vivo environment plays a crucial role in influencing the druggability of target proteins and their therapeutic responses, and that in vivo drug screening should be employed for drug development studies to circumvent limitations of assays based on simplified laboratory assays.

PRL3 is specifically overexpressed in 80.6% of randomly analyzed human tumors (across 11 cancer types), but not in any matched normal tissue examined, making it an attractive general tumor antigen to treat many human cancers. Moreover, PRL3-matched normal tissues examined, making it an attractive general tumor antigen to treat many human cancers. Moreover, PRL3-matched normal tissues were not observed in any tumor types.

Conditions and media
PRL3 surface was determined by flow cytometry using an antibody against PRL3 (clone 318) and secondary antibody (goat anti-mouse IgG, H+L, 1:4000, Thermo). The antibody was diluted in serum-free media (Gibco) containing 1% pre-warmed bovine serum albumin (BSA; Sigma). Antibody binding was detected by PIE Detection Kits (DiaSorin). Data were acquired using a BD LSR Fortessa flow cytometer (BD Biosciences) and analyzed using FlowJo software (TreeStar). The percentage of PRL3+ cells was calculated by summing the number of events in the PRL3+ gate and dividing by the total number of events in the sample.

Table 1 Summary of PRL3 expression across different tumor types

| Tumor type   | PRL3+ | PRL3- | Total | % PRL3+ |
|--------------|-------|-------|-------|---------|
| Liver        | 16    | 4     | 20    | 80      |
| Lung         | 9     | 1     | 10    | 90      |
| Colon        | 7     | 3     | 10    | 70      |
| Breast       | 9     | 1     | 10    | 90      |
| Stomach      | 12    | 2     | 14    | 86      |
| Thyroid      | 11    | 1     | 12    | 92      |
| Pancreas     | 6     | 1     | 7     | 86      |
| Kidney       | 13    | 5     | 18    | 72      |
| AML          | 6     | 6     | 12    | 50      |
| Bladder      | 24    | 10    | 34    | 71      |
| Prostate     | 4     | 0     | 4     | 100     |
| Total        | 117   | 34    | 151   | 80.6    |

PRL3 phosphatase of regenerating liver 3

Despite potent anti-tumor activity in vivo, PRL3-zumab was generated in-house. PRL3-zumab was engineered based on the original framework of murine anti-PRL3 mAb (clone 318) and extensively validated for specific PRL3 binding.41, GAPDH (clone MAB374) antibody was purchased from Millipore. PFP (clone B-2) and actin (clone H-196) antibodies were purchased from Santa Cruz Biotechnology. TSG101 (14497-1-AP) antibody was purchased from Proteintech. Aflx (clone 3A9) and fibrillin (clone C13C3) antibodies were purchased from Cell Signaling Technologies. Calnexin (cat# 61050-1-Ig) and meprin p62 (cat# 610497), and pawlin (cat# 610501) antibodies were purchased from BD Biosciences. Horse radish peroxidase (HRP)-conjugated goat anti-mouse, anti-rabbit, and anti-human (H + L) secondary antibodies and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse antibody were purchased from Jackson ImmunoResearch. FITC-conjugated goat anti-human antibody was purchased from Life Technologies. CD35/NKp46 (clone 29A1.4), B220/CD45R (clone RA3-6B2), and CD86 (clone GL1) were purchased from BD Pharmingen. F4/80 antibody (clone REA126) was purchased from Miltenyi Biotec. Cetuximab was purchased from Oncogene, Inc. Anti-CD16/CD32 (clone RA3-4G2), polyclonal human IgG, and polyclonal mouse IgG were purchased from Bio X Cell. Additional antibody details and working dilutions used for various applications are provided in Supplementary Table 2.

Preparation of tissue and cell lysates. Excised tissue samples (5 mm3) were suspended in RIPA lysis buffer (150 mM NaCl, 1.0% IGEPAL CA-630, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 50 mM Tris, pH 8.0) supplemented with a protease and phosphatase cocktail (Roche) for 15 min at 4 °C and disrupted completely with a tissue homogenizer (Polytron). Lysates were analyzed by centrifugation at 13,000 × g for 40 min at 4 °C. For cultured cells, 106 cells were lysed in lysis buffer and clarified as described above. Protein concentrations of both tissue and cell lysates were estimated using a bicinchoninic acid assay kit (Pierce). The addition of 2× Laemmli buffer containing dithiothreitol (50 mM final concentration), samples were boiled and used immediately for Western blotting or stored at −80 °C until use.

Isolation of exosomes. Exponentially growing cells in T-75 flasks were cultured in complete media till 70–80% confluence and washed twice with phosphate-buffered saline (PBS) before incubation in serum-free media (10 ml per flask) for 24 h. For transient transfection studies, 106–107 cells were transfected with pEGFP-C1 or pEGFP-C1-PRL-3 plasmids pre-mixed with 25 µl of jetPRIME reagent and added to exponentially growing cells (20 µg) resuspended in PBS were incubated in serum-free media for another 24 h. We used serum-free media in these cellular studies to avoid the contamination of bovine exosomes abundantly present in FBS. The harvested culture medium was first subjected to sequential centrifugation at 300 × g for 5 min to remove floating cells and at 2000 × g for 20 min to remove cell debris. The clarified culture medium (~50–100 µl) was then concentrated to ~250 µl using Amicon Ultra centrifugal filters (100 K MWCO; EMD Millipore), mixed thoroughly with 0.5 volumes of Isolation Reagent (Thermo Fisher Scientific), and incubated for 16 h at 4 °C. Exosomes were finally pelleted by centrifugation at 10,000 × g for 60 min at 4 °C, resuspended in PBS, and analyzed immediately or stored at −20 °C till use.

Proteinase K treatment. Exosomes (20 µg) resuspended in PBS were incubated with 2 µg ml−1 Proteinase K (Invitrogen) in the presence of 5 mM CaCl2 for 20 min at 37 °C. The proteinase activity was then inhibited by adding 5 mM phe nylmethylsulfonyl fluoride for 5 min on ice and immediately analyzed.

Western blotting. Tissue, cellular, and exosomal lysates were resolved on SDS-polyacrylamide gels and transferred to nitrocellulose membranes before incubation with blocking buffer (5% skim milk in 20 mM Tris, pH 7.6, 140 mM NaCl, 0.2% Tween-20) for 1 h, followed by overnight incubation at 4 °C with primary antibodies diluted in blocking buffer (working dilutions given in Supplementary Table 2). After thorough washing with TBS-T buffer (20 mM Tris, pH 7.6, 140 mM NaCl, 0.2% Tween-20), membranes were incubated with HRP-conjugated secondary antibodies for 1 h, thoroughly washed with TBS-T, and visualized using a
chemiluminescent substrate (Millipore). Uncropped and unprocessed scans of all blots are provided in the Source Data file.

Mouse tumor models and treatments. Eight-week-old male NCr nude mice (InVivos Pte Ltd, Singapore) were used for all mouse models in this study. Mice were anesthetized intraperitoneally with a cocktail comprising ketamine (150 mg kg\(^{-1}\)) and xylazine (10 mg kg\(^{-1}\)). Abdomens of anesthetized mice were opened in layers by a 1-cm midline incision starting just below the xiphoid sternum. For orthotopic liver tumor models, the liver was exposed and HCC cells (3 \(\times\) 10\(^5\) for MHCC-LM3, 5 \(\times\) 10\(^5\) for Hep3434 and Hep344-PRL3) were inoculated in a total volume of 50 μl into the subcapsular layer of the left lobe of livers. For orthotopic gastric tumor models, 3 \(\times\) 10\(^3\) SNU-448 cells were inoculated in a total volume of 50 μl into the subserous layer of the exposed stomach. The abdominal wall was then sutured back in layers. Because of different growth rate of individual tumors, the duration of experiments were 5 weeks (35 days) for MHCC-LM3 tumors, 4 weeks (28 days) for the subcutaneous tumors in mice or 3 days for SNU-448 tumors by a 1-cm midline incision starting just below the xiphoid sternum. For other studies before being cut into 2–4 mm pieces for dissociation using a human Tumor Dissociation Kit and gentle MACS Octo Dissociator (Miltenyi Biotec) following manufacturer's instructions. Briefly, 2 \(\times\) 10\(^5\) cells were seeded in complete media into triplicate wells of a 96-well plate and allowed to attach overnight. The medium was then replaced with complete media containing 3% BSA in PBS containing 0.05% Tween-20 (PBS-T) prior to incubation with 1 ng of GST-PRL1, GST-PRL2, or GST-PRL3 were blocked with 3% bovine serum albumin (BSA) in PBS containing 0.05% Tween-20 (PBS-T) prior to incubation with 200 ng PRL3-zumab, human IgG for 2 h at 37 °C. After washing three times with PBS-T, the plates were air-dried at room temperature. Total proteins were extracted and subjected to Western blot analysis. Fresh-frozen multiple cancer patient samples were collected and stored at liquid nitrogen immediately after surgery. Human tissue samples were approved by the Institutional Review Board of the National University of Singapore and the National Healthcare Group, Singapore.

Cell viability assay. Cell viabilities were assessed using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2,5-diphenyltetrazolium)-based CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega) according to the manufacturer's instructions. Briefly, 2 \(\times\) 10\(^3\) cells were seeded in complete media triplicate wells of a 96-well plate and allowed to attach overnight. The medium was then replaced with complete media containing PBS (0.1%; control), PRL3-zumab (5 or 50 μg mL\(^{-1}\)) and the indicated concentrations of cetuximab (chimeric anti-EGFR mAb), PRL3-zumab (humanized anti-PRL3 mAb), m318 (murine anti-PRL3 mAb), or polyclonal human or mouse IgG in a 1:200 dilution. Brieﬂy, cells were washed twice with PBS and incubated with serum-free RPMI at 37 °C and 5% CO\(_2\) for the indicated durations prior to harvest. Harvested cells were washed once with PBS, counted, resuspended in full RPMI media, and kept on ice till analysis. For analysis of cultured cells in vitro, exponentially growing cells at 80% confluence in T-75 flasks were washed once with PBS and incubated with 2 ml non-enzymatic cell dissociation buffer (Sigma-Aldrich) for 5 min to dislodge the adherent cells into suspension. Incubation experiments, cells were washed twice with PBS and incubated with serum-free RPMI at 37 °C and 5% CO\(_2\) for the indicated durations prior to harvest. Harvested cells were washed once with PBS, counted, resuspended in full RPMI media, and kept on ice till analysis. For cell surface analysis by flow cytometry, 4 \(\times\) 10\(^3\) dissociated cells were washed with PBS and resuspended in PBS containing 1:1000 dilution of LIVE/DEAD Fixable Near-Infrared stain (Thermo Fisher Scientific). Cells were then washed and incubated with 2 μg of 4°C, the cells were washed twice with 1 mL Annexin V binding buffer (ABB; 10 mM HEPES, pH 7.4, 150 mM NaCl, 2.5 mM Ca\(_{2+}\)) and resuspended in 100 μL ABB containing 2.5 μL Annexin-V FITC (Roche Diagnostics) or Annexin-V Alexa Fluor 350 (Thermo Fisher Scientific). After a 15 min incubation at 4°C, cells were washed once with ABB and finally resuspended in 150 μL ABB prior to analysis. All samples were run on a BD LSRII flow cytometer and analyzed using the Flowing Software 2 (Turku Center for Biotechnology) or FlowJo software (FlowJo).

Immunoprofiling of tumor-infiltrating cells. Single-cell suspensions of tumors isolates were first stained with Zombie UV Fixable Viability dye (BioLegend) for 30 min at 4°C. Non-specific labeling was blocked with anti-CD16/32 (clone 2.4G2; BD Biosciences) for 30 min at 4°C before multiplex labeling for 30 min at 4°C with the following antibodies from BioLegend: AF488 anti-mouse CD45 (clone 30-F11; 1:15 dilution), Brilliant Violet 785 anti-mouse CD4 (clone 145-2C11; 1:30 dilution), Brilliant Violet 785 anti-mouse CD45RB/B220 (clone RA3-6B2; 1:60 dilution), Brilliant Violet 421 anti-mouse CD335 (clone 29A1.4; 1:15 dilution), PE/Dazzle 594 anti-mouse CD11b (clone M1/70; 1:60 dilution), APC/Cy7 anti-mouse F4/80 (clone BM8; 1:30 dilution), PE/Cy7 anti-mouse Ly-6G (clone IAB; 1:80 dilution), and PE/Cy7 anti-mouse Ly-6C (clone HK1.4; 1:60 dilution). Lineage markers (Lin) were defined as CD3, CD335, and B220. All samples were run on a BD LSRII flow cytometer and analyzed using the FlowJo software (FlowJo). The gating strategies used to identify Ly-6C/488*/Ly-6G/circulating myeloid cell populations are provided in Supplementary Fig. 9.

Generation of anti-PRL3 (scFv-CH2-Mab)-minibody. The DNA sequence of PRL3-zumab antibody was sent to ImaginAb (CA) for the synthesis of the anti-PRL3 (scFv-CH2-Mab)-"minibody" ("PRL3-minibody") using their proprietary in-house methodology. PRL3-minibody is a smaller (∼80 kDa) version of PRL3-zumab, devoid of Fc C\(_\text{II}\) and C\(_\text{III}\) domains but validated to retain specific binding towards the conserved PRL3-zumab epitope (Supplementary Fig. 4a, b).

Enzyme-linked immunosorbent assay. Ninety-six-well plates coated overnight with 1 ng of GST-PRL1, GST-PRL2, or GST-PRL3 were blocked with 3% bovine serum albumin (BSA) in PBS containing 0.05% Tween-20 (PBS-T) prior to incubation with 200 ng PRL3-zumab, PRL3-minibody, or human IgG for 2 h at 37 °C. After extensive washing in PBS-T, HRP-conjugated anti-human antibody was added for 1 h at 37 °C. Colorimetric development was performed using a TMB substrate kit (Thermo Fisher Scientific) and stopped by acidification with M H\(_2\)SO\(_4\). Absorbance was measured at 450 nm using a plate reader (Tecan).

Immunofluorescence analysis. Fresh-frozen specimens of MHCC-LM3 orthotopic liver tumors were sectioned into 10 μm slices using a cryostat (Leica) at 16 °C and transferred onto polyo-1-lysine-coated slides (VWR). For cultured cells, cells were seeded onto glass coverslips in complete media 24 h prior to fixation. Slides or coverslips were fixed with 4% paraformaldehyde washed twice with PBS for 10 min at 4°C, or citiplastin (Hospital; 2 or 10 µg mL\(^{-1}\)) and left to incubate for another 48 h at 37°C in 5% CO\(_2\) atmosphere. The media were subsequently aspirated and replaced with 150 μL fresh media containing MTs (Promega) and formazan development was done for 2 h at 37°C. For mouse tissue samples, frozen sections were cut on a cryostat (Leica) at 16°C, mounted in Tissue-Tek O.C.T. Compound (Sakura Finetek USA, Inc.) and stored at −20°C. Tissue sections were dewaxed, hydrated and stained with hematoxylin and eosin. Immunohistochemistry was performed using a DAKO autostainer (DAKO) with the following antibodies from BioLegend: AF488 anti-mouse CD45 (clone 30-F11; 1:30 dilution), PE/Cy7 anti-mouse Ly-6G (clone IAB; 1:80 dilution), and PE/Cy7 anti-mouse Ly-6C (clone HK1.4; 1:60 dilution). Lineage markers (Lin) were defined as CD3, CD335, and B220. All samples were run on a BD LSRII flow cytometer and analyzed using the FlowJo software (FlowJo).
log-rank test was used to assess significant differences in the Kaplan–Meier analysis of overall survival between untreated and treated mouse groups. GraphPad Prism v4.0 (GraphPad Software) was used for statistical calculations, and P values <0.05 were considered statistically significant.

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

**Data availability**

All data generated or analyzed during this study are included in this published article (and its supplementary information files). The source data underlying all Main and Supplementary Figures are provided as a Source Data file.

Received: 11 April 2018 Accepted: 23 April 2019 Published online: 06 June 2019

**References**

1. Imai, K. & Takaoka, A. Comparing antibody and small-molecule therapies for cancer. *Nat. Rev. Cancer* 6, 714–727 (2006).

2. Strebhardt, K. & Ullrich, A. Paul Ehrlich Cancer Biol. Ther.

3. Zeng, Q. et al. Phosphatase associated with metastasis of colorectal cancer. *Cancer Metastas. Rev.* 27, 231–252 (2008).

4. Saha, S. et al. A phosphatase associated with metastasis of colorectal cancer. *Science* 294, 1343–1346 (2001).

5. Bessette, D. C., Qiu, D. & Pallan, C. J. PRTL PTPs: mediators and markers of cancer progression. *Cancer Metastas. Rev.* 27, 341–355 (2014).

6. Guo, K., Li, J., Tang, J., Tan, C. & Hong, C. Targeting intracellular oncoproteins with antibody therapy or vaccination. *Sci. Transl. Med.* 3, 99ra85 (2011).

7. Guo, K. et al. The first chimeric antibody in targeting intracellular PRTL-3 oncoprotein for cancer therapy in mice. *Oncotarget* 3, 158–171 (2012).

8. Liu, M. et al. Oncogenic roles of PRTL-3 in FLT3-ITD induced acute myeloid leukemia. *EMBO Mol. Med.* 5, 1351–1366 (2013).

9. Thura, M. et al. PRTL-3-zumab, a first-in-class humanized antibody for cancer therapy. *JCI Insight* 2, e87607 (2016).

10. Ferrone, S. Hidden immunotherapy targets challenge dogma. *Sci. Transl. Med.* 3, 99ra38 (2011).

11. Werner, L. M., Murray, J. C. & Shuptrine, C. W. Antibody-based immunotherapy of cancer. *Cancer Cell* 148, 1081–1084 (2012).

12. Wichmann, C., van Dijk, D., O’Brian, C. A., Bucana, C. D. & Fidler, I. J. Orthotopic and ectopic organ environments differentially influence the sensitivity of murine colon carcinoma cells to doxorubicin and 5-fluorouracil. *Int. J. Cancer* 52, 98–104 (1992).

13. Wang, Y., Wang, X., Ferrone, C. R., Schwab, J. H. & Ferrone, S. Intracellular antigens as targets for antibody based immunotherapy of malignant diseases. *Mol. Oncol.* 9, 1982–1993 (2015).

14. Hong, C. W. & Zeng, Q. Awaiting a new era of cancer immunotherapy. *Cancer Res.* 72, 3715–3719 (2012).

15. Aule, J. F. & Bucana, C. D. The blockade of Fc receptor- mediated signaling regulates the expression of the B7 antagonist B7-H1 in tumor cells as compared with strainL cells. *Cancer Res.* 55, 1386–1393 (1995).

16. Logozzi, M., De, M., Lugini, L., Borghi, M. & Calabrò, L. High levels of exosomes expressing CD63 and caveolin-1 in plasma of melanoma patients. *PLoS ONE* 4, e5219 (2009).

17. Tanaka, T. & Rabbitts, T. H. Interfering with RAS-effector protein interactions prevent RAS-dependent tumour initiation and causes stop-start control of cancer growth. *Oncogene* 29, 6064–6070 (2010).

18. Shin, S. M. et al. Antibody targeting intracellular oncoprotein Ras mutants exerts anti-tumour effects after systemic administration. *Nat. Commun.* 8, 15090 (2017).

19. Zhang, J. F. et al. A cell-penetrating whole molecule antibody targeting intracellular HBs suppresses hepatitis B virus via TRIM21-dependent pathway. *Theranostics* 8, 549–562 (2018).

20. Li, J. et al. Generation of PRL-3- and PRL-1-specific monoclonal antibodies as potential diagnostic markers for cancer metastases. *Clin. Cancer Res.* 11, 2195–2204 (2005).

**Acknowledgements**

This work was supported by research grants from the Agency for Science, Technology and Research (A*STAR), Singapore. We are grateful to Dr. Eng Chon Boon and the National University Hospital Tissue Repository, Singapore, for providing clinical samples, as well as to the Advanced Molecular Pathology Laboratory (IMCR, A*STAR) for pathological analysis and H&E staining of the patient kidney samples. We are appreciative to Prof. John Connolly and Dr. Srimat Narayanayam for their support in flow cytometry analysis, and to Dr. Manikanuk Lakshmanan and Mr. Anandhukumar Raju.
for assistance with clinical samples. We also thank Professor Sir David Lane (Chief Scientist, A*STAR) for assisting with PRL3-minibody generation with ImaginAb, Inc., USA.

Author contributions
M.T., A.Q.A., and Q.Z. designed the experiments and prepared the manuscript. M.T., A.Q.A., A.G., J.S.P.Y., J.L., J.X.E.S., and N.Y.Z.T. performed the experiments. C.E.C., S.C.L., K.M.H., Y.K.G., W.P.Y., J.S., W.J.C., C.H., J.B.Z., L.Z.W., J.S.P.Y., S.M.Y., H.S.S.H., E.C., S.P.C., J.N., M.C.H.N., C.C., E.S.A.Y., I.B.H.T., J.F.T., and B.C.G. provided materials and analyzed the results. M.T., A.Q.A., J.S.P.Y., and Q.Z. proofread and finalized the manuscript. All authors approved the manuscript.

Additional information
Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-019-10127-x.

Competing interests: Q.Z. is the founder of Intra-Immu SG Pte Ltd., an Agency of Science, Technology and Research (A*STAR) spin-off company granted licensing rights for the PRL3-zumab IP portfolio. The other authors declare no competing interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

Journal peer review information: Nature Communications thanks Vassiliki Boussiotis and other anonymous reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2019