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Large Molecule Therapeutics

Molecular Cancer Therapeutics

A Novel Neutralizing Antibody Targeting Pregnancy-Associated Plasma Protein-A Inhibits Ovarian Cancer Growth and Ascites Accumulation in Patient Mouse Tumorgrafts

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Abstract

The majority of ovarian cancer patients acquire resistance to standard platinum chemotherapy and novel therapies to reduce tumor burden and ascites accumulation are needed. Pregnancy-associated plasma protein-A (PAPP-A) plays a key role in promoting insulin-like growth factor (IGF) pathway activity, which directly correlates to ovarian cancer cell transformation, growth, and invasiveness. Herein, we evaluate PAPP-A expression in tumors and ascites of women with ovarian cancer, and determine the antitumor efficacy of a neutralizing monoclonal PAPP-A antibody (mAb-PA) in ovarian cancer using primary patient ovarian tumorgrafts ("Ovatars"). PAPP-A mRNA expression in patient ovarian tumors correlated with poor outcome and was validated as a prognostic surrogate in Ovatar tumors. Following confirmation of mAb-PA bioavailability and target efficacy *in vivo*, the antitumor efficacy of mAb-PA in multiple Ovatar tumor models was examined and the response was found to depend on PAPP-A expression. Strikingly, the addition of mAb-PA to standard platinum chemotherapy effectively sensitized platinum-resistant Ovatar tumors. PAPP-A protein in ascites was also assessed in a large cohort of patients and very high levels were evident across the entire sample set. Therefore, we evaluated targeted PAPP-A inhibition as a novel approach to managing ovarian ascites, and found that mAb-PA inhibited the development, attenuated the progression, and induced the regression of Ovatar ascites. Together, these data indicate PAPP-A as a potential palliative and adjunct therapeutic target for women with ovarian cancer. *Mol Cancer Ther; 14(4); 973–81. ©2015 AACR.*

Introduction

Epithelial ovarian cancer is the leading cause of death among gynecologic malignancies (1). Although generally curable if detected at an early stage, the symptoms of ovarian cancer are vague and patients often present with advanced disease. The current standard for ovarian cancer includes primary surgical intervention to remove macroscopic disease burden (both solid tumor and any accompanying ascites) proceeded by a platinum-based chemotherapy regimen to abrogate any remaining microscopic deposits. Unfortunately, the majority of patients will relapse with platinum-resistant disease and develop intractable ascites, thereby contributing to the historically poor median overall survival (OS) rate. Thus, there is a critical need to identify novel targets and ways to predict response of the individual patient to new therapeutic agents to improve outcomes for women with this disease.

The role of insulin-like growth factors (IGF) in the development and progression of a broad range of epithelial cancers, including ovarian cancer, is well documented (2–4), and investigational compounds targeting the IGF axis have been developed for cancer therapeutics. In particular, monoclonal antibodies directed against the IGF-I receptor (IGF-IR), which transduces IGF-I and IGF-II signaling in cells, have been evaluated. Although conceptually sound, the clinical experience to date using these antibodies in unselected patients with various cancers has been largely disappointing. This can be explained by the broad-based activity of said antibodies (IGF-IR is ubiquitous and serves essential functions in normal tissues), lack of effect on IGF-II mitogenic signaling through insulin receptor isoform A (InsR-A), and secondary hormonal and metabolic derangements (3–7). Nevertheless, there remains the possible benefit to patient subgroups and, therefore, a need of predictive biomarkers to identify those patients who would be most likely to respond to specific IGF-targeted therapies. More importantly, alternative means of inhibiting IGF signaling to avoid the above side effects is desirable. We propose targeting pregnancy-associated plasma protein-A (PAPP-A).

PAPP-A is a novel metalloprotease of the metzincin superfamily (8). PAPP-A enhances IGF action through specific cleavage of inhibitory IGF binding proteins, primarily IGFBP-4, resulting in increased IGF bioavailability (reviewed in ref. 9). The secreted protease is tethered to the surface of the secreting and neighboring cells, thereby acting in an autocrine/paracrine

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manner to increase local IGF available for IGF-IR activation. It also has the potential to increase local IGF-II available for activation of InsR-A, an insulin receptor that is prevalent in tumor tissue and mediates a mitogenic signal (10). Conversely, therapeutic inhibition of PAPP-A proteolytic activity would effectively suppress both IGF-IR and InsR-A signaling, whereas insulin signaling through the classic InsR-B would not be affected by this approach. Thus, there are several advantages of targeting PAPP-A: It is a novel enzyme lending itself to specificity; it is present extracellularly, and therefore accessible; its expression is both condition- and cell-specific, and therefore selective; and its inhibition would suppress both IGF-I and IGF-II (but not insulin) signaling, thereby enhancing efficacy but limiting metabolic toxicity.

There are compelling data supporting PAPP-A inhibition of IGF signaling in ovarian cancer as a viable therapeutic approach. Several ovarian cancer cell lines and primary cultures express IGF-IR and have been shown to be responsive to IGFs (11, 12). In addition, IGF-II demonstrates mitogenic signaling through the InsR-A in human ovarian cancer cells (13). Wang and colleagues (14) found that an IGF-IR antibody inhibited ovarian cancer tumor growth in a xenograft model, and Gest and colleagues (15) suggest a role for IGF signaling in ovarian cancer aggressiveness. Moreover, we have recently shown that a relatively nontumorigenic ovarian cancer cell line becomes highly tumorigenic upon overexpression of PAPP-A (16). Conversely, decreased PAPP-A expression was associated with lowered invasive potential and tumor growth rate of an ovarian cancer cell line *in vivo* (17). Unfortunately, screening for PAPP-A expression in primary ovarian cancer has been limited (18, 19).

A substantial barrier to the study of ovarian cancer is the paucity of translationally and clinically relevant models. The development of primary patient ovarian tumorgrafts ("Ovatars"), with availability of source patient biospecimens (germline DNA, serum, frozen and formalin-fixed paraffin-embedded tissue) and prospective clinical annotations, helps to overcome these hurdles. We have shown that intraperitoneally derived Ovatars recapitulate patient tumor in terms of histologic, genomic, transcriptomic, and therapeutic heterogeneity (20). Thus, Ovatars represent a practical medium to study the effects of novel targets in ovarian cancer. Rather than selecting for clonal population of patient-derived cells able to grow *in vitro*, the generation of individualized orthotopic models allows for development and interaction of the tumor cells with the stroma in an environment similar to the source patient (20–22). As a result, experiments in Ovatars are more likely to produce clinically-relevant outcome parameters. To this end, we examined the potential role of PAPP-A as a prognostic surrogate of clinical outcome and predictive index of anti–PAPP-A–targeted therapy in patient ovarian cancer tumors and their respective Ovatars. Herein, we describe the efficacy of a novel PAPP-A–neutralizing antibody to limit tumor growth, prevent ascites accumulation and reverse platinum resistance in Ovatars.

Materials and Methods

Neutralizing PAPP-A monoclonal antibody

We have developed a high-affinity IgG monoclonal antibody against a substrate-binding exosite of PAPP-A required for proteolysis of IGFBP-4 (23). The development and characterization of this antibody and its effectiveness in inhibiting IGFBP-4 proteolysis and xenograft tumor growth has been published recently (24).

Ovatar model

The generation and expansion of viable ovarian tumor tissue obtained from consenting patients at the time of surgery has been described previously (20). Briefly, fresh patient tumor tissue was injected i.p. into severe combined immunodeficient (SCID) mice (Harlan). Upon engraftment, solid tumor (surgically resected and minced) or ascites was reimplanted into 20 to 80 mice, depending on the experiment, to generate biologic Ovatar replicates for *in vivo* experiments. The use of all human subject material was approved by the Institutional Review Board of Mayo Clinic. All animal studies were approved by the Institutional Animal Care and Use Committee of Mayo Clinic.

Treatments were initiated upon confirmation of tumors measuring ≥ 0.2 cm^2 cross-sectional area or the presence of ascites as measured by transabdominal ultrasound (SonoSite S-series, SonoSite Inc.). Unless otherwise indicated, mice were treated weekly with monoclonal PAPP-A antibody (mAb-PA; 30 mg/kg) or IgG2a isotype control (Bio X Cell) via intraperitoneal delivery. For the platinum studies, Ovatars were randomized to receive i.p. saline or carboplatin plus paclitaxel (CP; NOVAPLUS) at 50 and 15 mg/kg, respectively, as described previously (20). Disease burden was assessed in tumor-bearing animals up to three times per week. After 4 weeks (or if clinical endpoints of tumor size, ascites burden, or morbidity were reached), mice were euthanized and blood and tumor tissue harvested. Final tumor weights were recorded and tumor sections snap frozen in liquid nitrogen. Where appropriate, ascites was collected, centrifuged, and acelluar and cellular components independently stored at -80°C. Personnel involved with acquisition of ultrasound measurements and subsequent tumor and/or ascites analyses were blinded to the treatments.

Microarray

For analysis of public microarray datasets, normalized gene-expression data were obtained from The Cancer Genome Atlas (TCGA) Research Network and Gene Expression Omnibus (GEO) database for the following independent studies: GSE13876, GSE14764, GSE49997, and GSE9891. Patient tumors within each cohort were ranked according to *PAPPA* expression and split evenly into two cohorts, defining the top 50% as "*PAPPA* high" and the bottom 50% as "*PAPPA* low." Ovatar tumors ($n = 118$) were analyzed by Affymetrix HG U133 plus 2.0 arrays at the Mayo Medical Genome Facility according to the manufacturer's protocol. Gene-expression arrays were preprocessed and normalized by frozen multichip analysis (25). Patients were ranked according to their matched Ovatar *PAPPA* expression, split evenly into two groups ("*PAPP-A* high" vs. "*PAPP-A* low") and assessed for outcome.

Immunohistochemistry

Formalin-fixed paraffin-embedded Ovatar tumor tissue samples were processed and immunostained for PAPP-A using a recombinant anti-human PAPP-A monoclonal antibody as previously described (26)

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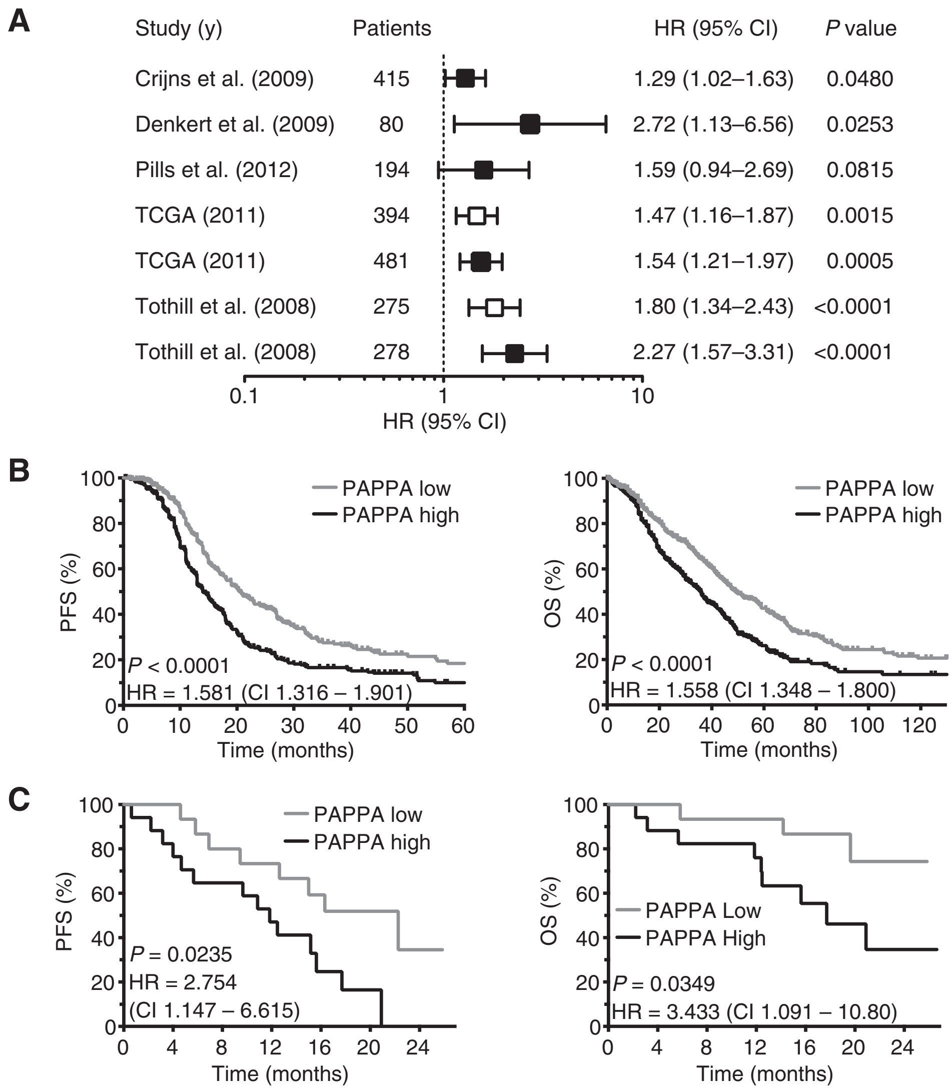

Figure 1.
PAPPA expression correlates with poor outcome in patients with ovarian cancer and represents a prognostic surrogate in Ovatar tumors. A, the Forest plot of PFS (open) and OS (filled) by *PAPPA* expression in multiple ovarian carcinoma tumor cohorts, showing HRs (PAPPA low/high) and 95% CIs where an HR >1 implies a higher risk of recurrence and mortality in the PAPPA high group. Kaplan–Meier analyses of the combined cohorts depicting PFS and OS in the PAPPA low (gray line) versus PAPPA high (black line) for patient (B) and Ovatar (C) tumor expression.

Human PAPP-A ELISA

Snap-frozen Ovatar tumor tissues were pulverized in liquid nitrogen using the Cellcrusher. PAPP-A protein levels in pulverized tumor tissue [lysed in M-PER extraction buffer (Thermo Scientific)] or acellular ascites were quantified using a highly sensitive PAPP-A ELISA (picoPAPP-A) generously provided by Ansh Laboratories. Of special note, this assay does not recognize mouse PAPP-A.

IGFBP-4 proteolysis

Aliquots of tumor lysates or cell-free ascites were incubated overnight at 37°C with IGFBP-4 and IGF-II (IGFBP-4 must bind IGF to be susceptible to cleavage by PAPP-A; ref. 27). IGFBP-4 proteolysis was assessed by Western blot analysis using primary antibodies toward the C- and N-termini of IGFBP-4 (Abcam) and fluorescently labeled secondary antibodies (LI-COR). Images were captured using the LI-COR Odyssey scanner and intensities quantitated using ImageJ software (28). IGFBP-4 proteolysis was also assessed using ELISA kits for total and intact IGFBP-4 (kindly provided by Ansh Laboratories). Total IGFBP-4 minus intact IGFBP-4 provides a quantitatively accurate measure of proteolyzed IGFBP-4.

IgG2 immunofluorescence

Cryosections (5 μm) of Ovatar tumor were fixed in methanol and dried to glass slides. Sections were rehydrated in PBS, blocked in protein-free buffer (Dako), and penetrated mAb-PA detected using a FITC-labeled anti-mouse IgG2.

IGF-I and IGFBP assessment

Total mouse IGF-I and total and active human IGF-I were measured using ELISA kits from Ansh Laboratories. The serum IGFBP profile was assessed by Western ligand blot analysis using radiolabeled IGF-I, as described previously (29).

Statistical analyses

Statistical significance between two groups was tested using ANOVA with the Bonferroni *post hoc* test for multiple comparison analysis using GraphPad Prism 6.0. Univariate analyses for

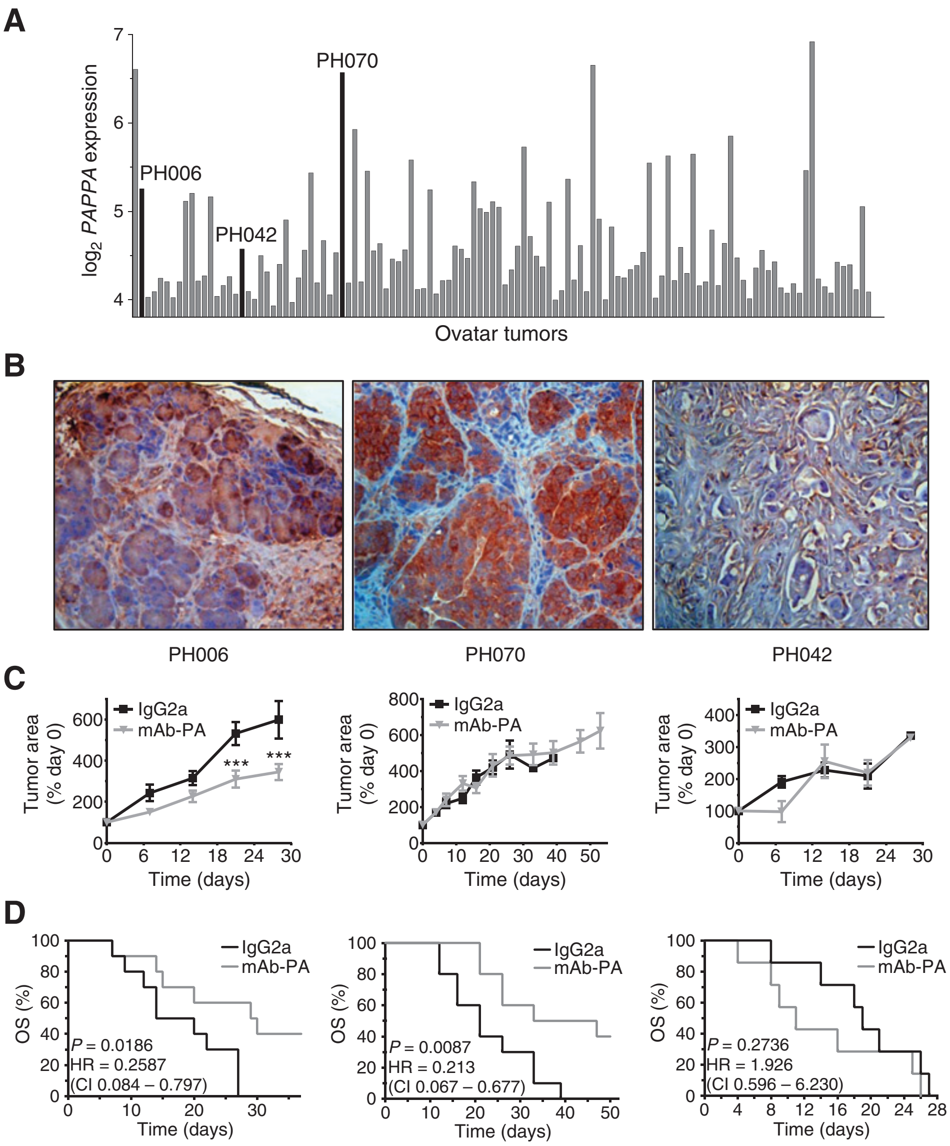

Figure 2.
Antitumor efficacy of mAb-PA in Ovatars is dependent on PAPP-A levels. A, *PAPPA* expression in a cohort of Ovatar tumors ($n = 118$). Three Ovatar tumors (PH006, PH070, and PH042) were selected for *in vivo* testing (denoted by black bars). B, PAPP-A immunostaining of tumors from Ovatar PH006, PH070, and PH042. Tumor growth (normalized to pretreatment day 0; C) and corresponding Kaplan–Meier analysis (D) depicting OS in the mAb-PA–treated (gray line) versus IgG2a-treated (black line) PH006 ($n = 7$/group), PH070 ($n = 10$/group), and PH042 ($n = 10$/group) Ovatar models; error bars, SEM; asterisks denote statistical significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

progression-free survival (PFS) and OS were performed using the Kaplan–Meier method and corresponding log-rank test for intergroup differences. All analyses were conducted using JMP 9.0 (SAS Institute).

Results

PAPP-A expression correlates with poor outcome in ovarian cancer tumors

To date, the prognostic value of PAPP-A expression in ovarian cancer tumors has not been reported. As an initial screen, five publicly available datasets were interrogated (TCGA, GSE13876, GSE14764, GSE49997, and GSE9891) for *PAPPA* gene expression split evenly into two cohorts, defining the top 50% as "*PAPPA* high" and the bottom 50% as "*PAPPA* low," and correlated to patient outcome. Univariate HRs with corresponding 95% confidence intervals (CI) for PFS and OS were calculated and depicted in a forest plot (Fig. 1A). These data support high *PAPPA* as a direct correlate of poor outcome in terms of PFS and OS in ovarian cancer tumors. Moreover, univariate analysis of the combined cohorts yielded highly significant differences in PFS (HR, 1.581; 95% CI, 1.316–1.901; $P < 0.001$) and OS (HR, 1.558; 95% CI, 1.348–1.800; $P < 0.0001$; Fig. 1B).

We recently demonstrated that Ovatar response to standard carboplatin/paxlitaxel chemotherapy recapitulates donor patient response (20). To further validate Ovatars as molecular

Table 1. PAPP-A expression in Ovatar tumors and ascites

Avatar	mRNA[a]	Tumor[b]	Ascites[c]
PH006	Medium	0.7	—[d]
PH070	High	8.5	0.3
PH042	Low	nd	nd

NOTE: PAPP-A protein levels in Ovatar tumors and ascites as measured by ultrasensitive PAPP-A ELISA.
Abbreviation: nd, not detectable.
[a]Relative mRNA expression (Fig. 2A).
[b]ng/50 μg tumor.
[c]ng/mL ascites.
[d]No ascites available.

surrogates, we sought to determine whether Ovatar *PAPPA* expression correlates to donor patient outcome. Patients were evenly stratified into two groups according to their Ovatar *PAPPA* expression as described above (*PAPPA* High vs. Low) and univariate analysis revealed significant differences in PFS (HR, 2.754; 95% CI, 1.147–6.615; $P = 0.0235$) and OS (3.433; 95% CI, 1.091–10.80; $P = 0.0349$; Fig. 1C).

Targeted PAPP-A inhibition confers antitumor efficacy in Ovatars

The following three Ovatars were selected from a cohort of 118 models, based on relative *PAPPA* expression, to test the effects of an inhibitory monoclonal antibody specific to PAPP-A (mAb-PA): PH006 (moderate expression, PH042 (low expression), and PH070 (high expression). Elevated *PAPP-A* expression was observed in 15% to 20% of ovarian tumors and was reflected in the Ovatars (Fig. 2A). In addition, PAPP-A protein levels were determined by ELISA (Table 1) and immunohistochemistry (Fig. 2B) and found to correlate with *PAPPA* gene expression.

For the first experiment, Ovatar PH006 mice were treated with mAb-PA at varying doses (10 vs. 30 mg/kg, once vs. twice weekly) for a total of 4 weeks. At necropsy, all mice presented with multilobular tumors adherent to the pelvic floor. Final tumor weight was significantly reduced in mice receiving 30 mg/kg mAb-PA compared with IgG2a isotype control; there was no significant difference between the once versus twice weekly 30 mg/kg dose. In subsequent studies, significant inhibition of tumor growth (Fig. 2C) and increased OS ($P = 0.0119$; HR, 0.26; Fig. 2D) was demonstrated in Ovatar PH006 mice that received mAb-PA once weekly at 30 mg/kg compared with mice given IgG2a.

Treatment of Ovatar PH070 mice with mAb-PA once weekly at 30 mg/kg mAb-PA did not inhibit tumor growth (Fig. 2C), but significantly increased OS ($P = 0.009$; HR, 0.21; Fig. 2D). Investigation into the patient history of PH070 revealed that this Ovatar model recapitulated the clinical disease in terms of bowel adhesions and ascites development. PH070 Ovatars treated weekly with 30 mg/kg mAb-PA demonstrated decreased adhesions and ascites development (data not shown). Assessment of Ovatar tumors indicated penetrance of the mAb-PA (Fig. 3A) and effective inhibition of PAPP-A proteolytic activity toward IGFBP-4 (Fig. 3B).

As a confirmatory measure of PAPP-A as a biomarker of therapeutic efficacy, we also evaluated the response of Ovatar PH042, which had relatively low PAPP-A mRNA expression and little or no PAPP-A protein (Table 1, Fig. 2B). mAb-PA did not significantly affect tumor progression (Fig. 2C) or outcome (Fig. 2D) in this Ovatar.

No apparent secondary effects of mAb-PA were observed

In all Ovatar models analyzed there were no significant differences in circulating levels of IGF-I (128 ± 7 ng/mL for mAb-PA vs.120 ± 10 ng/mL for IgG2a) or IGFBPs, the latter assessed by Western ligand blotting (data not shown).

mAb-PA treatment reduces ascites

To investigate the effect of mAb-PA on ascites accumulation and progression, it was determined that ultrasound was an accurate and precise measure of actual tumor weight and ascites volume (Supplementary Fig. S1). PH070 Ovatars were analyzed following tumor cell implantation. Ascites-free survival (AFS)

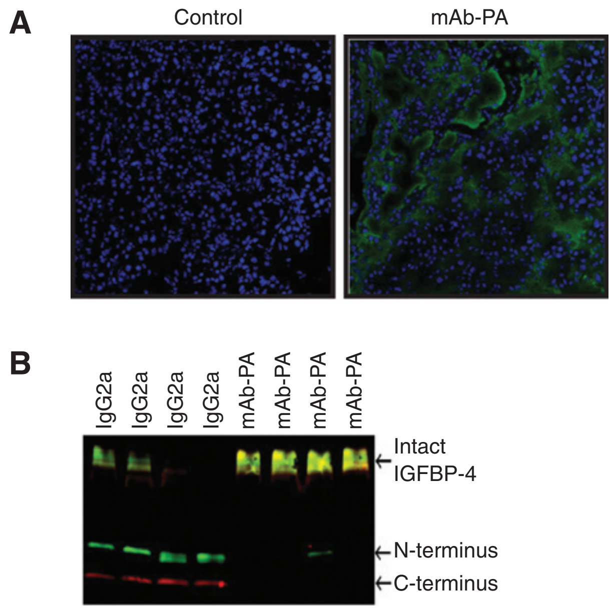

Figure 3.
Bioavailability and target efficacy of mAb-PA in Ovatar tumors. A, immunofluorescent staining for the nucleus (blue) and IgG2a (green) in mAb-PA–treated (right) versus untreated (left) PH070 Ovatar tumors, indicating penetrance of the antibody into the tumor. B, Western blot analysis of intact (top, yellow) and proteolyzed (bottom, green/red) IGFBP-4 in IgG2a and mAb-PA–treated Ovatar tumors, indicating effective inhibition of PAPP-A activity in the tumor by mAb-PA.

was significantly increased ($P < 0.0001$; HR, 0.2351) and time for tumor-to-ascites appearance was significantly delayed ($P < 0.0001$) with mAb-PA treatment (Fig. 4A). To assess the effect of mAb-PA on ascites progression, a fraction of ascites containing active tumor cells from Ovatar PH070 was injected i.p. into mice, and mAb-PA or IgG2a treatments initiated 4 days later. OS was significantly increased ($P = 0.0458$; HR, 0.310) and ascites volume significantly decreased ($P = 0.0317$) in response to mAb-PA treatment (Fig. 4B). Moreover, mAb-PA treatment was found to induce ascites regression ($P = 0.0157$; Fig. 4C).

PAPP-A levels in ovarian cancer patient ascites

The ascites collected from PH070 Ovatars had measureable levels of human PAPP-A, indicating production by the patient tumor. Although ascites from patient PH070 was not available, PAPP-A levels were evaluated in a large cohort of additional patient ascites. All patient ascites ($N = 33$) had high levels of PAPP-A, with mean $\pm$ SEM of 45 ± 3 ng/mL (normal serum levels < 1 ng/mL). Greater than 90% of the endogenous IGFBP-4 in the ascites was proteolyzed, and levels of bioactive IGF-I were 2% to 50% that of total IGF-I (mean $\pm$ SEM, 18 ± 3%). Proteolytic activity in patient ascites could be inhibited with mAb-PA (Fig. 5), demonstrating that PAPP-A is responsible for IGFBP-4 cleavage in this fluid.

Combination mAb-PA and adjunct carboplatin/paclitaxel therapy

Patient PH070 had chemoresistant ovarian cancer (20). To determine whether targeted inhibition of PAPP-A could affect platinum resistance, standard platinum chemotherapy (CP)

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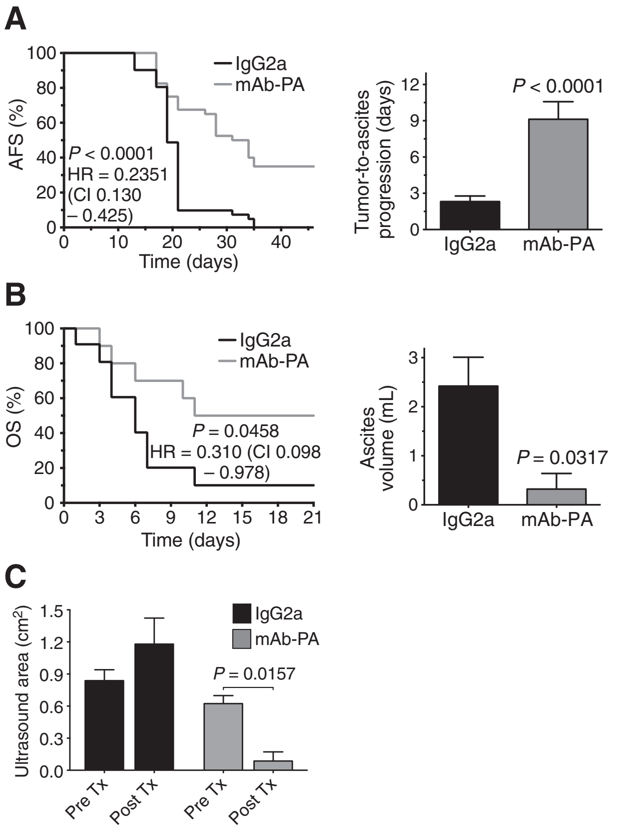

Figure 4.
Targeting PAPP-A in Ovatar models with ascites development. A, PAPP-A blockade inhibits ovarian ascites development. Left, Kaplan–Meier analysis depicting AFS in mAb-PA–treated (gray line) versus IgG2a-treated (black line) PH070 Ovatars ($n = 40$/group). Right, time (days) from solid tumor detection to the development of ascites in mAb-PA versus IgG2a-treated mice. B, PAPP-A blockade attenuates ovarian ascites progression. Left, Kaplan–Meier analysis depicting OS in PH070 Ovatars ($n = 11$/group) wherein treatment (mAb-PA vs. IgG2a) was initiated at the time of confirmed (ultrasound) subclinical ascites burden (day 0). Right, final ascites tumor burden depicted as volume in mL at the time of necropsy. C, PAPP-A blockade induces ovarian ascites regression. Ascites burden as measured by ultrasound (area in cm^2) before treatment (Pre Tx) and after treatment (Post Tx) with mAb-PA versus IgG2a ($n = 5$/group).

combined with mAb-PA therapy was tested (Fig. 6A). The combination CP plus mAb-PA versus CP plus IgG2a was initiated upon ultrasound confirmation of engrafted PH070 Ovatar tumors. Chemotherapy was ceased after 4 weeks and Ovatars continued to receive weekly treatments of mAb-PA or IgG2a for up to 100 days after initial treatment. As expected, CP treatment alone did not regress tumors below baseline during chemotherapy and the addition of IgG2a did not alter tumor growth trajectory. However, mAb-PA treatment enhanced sensitivity to platinum-based chemotherapy, as further supported by the highly significant reduction in final tumor weight (Fig. 6B).

Discussion

In this study, we demonstrate the efficacy of a novel neutralizing antibody against PAPP-A (mAb-PA) in primary patient ovarian tumorgrafts (Ovatars) to significantly: (i) inhibit intraperitoneal ovarian cancer tumor growth, (ii) delay ascites development, (iii) inhibit ascites accumulation, and (iv) induce ascites regression. Ovatars were selected for predictive response to treatment based on elevated PAPP-A expression in matched primary patient tumors. In contrast with the adverse effects reported in response to IGF-IR–directed antibodies (3–7), secondary endocrine disruption resulting from mAb-PA monotherapy was not evident. Furthermore, the addition of mAb-PA to standard frontline carboplatin/paclitaxel chemotherapy markedly improved tumor regression to effectively sensitize chemoresistant Ovatars.

Importance of the Ovatar model

Orthotopic implantation of patient ovarian cancer tissue in SCID mice results in the formation a primary tumor that is commonly localized to the mouse pelvis and/or ovaries. As these primary tumors develop and progress, cancer cell growth, proliferation, and metastatic dissemination within the peritoneal cavity is comparable with that of the patient. We have previously demonstrated that Ovatar tumors maintain the histopathologic and molecular diversity (genomic, transcriptomic, and proteomic) of the donor patient tumor (20). Moreover, Ovatars recapitulate the patient experience in terms of metastasis and ascites-related complications. Indeed, the Ovatar models (PH006, PH042, and PH070) used herein, exhibited markedly different characteristics across models while conserving the patient disease phenotype. For example, Patient PH006 presented with a solid stage III tumor with no ascites or signs of metastases at the time of primary debulking surgery whereas patient PH070 presented with adhesions, bowel involvement, and ascites. Thus, these orthotopic Ovatars more accurately resemble the patient experience in terms of clinical complications as compared with subcutaneous xenografts, and present a more relevant and directly translatable medium toward analyzing ovarian cancer disease progression and metastasis. More importantly, therapeutic response in Ovatars (standard and targeted therapeutics) should more likely reflect how the patient would respond, and, therefore, is a step closer toward translation into clinical benefit.

Necessity of identifying anti–PAPP-A therapy biomarkers

Arguably the greatest impedance to the advancement of IGF-IR–directed antibodies is a lack of predictive biomarkers, as the clinical value of IGF-IR remains controversial (2, 30). In this study, we used patient tumor *PAPP-A* gene expression to select candidate

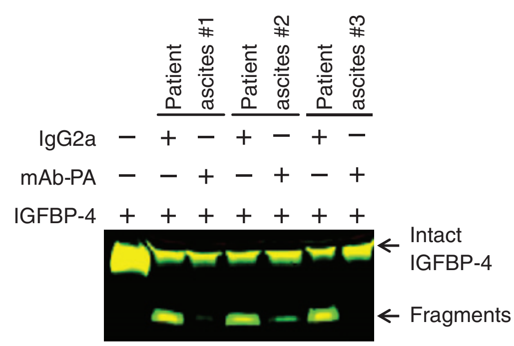

Figure 5.
mAb-PA inhibits IGFBP-4 proteolysis in patient ascites. Aliquots of ascites from three different patients were incubated with IGFBP-4 and IgG2a or mAb-PA. Western blot analysis indicates effective inhibition of IGFBP-4 proteolysis by mAb-PA.

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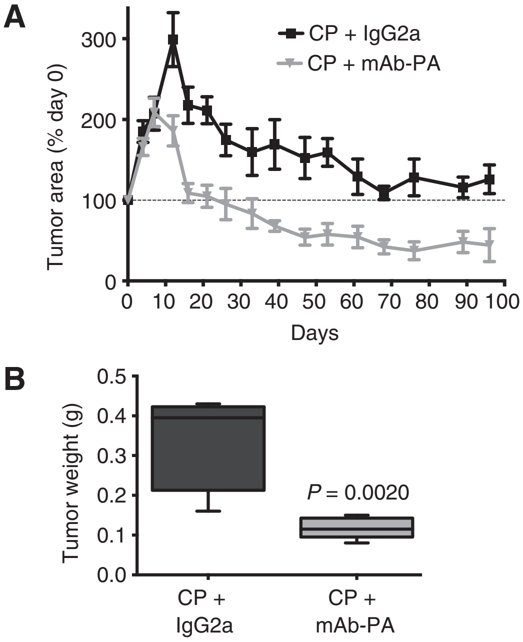

Figure 6.
Adjuvant mAb-PA therapy sensitizes platinum-resistant Ovatars. A, tumor growth in response to platinum chemotherapy combined with mAb-PA [CP + mAb-PA, gray line] versus IgG2a [CP + IgG2a, black line] ($n = 10$/group). B, final tumor burden (solid tumor) at the time of necropsy.

Ovatar models for testing mAb-PA therapy. DNA microarray data identified a subset of patient tumors (15%–20%) reporting high *PAPP-A* expression. Elevated PAPP-A protein expression in both solid tumors and in ascites was confirmed using a highly sensitive PAPP-A ELISA. Strikingly, 100% of patients' ascites tested reported high PAPP-A, and, with the exception of a single sample, levels were increased $\geq$100-fold compared with the average serum level of nonpregnant women. Bioactive IGF-I accounts for less than 1% of total IGF-I and high levels of free IGF-I are associated with disease progression (12). Virtually, all of the endogenous IGFBP-4 in patient ascites samples were found to be proteolyzed and, as a result, bioactive IGF-I levels were 2% to 50% of total IGF-I. Furthermore, we verified that PAPP-A present in the ascites remains proteolytically active. This was important as the expression of a naturally occurring, irreversible PAPP-A inhibitor (proMBP) has been shown to be increased in ovarian tumors and transformed ovarian epithelial cells (19).

Advantages of targeting PAPP-A via mAb-PA

The mAb-PA presented herein is uniquely specific to PAPP-A as it targets a substrate-binding exosite required for IGFBP-4 proteolysis (23). Importantly, the epitope of mAb-PA is not present in other enzymes, including homologous PAPP-A2 (primarily an IGFBP-5 protease; ref. 31). The specificity of this anti–PAPP-A therapy would, therefore, serve to improve the safety and tolerability via reducing off-target and potentially harmful side effects, and target inhibition to the site(s) of local (supraphyiologic) PAPP-A expression, in this case the tumor. This is in contrast with strategies targeting IGF-IR, IGF ligands, and IGFBPs that are ubiquitous, and therefore nonspecific to site or condition. Another advantage of mAb-PA is its potential to reduce both IGF-I and IGF-II binding to IGF-IR, IGF-IR:InsR hybrids, and InsR-A, while sparing any effect on insulin-stimulated InsR-B signaling. Therefore, there should be preferential reduction in proliferative/metastatic effects versus metabolic dysregulation.

Compensatory increases in circulating growth hormone (GH) and IGF-I have been observed with IGF-IR antibodies (4). There did not appear to be any secondary consequences of PAPP-A inhibition in the Ovatars resulting from mAb-PA. There were no apparent effects on either serum levels of IGF-I or profiling of IGFBPs, thus indirectly supporting that there were no effects on GH, which regulates serum IGF-I and IGFBP-3 (32). GH can also induce insulin resistance (33). We did not perform specific insulin sensitivity tests in Ovatars treated with mAb-PA, but previous studies in PAPP-A knockout mice did not indicate insulin resistance (34).

Ascites attenuation in response to mAb-PA treatment

Perhaps the most exciting finding in this study was the effect of mAb-PA therapy on inhibiting ascites development and accumulation as well as promoting regression of established ascites. Ascites produces significant morbidity in ovarian cancer, and palliative treatment options to reduce ascites burden are limited (35). Paracentesis via percutaneous drainage is frequently used for short-term symptom relief. Unfortunately, this invasive procedure carries many risks and frequently requires hospitalization. Thus, our findings suggest the potential use of mAb-PA monotherapy for palliative benefit, with minimal risk, toxicity, and discomfort.

In conjunction with previously cited work (16, 17), our Ovatar data implicate PAPP-A attenuation (expression/activity) as a potential strategy to limit and/or suppress ovarian cancer metastasis. Indeed, PAPP-A has recently been identified as a metastasis-related target gene (36, 37). It is of interest that ascites, which promotes tumor growth and metastases, has high levels of PAPP-A. Furthermore, ascites has been found to be rich in proinflammatory cytokines (38), which have been shown to upregulate PAPP-A expression in several cell types (39, 40). Further studies will be necessary to establish a role for PAPP-A in ovarian cancer metastases.

mAb-PA in platinum-resistant ovarian cancer

The application of potentially harmful and cytotoxic-targeted therapeutics in combination with standard chemotherapy in the frontline and/or maintenance setting has been rationalized by the promise of extending historically poor progression-free disease intervals and improving OS. For a subset of primary ovarian cancer tumors with elevated PAPP-A expression, anti–PAPP-A therapy presents as a viable adjuvant option in terms of favorable pharmacokinetic (e.g., easily administered, extensive tumor uptake, long half-life, little to no toxicity) and pharmacodynamic (e.g., low K_d, low IC_{50}) attributes. Increased IGF signaling can potentially limit the efficacy of cytotoxic agents, and IGF-IR inhibition to overcome platinum resistance in ovarian cancer is by no means a novel concept (41, 42). PAPP-A is proposed as a better therapeutic target with greater tumor specificity and lower risk of side effects than other IGF system targets.

Conclusion

Identification of women with ovarian cancer who are most likely to respond is critical to the success of PAPP-A inhibition in clinical trials. In this study, we illustrate the potential power of patient selection through appropriate and easily obtainable biomarkers of PAPP-A in terms of expression and bioactivity, the efficacy of a novel neutralizing monoclonal antibody against PAPP-A, and proof-of-concept in a relevant patient Ovatar model supporting a multitude of therapeutics applications for PAPP-A as a promising new target in ovarian cancer.

Disclosure of Potential Conflicts of Interest

M.A. Becker has ownership interest in Ovarian Cancer Tumorgrafts—Avatar System. P. Haluska has ownership interest in Mayo Ovarian Avatar-IP and is a consultant/advisory board member for Ansh. C. Oxvig has ownership interest in a patent application. C.A. Conover reports receiving a commercial research grant from Ansh Laboratories. No potential conflicts of interest were disclosed by the other author.

Authors' Contributions

Conception and design: M.A. Becker, C. Oxvig, C.A. Conover
Development of methodology: M.A. Becker, P. Haluska Jr, C. Oxvig
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.A. Becker, P. Haluska Jr, L.K. Bale
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.A. Becker, P. Haluska Jr, C. Oxvig
Writing, review, and/or revision of the manuscript: M.A. Becker, P. Haluska Jr, C. Oxvig, C.A. Conover
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.A. Becker, L.K. Bale
Study supervision: M.A. Becker, C.A. Conover

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Molecular Cancer Therapeutics

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