



gC1qR/HABP1/p32 Is a Potential New Therapeutic Target Against Mesothelioma

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Mesothelioma is an aggressive cancer of the serous membranes with poor prognosis despite combination therapy consisting of surgery, radiotherapy, and platinum-based chemotherapy. Targeted therapies, including immunotherapies, have reported limited success, suggesting the need for additional therapeutic targets. This study investigates a potential new therapeutic target, gC1qR/HABP1/p32 (gC1qR), which is overexpressed in all morphologic subtypes of mesothelioma. gC1qR is a complement receptor that is associated with several cellular functions, including cell proliferation and angiogenesis. *In vitro* and *in vivo* experiments were conducted to test the hypothesis that targeting gC1qR with a specific gC1qR monoclonal antibody 60.11 reduces mesothelioma tumor growth, using the biphasic mesothelioma cell line MSTO-211H (MSTO). *In vitro* studies demonstrate cell surface and extracellular gC1qR expression by MSTO cells, and a modest $25.3 \pm 1.8\%$ ($n = 4$) reduction in cell proliferation by the gC1qR blocking 60.11 antibody. This inhibition was specific for targeting the C1q binding domain of gC1qR at aa 76–93, as a separate monoclonal antibody 74.5.2, directed against amino acids 204–218, had no discernable effect. *In vivo* studies, using a murine orthotopic xenotransplant model, demonstrated an even greater reduction in MSTO tumor growth (50% inhibition) in mice treated with the 60.11 antibody compared to controls. Immunohistochemical studies of resected tumors revealed increased cellular apoptosis by caspase 3 and TUNEL staining, in 60.11 treated tumors compared to controls, as well as impaired angiogenesis by decreased CD31 staining. Taken together, these data identify gC1qR as a potential new therapeutic target against mesothelioma with both antiproliferative and antiangiogenic properties.

Keywords: mesothelioma, complement, gC1qR/HABP1/p32, monoclonal antibody therapy, therapeutic target

INTRODUCTION

Mesothelioma is an aggressive cancer of the serous membranes, typically those lining the pleural space (1, 2). It is chiefly caused by exposure to and inhalation of asbestos. Treatment outcomes continue to be poor, despite multimodal therapy consisting of surgery, chemotherapy, and radiation (3–5). An estimated 38,400 individuals die globally each year from mesothelioma (6), and the incidence is expected to rise in the US as a result of asbestos exposure following destruction of the World Trade Center in New York, NY in 2001 (7). Novel therapies, especially targeted therapies, are needed to improve treatment outcomes and reduce off-target side effects (8, 9).

The complement system is emerging as a novel target in cancer therapy. Complement is involved not only in shaping the inflammatory tumor microenvironment, but also in tumor growth and spread (10). In this regard, the complement component C1q is increasingly recognized as a tumor-promoting factor. It has been reported to enhance cancer cell adhesion, migration, proliferation, and angiogenesis (11–13).

We have identified gC1qR (also known as p32/HABP1) as the major cellular binding site for C1q (14). Marked upregulation of gC1qR expression has been observed in cancers of epithelial cell origin including breast, colon, and lung cancers (15, 16). In patients with breast cancer (17, 18), prostate cancer (19), and serous ovarian adenocarcinoma (20), as well as endometrial cell cancer (21), overexpression of gC1qR has been associated with poor prognosis. In addition, gC1qR is being considered as a potential molecular target for delivery of cytotoxic agents (22, 23) in breast cancer.

gC1qR, is a multicompartamental cellular protein (24), with expression in mitochondria, the cytosol, and at the cell surface. In addition, gC1qR is cleaved from cell membrane for release into the extracellular milieu by enzymes such as the membrane-type metalloproteinase MT1MMP (25, 26). gC1qR shedding by cancer cells has been hypothesized to form a biochemical shield to protect malignant cells from complement mediated attack and produce inflammatory mediators to promote and enhance cancer metastasis (27). Interestingly, gC1qR has been described to exert both pro-proliferative and antiproliferative properties in cancer (24). Many cancer cells have been reported to express gC1qR with varying biological effects (11, 13, 28).

We recently described the overexpression of gC1qR in all mesothelioma subtypes, including epitheloid, sarcomatoid, and biphasic phenotypes (29). This finding suggests that gC1qR may represent a novel therapeutic target against mesothelioma. The present study tested this hypothesis in *in vitro* and *in vivo* experiments using a biphasic cultured human mesothelioma cell line, MSTO-211H (MSTO). The data demonstrate that targeting gC1qR with monoclonal antibody 60.11 reduces cell proliferation *in vitro* and tumor growth *in vivo*, associated with increased apoptosis and decreased angiogenesis, and provide proof of concept for further exploration of gC1qR directed therapy in mesothelioma.

METHODS

Materials

The following materials were purchased from the sources indicated: MSTO-211H biphasic mesothelioma cell line (MSTO; ATCC, Manassas, VA); RPMI 1640, 100× penicillin/streptomycin, and 0.05% trypsin-EDTA (GIBCO-Invitrogen, Grand Island, NY); heat-inactivated fetal bovine serum (FBS; Hyclone, Logan, UT); coating buffer (CB) comprised of 35 mM sodium bicarbonate and 15 mM sodium carbonate, aqueous; tris-buffered saline (TBS) comprised of 20 mM tris-HCl, 150 mM sodium chloride, and 0.05% tween, aqueous; ChromPure human IgG Fc fragments (Jackson ImmunoResearch, West Grove, PA); para-nitrophenyl phosphate (pNPP; Pierce, Rockford, IL); and Dulbecco's PBS (Mediatech Inc., Manassas, VA).

Monoclonal Antibodies (mAbs)

mAbs to gC1qR were generated as described (30). The 60.11 therapeutic antibody is directed against the C1q binding domain of gC1qR, amino acids 76–93. mAb 74.5.2 recognizes amino acids 204–218, which constitutes the binding site for high molecular weight kininogen. AlexaFluor 488-conjugated secondary antibodies (Invitrogen, Carlsbad, CA) and non-immune mouse IgG MOPC 21 (Sigma-Aldrich) were purchased.

Cell Culture

MSTO cells were cultured in RPMI supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin, in T175 culture flasks. Cultures were maintained at 37°C, 100% humidity, 5% CO₂, and subcultured when cells reached ~90% confluence.

Recombinant gC1qR

The strategy for the construction of a plasmid containing the full-length (mature form or wild type, WT) and purification of the glutathione-S-transferase (GST)-gC1qR fusion products has been described in detail (14). The GST-gC1qR fusion product is cleaved by thrombin (3.2 µg/ml) and the GST-free gC1qR protein is purified on fast protein liquid chromatography (FPLC, Pharmacia) using a Mono-Q ion exchange column. The single peak containing the gC1qR is pooled, concentrated to 1–2 mg/ml, and stored at –80°C in the presence of 50 nM PPACK (D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone), a specific thrombin inhibitor (Sigma Aldrich).

Detection of Soluble gC1qR

A qualitative direct ELISA was used to evaluate the presence of soluble gC1qR in MSTO culture medium. MSTO were seeded at 50,000 cells per well and grown for 48 h. Culture supernatants were harvested and centrifuged to remove cellular debris, diluted 1:1 with CB, and incubated in a high-binding microtiter well-plate for 1 h at ambient temperature. Wells were blocked with 1% heat-inactivated bovine serum albumin (BSA) in TBS, 10 min. Immobilized gC1qR was detected with biotinylated immunoaffinity purified polyclonal antibody to gC1qR peptide (144–155) conjugated to alkaline phosphatase, and pNPP substrate. Absorbance was measured at 405 nm.

Soluble gC1qR in pleural fluid from patients with advanced malignant pleural mesothelioma was quantified using a commercial, quantitative human gC1qR ELISA kit (Hycult, Netherlands) (26). Deidentified patient samples ($n = 22$) were evaluated according to manufacturer instruction and in compliance with Memorial Sloan Kettering IRB approved protocols (#16-1547).

Immunofluorescence Microscopy

MSTO cells were seeded in 24-well-tissue culture-treated plates (50,000 cells per well) and grown to near-confluence. Cells were fixed (10 min) in 1% paraformaldehyde. Wells were blocked with 1% BSA and 1 μ g/ml Fc fragments (30 min). gC1qR expression was examined by incubation (30 min) with anti-gC1qR mAb 60.11 (5 μ g per well) followed by 30 min incubation with AlexaFluor 488-conjugated goat anti-mouse secondary antibody (5 μ g per well). Staining with non-immune rabbit IgG (NIRG) and AlexaFluor 488-conjugated goat anti-rabbit secondary antibody served as a negative control. DAPI (0.2 μ g per well) was used as a nuclear counterstain. Images were obtained using an Evos FL Imaging System at 10 \times magnification and normalized for background brightness. Additional brightness and contrast adjustments were applied uniformly to each image via Adobe Photoshop CS6.

Flow Cytometry

MSTO cells were detached from culture plates by incubation (30 min, room temperature) with 0.05% trypsin–0.01% EDTA in 0.01 M TBS, centrifuged (800 g, 5 min) and suspended using 10 mM EDTA in PBS, pH 7.4. Cellular Fc receptors were blocked with 5 μ g Fc fragments per 500,000 cells. gC1qR expression was detected with mouse anti-gC1qR mAb 74.5.2, and MOPC 21, as negative control. The gC1qR 74.5.2 antibody was preferred for flow cytometry studies as it produced greater staining of cells than the 60.11 antibody. Primary antibodies were visualized with AlexaFluor 488-conjugated goat pAb to mouse IgG. Unstained cells were used as an additional negative control. Cells were fixed in 1% paraformaldehyde. All reagents were diluted in DPBS. Fluorescence was determined using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA).

MSTO Cell Adhesion Assay

Cells were cultured as described above and imaged at 24-h intervals via compound light microscopy (10 \times magnification) with a PAXcam 3 microscope camera and Pax-it 11 software (Paxcam, Villa Park, IL). MSTO cells were seeded at 50,000 cells/well, in the presence or absence of 5 μ g/ml recombinant gC1qR. To evaluate the effect of immobilized gC1qR on cell adhesion, microtiter wells were incubated overnight (37°C) with 20 μ g/ml recombinant gC1qR in CB and rinsed with PBS before exposure to cells.

MSTO Cell Proliferation Assay

MSTO cells were seeded in 24-well-cell culture plates at 50,000 cells per well and allowed to adhere overnight. Cultures were subsequently treated with 10 μ g/ml anti-gC1qR mAb 60.11 or 74.5.2. Untreated cultures were used as controls. Cell

proliferation was determined at 24 h intervals. At the desired times, cells were removed from culture wells using 0.05% trypsin-EDTA, stained with trypan blue dye, and counted using a hemocytometer. Trypan blue positive cells were excluded from the count.

Orthotopic Pleural Mesothelioma Mouse Model

All procedures were performed under approved Institutional Animal Care and Use Committee protocols. Female severe combined immunodeficiency gamma mice (NSG (NOD, scid, gamma), Jackson Laboratories), 6–8 weeks old, were anesthetized using inhaled isoflurane and oxygen. Direct intrapleural injection of 1×10^6 GFP-Firefly Luciferase expressing MSTO-211H cells in 200 μ l serum-free media was administered to establish orthotopic malignant pleural mesothelioma tumors via a right thoracic incision, as previously described (31–33). For this proof of concept study, mice were divided into two treatment groups: vehicle ($n = 10$), and 60.11 antibody treatment ($n = 10$) (100 mg/kg, administered twice weekly by intraperitoneal injection beginning on day 8 after tumor implantation). Intraperitoneal injection of antibody was chosen over the intravenous route, as it allowed for the administration of greater fluid volume, which was required to reach the desired therapeutic antibody dose. Clinical assessments of animal distress (weight loss, disruption of locomotor coordination, hunching, lack of grooming, lethargy) were made and recorded daily to assess toxicity.

Tumor burden was measured beginning on day 8 after tumor implantation, and at regular intervals until day 23 using quantitative bioluminescence imaging (BLI). Mice were imaged with the Xenogen IVIS 100 Imaging System, 20 min following injection of a single intraperitoneal dose of 150 mg/kg D-Luciferin. Images were acquired for 5–30 s depending on signal strength. BLI data were analyzed using Living Image 2.60 software and BLI signal reported as total flux (Photons/s). Due to high tumor burden observed in the control group, the experiment was terminated at day 23. At time of sacrifice, blood was collected for cell counts and measurement of serum 60.11 antibody levels using a direct ELISA assay with immobilized recombinant gC1qR. Tumors were removed, fixed and processed for histologic (hematoxylin and eosin staining) and immunohistochemical evaluation.

Immunohistochemical Analysis

Tissue processing and immunohistochemical analysis was performed by the Molecular Cytology Core Facility of Memorial Sloan Kettering Cancer Center as previously described (31, 32). In brief, tissues were fixed in 4% formaldehyde and processed by paraffin embedding using a tissue processor (Leica ASP6025). Five micrometer sections were obtained and applied to superfrost plus slides. Immunohistochemical detection of Ki67, Cleaved Caspase 3, TUNEL, and CD31 was performed using a Discovery XT processor (Ventana Medical Systems). Slides were counterstained with hematoxylin and cover-slipped with Permount (Fisher Scientific).

Ki-67

The Discovery XT autostainer was programmed to incubate slides with primary rabbit polyclonal Ki-67 antibody (Abcam, catalog # ab16667) at 1 $\mu\text{g/ml}$ for 4 h, followed by incubation with secondary antibody (biotinylated goat anti-rabbit IgG; Vector labs) at a concentration of 5.75 $\mu\text{g/ml}$ for 30 min. Blocker D, Streptavidin-HRP, and DAB detection kit (Ventana Medical Systems) were used according to manufacturer instructions.

Cleaved Caspase 3

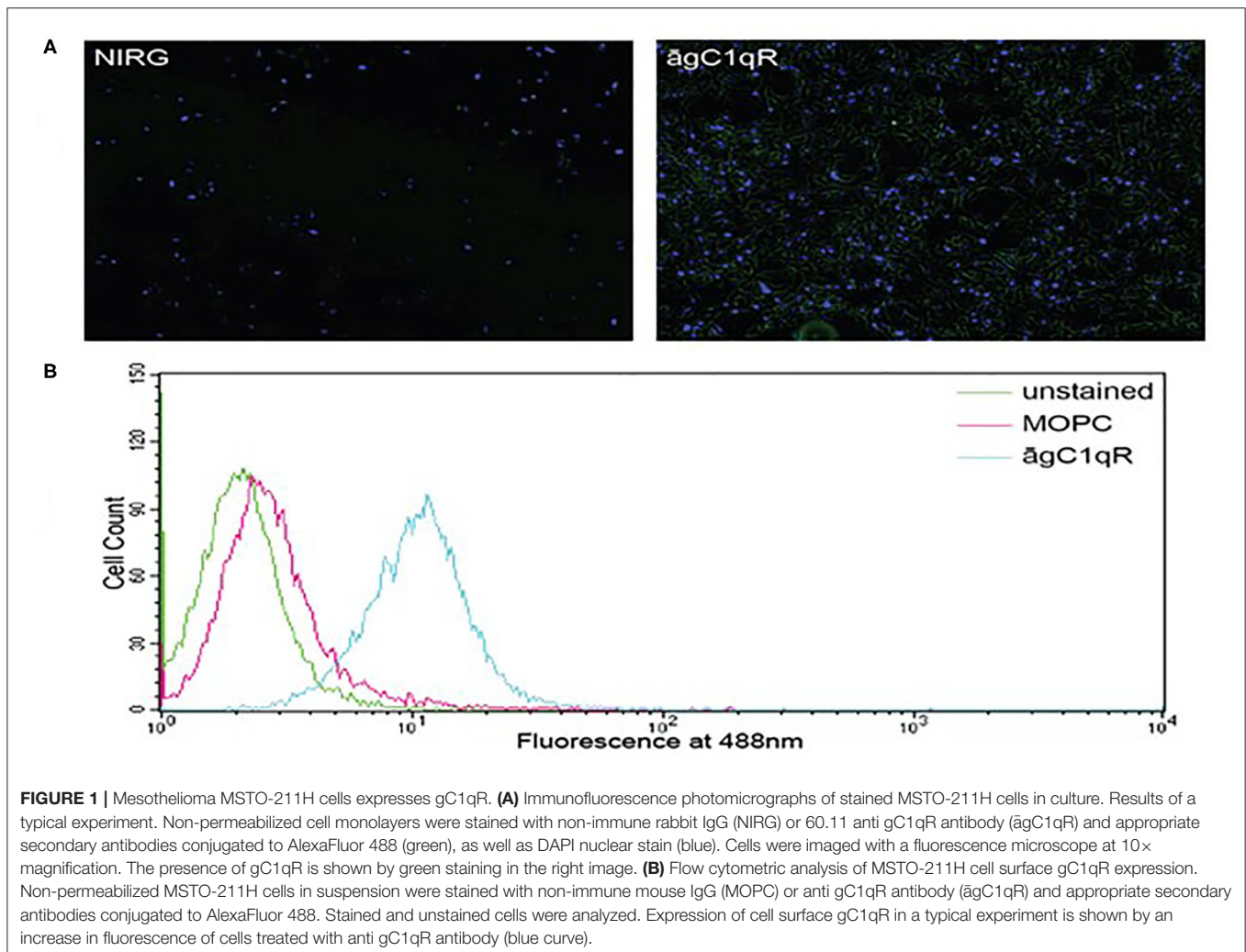
A rabbit polyclonal Cleaved Caspase 3 antibody (Cell Signaling, catalog # 9661) was used at 0.1 $\mu\text{g/ml}$ concentration. Slides were incubated in the Discovery XT autostainer for 3 h. Incubation with secondary antibody (biotinylated goat anti-rabbit IgG; Vector labs) at a concentration of 5.75 $\mu\text{g/ml}$ occurred for 20 min. Blocker D, Streptavidin-HRP, and DAB detection kit (Ventana Medical Systems) were used according to manufacturer instructions.

TUNEL

Terminal deoxynucleotidyl dUTP nick end labeling (TUNEL) analysis was done as follows. Slides were manually deparaffinized in xylene, rehydrated in a series of alcohol dilutions (100, 95, and 70%) and tap water, and placed into the autostainer, where tissue sections were treated with Proteinase K (20 $\mu\text{g/ml}$ in PBS) for 8 min, and incubated with endogenous biotin blocking kit (Roche) for 12 min, followed by incubation with labeling mix: TdT (Roche, 1,000 U/ml) and biotin-dUTP (Roche, 4.5 nmol/ml) for 2 h. Detection was performed with Streptavidin-HRP and DAB detection kit (Ventana Medical Systems) according to the manufacturer's instruction.

CD31

Primary antibody, a rat anti-mouse CD31 antibody (Dianova, catalog # DIA-310) was used at 2 $\mu\text{g/ml}$. Slides were incubated in the autostainer for 6 h, followed by exposure to biotinylated rabbit anti-rat IgG (Vector, 1:200 dilution) for 60 min. Blocker D, Streptavidin-HRP, and DAB detection kit (Ventana Medical Systems) were used according to the manufacturer's instructions.



Quantitative Analysis of Immunohistochemical Staining

Quantitative analysis of immunohistochemical staining was performed on images generated by a slide scanner (Panoramic Flash 250, 3DHitech, Hungary) using Image J software.

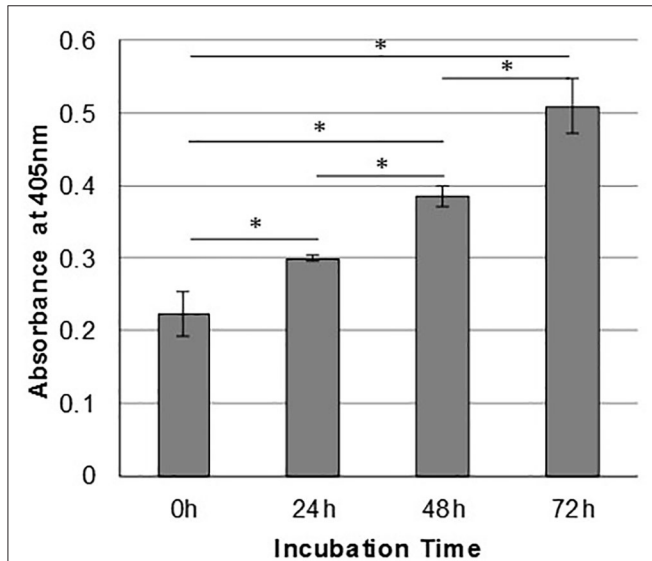


FIGURE 2 | Mesothelioma cells shed gC1qR into the extracellular milieu. MSTO-211H cells were cultured in complete medium, and supernatants were sampled at 24-h intervals. Relative concentrations of gC1qR in the supernatants at each time point were determined via a direct ELISA. Error bars represent mean substrate absorbance \pm standard deviation. $n = 2$ separate experiments performed in duplicate. *Significantly different results at $p < 0.05$.

Statistical Analysis

Data were analyzed with Student's *t*-test as applicable, and $p < 0.05$ were considered statistically significant. All statistical analysis, calculations, and graphing were performed in Excel (Microsoft, Redmond, WA).

RESULTS

In vitro Studies

In order to assess the effect of anti-gC1qR therapy on MSTO cell proliferation, *in vitro* studies first evaluated the expression of the target antigen by MSTO cells. Expression of gC1qR by MSTO cells was demonstrated by immunofluorescence microscopy (**Figure 1A**) and flow cytometry (**Figure 1B**). In addition, soluble gC1qR was detected in culture supernatants of MSTO cells. Progressively increasing amounts of gC1qR were shed into the cell culture medium over a 72 h time course (**Figure 2**). Soluble gC1qR was found also *in vivo*, in 10 of 22 pleural fluids from patients with malignant pleural mesothelioma, with a mean concentration of 1.11 ± 0.57 ng/ml ($n = 10$). Interestingly, soluble gC1qR and immobilized gC1qR, enhanced MSTO cell adhesion in culture (**Figure 3**). Cell proliferation was reduced $25.3 \pm 1.8\%$ ($n = 4$) by targeting gC1qR with mAb (60.11) (**Figure 4**). This inhibition was specific for mAb 60.11, directed against amino terminal amino acids 76–93, representing the C1q binding domain (28). As illustrated in **Figure 4**, gC1qR mAb 74.5.2, directed against aa 204–218, had a negligible effect ($2.9 \pm 2.2\%$ inhibition).

In vivo Studies

An orthotopic mouse model of malignant pleural mesothelioma was used to evaluate gC1qR blockade with mAb 60.11 on MSTO cell proliferation. The data are summarized in **Table 1**. Animals

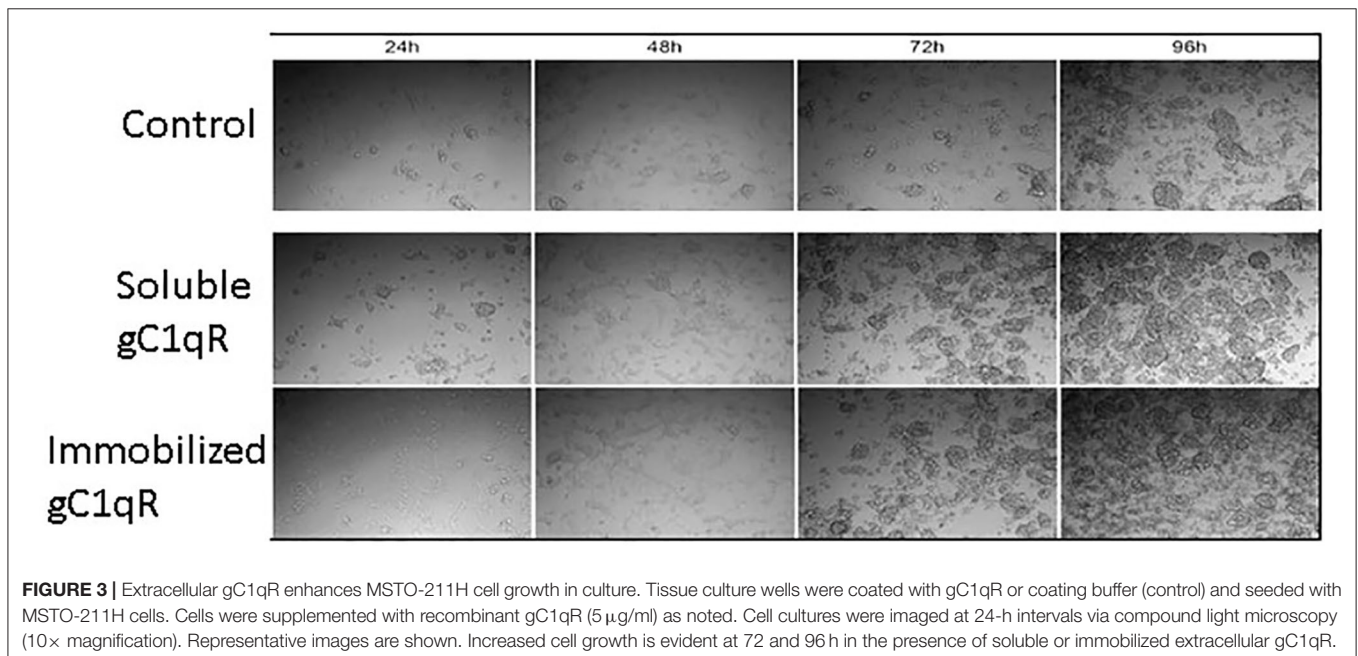


FIGURE 3 | Extracellular gC1qR enhances MSTO-211H cell growth in culture. Tissue culture wells were coated with gC1qR or coating buffer (control) and seeded with MSTO-211H cells. Cells were supplemented with recombinant gC1qR ($5 \mu\text{g/ml}$) as noted. Cell cultures were imaged at 24-h intervals via compound light microscopy ($10\times$ magnification). Representative images are shown. Increased cell growth is evident at 72 and 96 h in the presence of soluble or immobilized extracellular gC1qR.

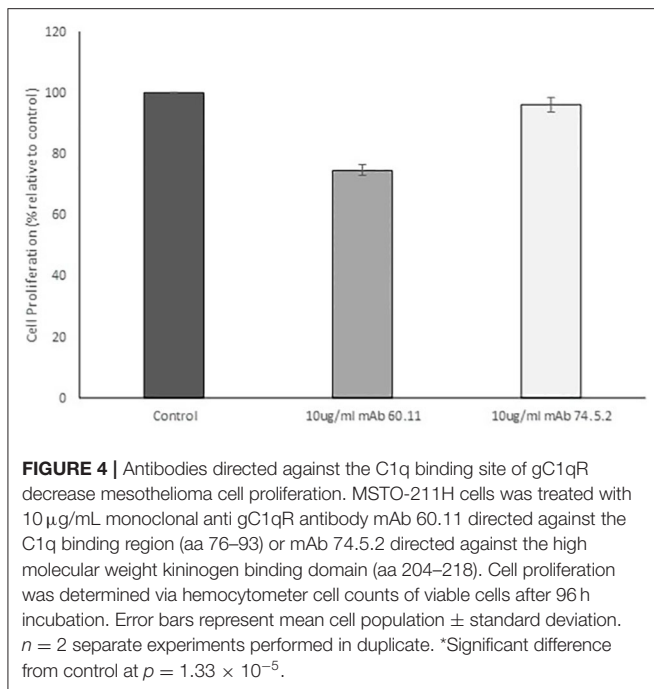


TABLE 1 | Targeted gC1qR (60.11) treatment reduces MSTO-211H tumor cell growth in an orthotopic murine xenotransplant model.

	Treatment Group		<i>p</i>
	Vehicle Control	60.11 Treatment	
Baseline	2.1 \pm 1.0	2.4 \pm 1.4	0.635
Week 1	18.8 \pm 6.1	9.6 \pm 3.6	0.0006
Week 2	61.1 \pm 25.6	34.6 \pm 19.6	0.018

BLI, Bioluminescence Imaging.

treated with the 60.11 antibody showed an approximately 50% reduction in tumor development compared to vehicle control. Serum 60.11 antibody concentrations measured on day of sacrifice ranged from 30 to 50 μ g/ml. 60.11 therapy was not associated with clinical changes such as weight loss (Table 2), disruption of locomotor coordination, hunching, and lack of grooming or lethargy. Comparison of peripheral blood cell counts showed no change in RBC and platelet counts between treatment groups, but a modest decrease in WBC counts, predominantly associated with a decrease in neutrophils in 60.11 treated mice, was observed (Table 2).

Figure 5 shows representative images of tumors resected from control and 60.11 treated mice. Although tumors from 60.11 treated mice were macroscopically smaller than those excised from control mice, no histologic differences were appreciated by H&E staining. Tumors consisted of densely packed MSTO cells with significant areas of necrosis. Immunohistochemical analysis (Figure 6) revealed an increase in early and late

TABLE 2 | Effect of 60.11 therapy on mouse weight and blood cell counts.

	Treatment Groups	
	Vehicle	60.11 treatment
Weight (g)	23.0 \pm 1.16	23.5 \pm 1.08
Cell Count		
RBC ($10^6/\mu$ l)	9.01 \pm 0.70	8.58 \pm 0.63
Hgb (g/dl)	15.01 \pm 1.15	14.23 \pm 1.01
HCT (%)	43.54 \pm 3.14	41.91 \pm 2.50
MCV (fl)	48.33 \pm 0.61	48.92 \pm 1.13
Platelets ($10^3/\mu$ l)	1579 \pm 121	1544 \pm 172
WBC ($10^3/\mu$ l)	5.02 \pm 1.05	3.65 \pm 1.02*
Neutrophils ($10^3/\mu$ l)	4.57 \pm 0.92	3.24 \pm 0.91*
Lymphocytes ($10^3/\mu$ l)	0.032 \pm 0.019	0.014 \pm 0.014*
Monocytes ($10^3/\mu$ l)	0.292 \pm 0.134	0.258 \pm 0.10
Eosinophils ($10^3/\mu$ l)	0.125 \pm 0.067	0.137 \pm 0.081
Basophils ($10^3/\mu$ l)	0.06 \pm 0.013	0.02 \pm 0.004

* $P < 0.05$.

apoptosis markers, cleaved caspase 3, and TUNEL, respectively, in tumors from 60.11 treated mice compared to controls. No difference in the tumor cell proliferation index (Ki67) was noted between treatment groups. Interestingly, 60.11 therapy was associated with decreased tumor CD31 staining, suggesting decreased angiogenesis. In addition, the CD31 positive vessels in tumors of 60.11 treated animals appeared generally small and poorly developed.

DISCUSSION

The data provide proof of concept that targeting the complement receptor, gC1qR, at the C1q binding site, may provide a potential novel therapeutic strategy in mesothelioma. The present study was developed based on our recent observation that gC1qR is overexpressed in malignant pleural mesothelioma (29), and reports from breast and lung cancer models, indicating decreased tumor cell proliferation (34–36) using gC1qR-targeted strategies. *In vitro* studies with the biphasic mesothelioma cell line MSTO demonstrate the presence of targetable gC1qR on the cell surface and in the extracellular milieu. Interestingly, extracellular gC1qR enhanced MSTO cell adhesion and proliferation *in vitro*, and may have similar direct effects *in vivo*. Additional postulated roles for extracellular gC1qR include shielding of the tumor from classical complement mediated attack, and activation of the kallikrein system with bradykinin generation and vascular leakage that may contribute to tumor metastasis (27).

The ability of tumor cells to adhere to tissue surfaces is a key element in metastasis formation (37). Thus, the enhanced ability of MSTO cells to adhere to immobilized gC1qR supports the hypothesis that gC1qR in the tumor microenvironment may contribute to tumor progression via autocrine or paracrine effects. This observation requires further

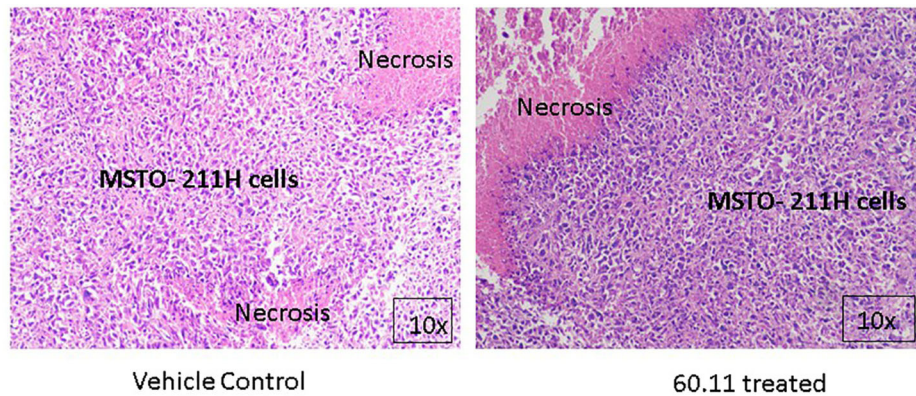


FIGURE 5 | MSTO-211H tumors resected from control and 60.11 treated mice are histologically similar. Representative histologic (10×) images of tumors stained with hematoxylin and eosin show tightly packed MSTO-211H cells and areas of necrosis. No histologic differences in tumor morphology were apparent between control and treatment groups.

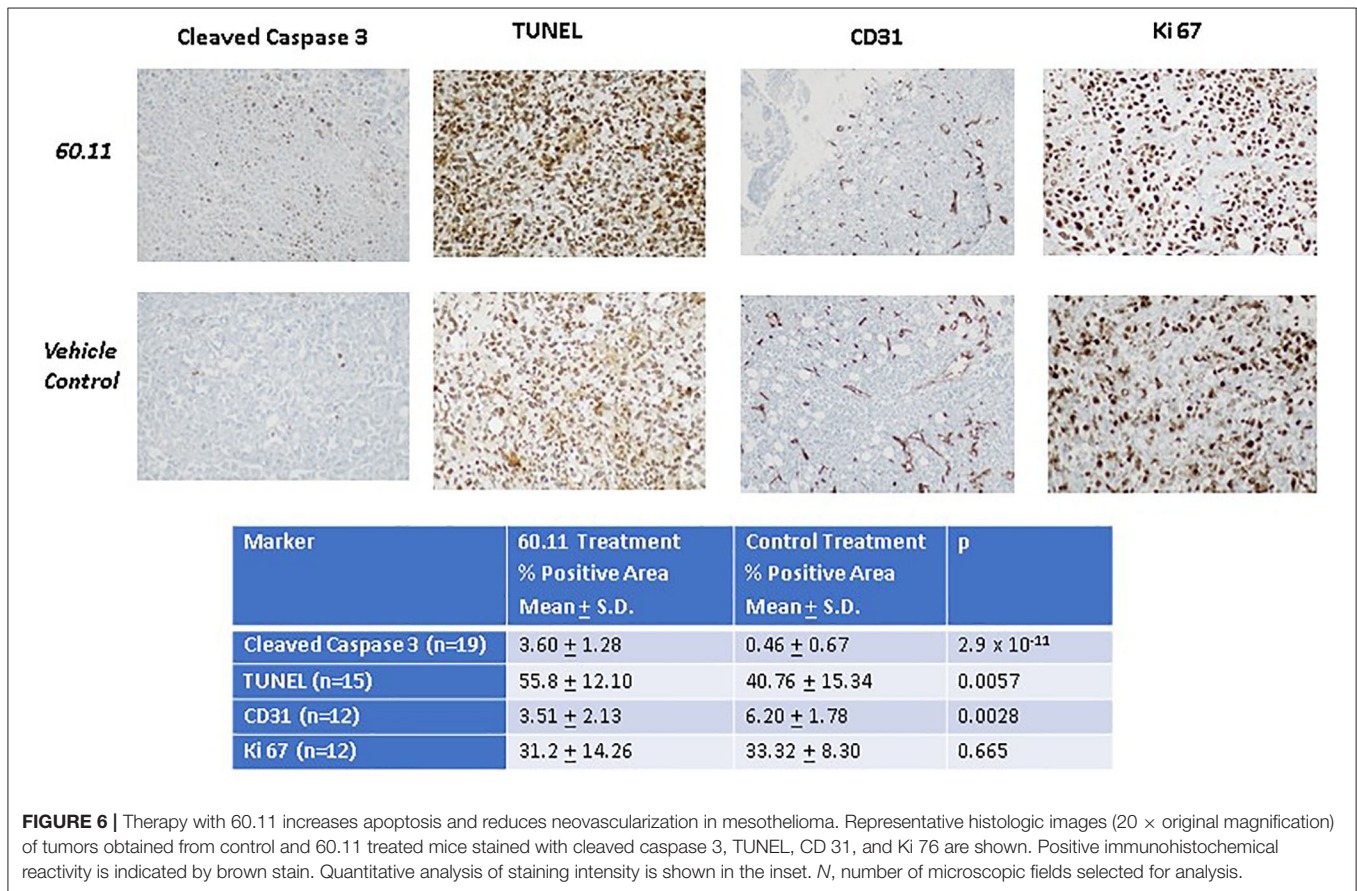


FIGURE 6 | Therapy with 60.11 increases apoptosis and reduces neovascularization in mesothelioma. Representative histologic images (20 × original magnification) of tumors obtained from control and 60.11 treated mice stained with cleaved caspase 3, TUNEL, CD 31, and Ki 76 are shown. Positive immunohistochemical reactivity is indicated by brown stain. Quantitative analysis of staining intensity is shown in the inset. *N*, number of microscopic fields selected for analysis.

study, since levels of soluble gC1qR, previously detected in blood and body fluids (26), as well as those noted in pleural effusions from patients with MPM, are significantly lower than those found to enhance cell proliferation *in vitro*. Concentrations in the tumor microenvironment, however, are likely different,

and greater in the microenvironment immediately adjacent to the tumor.

Targeting gC1qR, at the C1q binding site with mAb 60.11, reduced MSTO cell proliferation *in vitro*, and to a greater extent *in vivo*, using a clinically relevant orthotopic

pleural mesothelioma mouse model. This model resembles pleural mesothelioma in humans with associated extensive lymphangiogenesis, regional invasion, and shortened survival (31). Data from immunohistochemical studies comparing tumors from 60.11 treated and control mice reveal that inhibition of *in vivo* mesothelioma tumor growth is associated with both increased apoptosis and decreased angiogenesis.

One of the major ligands of gC1qR is C1q (14). The role of C1q in tumorigenesis is multifactorial. C1q is thought to promote tumor growth in part through its support of neovascularization (12). Indeed, in the present study, immunohistochemical analysis of tumors treated with 60.11 therapy demonstrate a decreased and abnormal microvasculature. Since mAb 60.11 is directed specifically against the C1q binding site of gC1qR, these findings support the hypothesis that gC1qR–C1q interactions in the tumor cell microenvironment contribute to mesothelioma tumor growth. Interestingly, recent immunohistochemical studies report the presence of C1q in mesothelioma (38). The observed anti-angiogenic effect of 60.11 therapy likely contributes to the overall *in vivo* effect of 60.11 therapy. This observation illustrates the complexity of tumor growth *in vivo*, and demonstrates that cell proliferation *in vitro* is not directly comparable to tumor growth *in vivo*.

Therapy with 60.11 was not associated with clinically discernable toxicity. Animal weights were similar between treatment and control groups. No difference in animal appearance, habitus or behavior was noted. However, a statistically significant decrease in total WBC was observed in the treatment group, which was attributed predominantly to a decrease in neutrophil count. Lymphocyte counts in the NSG mice are extremely low and differences between groups are therefore difficult to interpret. Given the observed significant anti-mesothelioma effect of 60.11 therapy, further exploration of both its therapeutic potential and toxicity profile are indicated.

The paucity of effective therapies for mesothelioma, including the limited success of mesothelin targeted therapy, immune checkpoint blockade, anti-angiogenesis therapies, and neoantigen based vaccines (39), necessitate the development of additional therapeutic strategies. This exploratory study provides the first *in vivo* proof of concept that targeting gC1qR at the C1q binding site can significantly reduce mesothelioma tumor burden by increasing tumor cell apoptosis and decreasing tumor angiogenesis. The study is limited by use of a single cell line and a single anti gC1qR targeting strategy. However, current results support further exploration of gC1qR as a potential new therapeutic target. Investigation of additional gC1qR targeting antibodies may further maximize treatment efficacy. For example, the 74.5.2 antibody, which has no effect on cell proliferation, is a potent inhibitor of vascular permeability,

blocking the binding of high molecular weight kininogen to vascular endothelial cells (40). In addition, combining gC1qR targeted therapy with chemotherapy or other targeted therapies should be evaluated in further preclinical studies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board/Privacy Board (FWA00004998). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. The animal study was reviewed and approved by Institutional Animal Care and Use Committee of Memorial Sloan Kettering Cancer Center (protocol no. 04-03-009).

AUTHOR CONTRIBUTIONS

EP, BG, and PA designed the study, supervised experiments, analyzed data, and wrote the manuscript. ES, QC, and YX conducted the *in vivo* studies. KM-T, NF, and AB performed immunohistochemical analyses. KS and EK performed *in vitro* studies. All authors participated in data review and review of the manuscript.

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Conflict of Interest: EP and BG receive royalty income from the commercialization of gC1qR antibodies, including 60.11, and hold a licensed patent for the use of gC1qR antibodies. PA has received research funding from ATARA Biotherapeutics and OSE Immunotherapies.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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