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Macrophage Scavenger Receptor A Promotes Tumor Progression in Murine Models of Ovarian and Pancreatic Cancer

Claudine Neyen,* Annette Plüddemann,* Subhankar Mukhopadhyay,* Eleni Maniati,† Maud Bossard,‡ Siamon Gordon,* and Thorsten Hagemann†

Alternatively activated macrophages express the pattern recognition receptor scavenger receptor A (SR-A). We demonstrated previously that coculture of macrophages with tumor cells upregulates macrophage SR-A expression. We show in this study that macrophage SR-A deficiency inhibits tumor cell migration in a coculture assay. We further demonstrate that coculture of tumor-associated macrophages and tumor cells induces secretion of factors that are recognized by SR-A on tumor-associated macrophages. We tentatively identified several potential ligands for the SR-A receptor in tumor cell–macrophage cocultures by mass spectrometry. Competing with the coculture-induced ligand in our invasion assay recapitulates SR-A deficiency and leads to similar inhibition of tumor cell invasion. In line with our in vitro findings, tumor progression and metastasis are inhibited in SR-A−/− mice in two in vivo models of ovarian and pancreatic cancer. Finally, treatment of tumor-bearing mice with 4F, a small peptide SR-A ligand able to compete with physiological SR-A ligands in vitro, recapitulates the inhibition of tumor progression and metastasis observed in SR-A−/− mice. Our observations suggest that SR-A may be a potential drug target in the prevention of metastatic cancer progression. The Journal of Immunology, 2013, 190: 3798–3805.

Solid tumors are composed of neoplastic cells, nonmalignant resident stromal cells, and migratory hematopoietic cells. Complex interactions between the cell types in this microenvironment regulate tumor growth, progression, metastasis, and angiogenesis (1). It is well established that stromal cells, including macrophages, within the microenvironment may contribute to tumor growth and spread (1). There is a body of preclinical and clinical evidence associating the abundance of tumor-associated macrophages (TAMs) with poor prognosis (2).

Macrophages exhibit marked phenotypic heterogeneity and have been broadly classified into M1 or M2 type (3, 4). M1 macrophages classically are activated by IFN-γ, with or without microbial products, produce large amounts of proinflammatory cytokines, express high levels of MHC molecules, and are implicated in the killing of pathogens and tumor cells (3). M2 macrophages modulate the inflammatory response, eliminate cell debris, and promote angiogenesis and tissue remodeling (3, 5). Stimulation with IL-4, IL-13, and IL-10 drives macrophages toward the M2 phenotype. The macrophages present in neoplastic tissues (TAMs) mainly display an M2-like phenotype with expression of classes of innate pattern recognition receptors, such as mannose receptor and scavenger receptor A (SR-A) (5, 6). However, the role of SR-A in TAMs is unclear.

Scavenger receptors are broadly defined by their ability to bind modified low-density lipoproteins and other polyanions, including proteins, lipids, carbohydrates, and nucleic acids (7). SR-A, in particular, binds a wide range of polyanionic ligands from artificial, microbial, and endogenous origin, including polyribonucleotides, polysaccharides, and glycated proteins, among which are extracellular matrix (ECM), proteoglycans, oxidized/modified lipids, and lipoproteins.

SR-A is restricted to the myeloid lineage and is expressed on most mature tissue macrophages and on bone marrow–derived dendritic cells and splenic dendritic cells but not on their immature precursor monocytes. In addition, SR-A is found on smooth muscle cells and on a small subpopulation of endothelial cells in the lung (8, 9).

SR-A, most well studied for its role in atherosclerosis, has been implicated in the metabolic changes that affect macrophages exposed to high fat loads and oxidative stress (10). Many of these SR-A functions may be relevant to other diseases with underlying metabolic and oxidative changes, including cancer (11, 12). SR-A-mediated adhesion of macrophages signals changes in oxidative output (13), and differential ligand binding to SR-A influences the inflammatory status of macrophages (14–16). Both phenomena may drive physiological changes in the microenvironment of SR-A–expressing macrophages.

We demonstrated previously that ovarian cancer cells switch cocultured macrophages to a phenotype similar to that found in ovarian tumors (6). Tumor cells caused upregulation of scavenger receptor SR-A, which is consistent with other publications identifying this receptor as a marker on alternatively activated mac-
raphages. We confirmed that SR-A was expressed on TAMs in ovarian cancer patients (6). Recent data published by Bak et al. (17) showed that targeting macrophages via the SR-A receptor in a murine orthotopic ovarian cancer model led to disease stabilization, assigning a pro-oncogenic role to SR-A. In addition, lack of SR-A was shown to improve stimulation of tumor immunity in vivo (18).

Therefore, we aimed to investigate the role of SR-A during tumor development and progression. In this article, we show that SR-A expression on macrophages is important for tumor progression and metastasis in vitro and in vivo. Upon coculture of tumor cells with macrophages, an SR-A ligand is secreted into the supernatant. Competition with the physiological SR-A ligand in vitro and possibly in vivo inhibits macrophage-induced tumor cell invasion, and tumor burden was significantly reduced specifically in SR-A−/− mice. We conclude that the macrophage SR-A and its uncharacterized ligand (s) contribute significantly to the host-tumor relationship.

Materials and Methods

Cell lines and reagents

Unless otherwise indicated, all reagents were purchased from Sigma (Poole, U.K.). Acetylated low-density lipoprotein (AcLDL) was from Molecular Probes (Eugene, OR), and PMH-Liposorb was from Calbiochem. 4F and scr4F peptides were generous gifts from Dr. Alan Fogelman (University of California Los Angeles, Los Angeles, CA). Cell lines were grown in RPMI 1640 medium supplemented with 10% FCS. All experiments were performed under endotoxin-free conditions. The murine cancer cell lines ID8 and Panc02 were cultured in DMEM supplemented with 10% FCS.

Coculture

Tumor–macrophage cocultures were performed as described previously (19). Briefly, luciferase-expressing tumor cells (2.5 × 105/well) and bone marrow–derived macrophages (BMMF; 5 × 105/Transwell insert) were grown without cell–cell contact in the upper compartment of a modified Boyden chamber, and invasiveness was measured by quantifying transmigrated tumor cells in the lower compartment using relative luciferase units. Cell viability was assessed using the Beckman Coulter ViCell XR Counter (Beckham, Fullerton, U.K.). To test for scavenging capacity in cocultures, supernatants were incubated repeatedly for 1 h on wild-type (wt) or SR-A−/− BMMF monolayers and then tested for SR-A ligand activity by ELISA.

SR-A ELISA

The SR-A–specific ELISA was carried out as described (20). Supernatants were coated onto ELISA plates overnight at a protein concentration of 10 μg/ml, and SR-A binding was detected using wt (129 ICR) or SR-A−/− BMMF lysates, followed by anti-SR-A mAb 2F8 and appropriate HRP-coupled secondary Ab. AcLDL was used as a positive control. For lipid depletion before ELISA, supernatants were processed with PMH-Liposorb resin (Calbiochem), following the manufacturer’s instructions.

Animals

The following knockout mouse strains were used: SR-A−/−, MARCO−/−, SR-A/MARCO double-knockout (db−/−), TLR2−/−, and TLR4−/−, all on a C57BL/6 background, along with their wt control Charles River strain. Bones from CD36−/− mice (21) were obtained from David Kluth (University of California, Los Angeles, CA). Cell lines were grown in RPMI 1640 medium supplemented with 10% FCS. All experiments were performed under endotoxin-free conditions. The murine cancer cell lines ID8 and Panc02 were cultured in DMEM supplemented with 10% FCS.

Next we confirmed that tumor invasiveness was due to expression of SR-A on macrophages and not on tumor cells (Supplemental Fig. 1B) and was not associated with other pattern recognition receptors, such as TLR2 and TLR4, which were shown to interact with SR-A. Loss of TLR2 or TLR4 did not influence tumor cell invasion (Fig. 1C) and neither did the loss of TLR3, TLR7, or TLR9 receptors, indicating that SR-A is sufficient on its own to promote tumor invasiveness and does not rely on innate immune signaling via TLR-dependent pathways.
Receptor competition with polyanionic ligands in the coculture assay

SR-A is an endocytic receptor and signals upon ligand binding. If ligand–receptor interaction was necessary for SR-A to exert its tumor-promoting effect, then it should be possible to block tumor invasion by outcompeting the tumor-promoting ligand with other known SR-A ligands. Therefore, we cocultured wt murine macrophages with ID8 tumor cells and used polyanionic ligands to compete with the coculture-induced SR-A ligand (Fig. 2). We screened a range of nonmicrobial polyanionic ligands and their closely related nonligand controls. First, we established concentration curves for the respective ligands (Fig. 2A). Although the addition of fucoidan, poly I, and poly G inhibited ID8 tumor cell invasion in a dose-dependent manner, the nonbinding control poly C had no effect. Addition of fucoidan, poly I, or poly G to ID8 or Panc02 cells alone did not induce invasion (Supplemental Fig. 1C), and previously published data showed that stimulation of tumor cells with proinflammatory LPS had no invasion-promoting effect (19).

To investigate whether SR-A activity is required at the early or late stages of tumor cell invasion, we added poly I to the ID8–macrophage coculture at different time points after the start of the coculture (Fig. 2B). Addition of poly I up to 6 h after the start of the experiment inhibited macrophage-induced tumor cell invasiveness. Addition of poly I at later time points had no effect, indicating that SR-A is required early during invasion (Fig. 2B). Similar results were obtained when we cocultured macrophages with Panc02 tumor cells (data not shown).

Receptor recognition of a coculture-induced ligand

The observation that the tumor-promoting effect of SR-A can be blocked by a range of SR-A ligands suggests the presence of a coculture-induced SR-A ligand at some stage during invasion. To assess the possibility of such an SR-A ligand, we cocultured macrophages with ID8 and Panc02 tumor cells or embryonic mouse fibroblasts (EMFs; benign control) and collected the supernatant after 24 h. Single-culture supernatant from the respective cells, wt

**FIGURE 1.** SR-A is necessary and sufficient to promote invasion in a macrophage–tumor cell in vitro invasion assay. (A) Wt or SR-A−/− BMMs were cocultured with ID8-Luc or Panc02-Luc cells in a modified Boyden chamber without direct cell–cell contact for 72 h. Invasion of ID8-Luc/Panc02-Luc cells was assessed by luciferase activity in the lower part of the chamber. SR-A−/− macrophages had a reduced ability to promote ID8-Luc and Panc02-Luc invasion (p < 0.05, t test). (B) Culture of ID8 or Panc02 cells alone or cocultured with wt, SR-A−/−, or CD36−/− macrophages. CD36−/− macrophages do not significantly reduce ID8-Luc invasion. (C) Culture of ID8 or Panc02 cells alone or cocultured with wt, SR-A−/−, TLR2−/−, TLR3−/−, TLR4−/−, TLR7−/−, or TLR9−/− macrophages. Data are mean ± SEM (n = 6). Representative data are shown from at least three independent experiments.

**FIGURE 2.** Competition with the coculture-induced ligand in the in vitro invasion assay by large polymeric SR-A ligands. (A) Macrophages and ID8 tumor cells were cocultured in the presence of fucoidan, poly I, poly G, or poly C. Addition of known SR-A ligands can inhibit tumor cell invasion (p < 0.01, t test). Poly C served as a negative control. Data are mean ± SEM (n = 6). Representative data are shown from at least three independent experiments. (B) Addition of 100 µg/ml of poly I up to 6 h after the start of the macrophage–ID8 coculture inhibits tumor cell invasion. When added after 9 h, there is no impact on the invasion assay.
and SR-A<sup>-/-</sup> macrophages, ID8, Panc02, and EMFs served as negative controls. We first screened all control and coculture supernatants for differential SR-A binding activity by ELISA (Fig. 3A) (32). This assay is nonspecific for the molecular nature of the ligand. Our results showed that SR-A recognized ligand(s) in coculture supernatants of wt or SR-A<sup>-/-</sup> macrophages with either ID8 or Panc02 but not in RPMI 1640 medium or supernatants from any single culture (Fig. 3A). SR-A–binding activity was also absent from macrophages cocultured with EMFs, proving that ligand induction is a specific feature of coculture with tumors. Ligand activity disappeared from wt–tumor cell supernatants after repeated passages on wt, but not on SR-A<sup>-/-</sup>, BMMΦ monolayers (Fig. 3B), further corroborating the presence of an SR-A ligand that can be scavenged from the coculture milieu in an SR-A–specific manner.

Studies indicated that the ligand was heat labile (56–C, 30 min), acid labile (pH 6), with an apparent molecular mass of 20–60 kDa, as determined by cut-off filters, and RNase and DNase stable (data not shown). In addition, lipid depletion of coculture supernatants significantly decreased ligand activity (Fig. 3C).

Having demonstrated the presence of SR-A ligand(s) in the supernatant of TAM–tumor cocultures, we undertook provisional steps to isolate and identify potential protein ligand(s) by SR-A–specific Far Western blot, followed by mass spectrometry analysis of SR-A–interacting proteins from corresponding gel bands (20). Potential candidates are listed in Supplemental Table I. Clustering of gene ontology terms associated with ligands in this list suggests enrichment in ECM proteins, proteins involved in wound healing and proteolysis, and proteins of inflammatory pathways (Supplemental Table II).

The large number of candidate ligands retrieved reflects the known promiscuous nature of scavenger receptors. In view of a possible therapeutic intervention, we reasoned that it would be more parsimonious to target the receptor rather than one of many potential ligands.

**SR-A deficiency delays tumor progression in the ID8 model and reduces lung metastasis in the Panc02 model**

Based on our in vitro results, we next investigated the role of SR-A during tumor progression in vivo. We previously reported a syngeneic model of ovarian cancer using a luciferase–labeled cell line ID8 (33). In this model, ID8 cells are injected i.p. and form local tumors in the peritoneal cavity. SR-A deficiency did not prevent the development of peritoneal tumors; however, tumor progression (growth of single tumors) was significantly slowed in ID8 or SR-A<sup>-/-</sup> mice compared with wt mice (Fig. 4A, 4B; p < 0.05, t test, n = 12). Interestingly, mice deficient in another class A scavenger receptor, MARCO, showed a significant increase in disease burden compared with wt mice (Fig. 4A) that was suppressed in db<sup>-/-</sup> mice, suggesting opposing effects of MARCO and SR-A. To exclude the contribution of nonhematopoietic SR-A receptor involvement in our observations, we generated SR-A<sup>-/-</sup>, MARCO<sup>-/-</sup>, and db<sup>-/-</sup> (SR-A<sup>-/-</sup>, MARCO<sup>-/-</sup>, or db<sup>-/-</sup> bone marrow in wt mice) and reverse (wt bone marrow in SR-A<sup>-/-</sup>, MARCO<sup>-/-</sup>, or db<sup>-/-</sup> mice) chimeras to study the contribution of hematopoietic/myeloid SR-A. SR-A<sup>-/-</sup> and db<sup>-/-</sup> chimeras, but not the respective reverse chimeras, showed a similar growth delay in the ID8 model as described above (Fig. 4C), proving that SR-A is specifically required on TAMs but not on other stromal cells.

The ID8 model only addresses the role of SR-A in primary tumor growth and not in metastasis to distant sites. Because our in vitro coculture assay showed reduced invasiveness in the absence of SR-A for ID8 and for the Panc02 pancreatic cancer cell line, we used the latter to study the role of SR-A during tumor progression/metastasis in vivo. In the Panc02 model, s.c. injection of tumor cells results in both primary tumors at the injection site and lung metastasis. We implanted 10<sup>6</sup> Panc02 cells s.c. into wt and SR-A<sup>-/-</sup> mice, and primary tumor growth and lung metastasis (tumor multiplicity) were assessed after 28 d, at which point there was no difference in the size of primary Panc02 tumors (data not shown). However, we observed a significant decrease in lung metastasis in SR-A<sup>-/-</sup> mice (Fig. 4D, 4E), suggesting that, in this model, SR-A does not affect local tumor growth but specifically promotes migration of tumor cells to distant sites. To differentiate between hematopoietic/myeloid SR-A contribution, we generated bone marrow chimeras. The respective SR-A<sup>-/-</sup> chimera, but not the reverse chimera, protected the mice from developing lung metastasis (Fig. 4F).

**Toward an SR-A–specific therapeutic intervention**

Because genetic ablation of SR-A resulted in reduced tumor invasiveness, we sought to determine whether SR-A could be therapeutically targeted in vivo to obtain similar results. Although an SR-A mAb is available (2F8), and Ab therapy of cancer has proven successful in several models (34), small molecule inhibitors remain advantageous over mAbs in ease of delivery and cost.

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**FIGURE 3.** Ligand-binding assay reveals SR-A ligand(s) in macrophage–tumor cell coculture supernatants. (A) An ELISA-based ligand-binding assay was used to screen for a possible SR-A ligand in coculture supernatants. Supernatant from ID8, Panc02, wt, or SR-A<sup>-/-</sup> macrophages or EMFs alone showed no ligand binding. Coculture supernatant from wt or SR-A<sup>-/-</sup> macrophages with ID8 or Panc02 cells showed a significant increase in SR-A–binding activity (p < 0.01, t test). There was no SR-A ligand binding detectable in the supernatant from macrophages with EMFs. AcLDL was used as a positive control. Data are mean ± SD (n = 12). Representative data are shown from at least three independent experiments. (B) SR-A–binding activity is lost after repeated passages of coculture supernatants on wt, but not SR-A<sup>-/-</sup>, BMMΦ monolayers. Coculture supernatants were incubated twice or five times for 1 h with wt or SR-A<sup>-/-</sup> BMMΦs and then SR-A ligand activity in the passed supernatant was assessed by ELISA as in (A). Data are represented as in (A). (C) Coculture supernatants contain a lipid ligand. Supernatants as in (B) were treated with PMH-Liposorb resin to remove all lipids or lipid-bound molecules and then tested for SR-A binding activity by ELISA as in (A).
Therefore, we investigated whether the small SR-A receptor inhibitor 4F could block tumor cell invasion in our model. 4F is a 16-aa amphipathic peptide that mimics the broad antiatherogenic and anti-inflammatory properties of apo A-I (35, 36), and it can compete with a range of SR-A ligands (20). In our in vitro invasion assay, 4F, but not the negative control scr4F, showed dose-dependent inhibition of coculture-induced ID8 or Panc02 invasion (Fig. 5A). A total of 100 μg/ml of 4F was sufficient to nearly completely block coculture-induced tumor cell invasion (Fig. 5B).

The SR-A inhibitor 4F prevents tumor progression in vivo

Because loss of SR-A seemed to prevent tumor growth and metastasis in vivo and blocking SR-A with physiological ligands reduced tumor invasiveness in vitro, we next assessed whether SR-A could be successfully targeted with 4F in vivo. We used the ID8 model to quantify tumor growth in the presence of the SR-A inhibitor 4F or its scrambled control peptide scr4F, showed dose-dependent inhibition of coculture-induced ID8 or Panc02 invasion (Fig. 5A). A total of 100 μg/ml of 4F was sufficient to nearly completely block coculture-induced tumor cell invasion (Fig. 5B).

Discussion

In the current study, we demonstrated that SR-A is required on macrophages to support tumor invasiveness during coculture in vitro and metastasis in vivo and that competition with SR-A ligand(s) can reduce macrophage-induced tumor cell invasion. We detected SR-A ligand-binding activity in the coculture medium, but not in single-culture controls, and identified a range of candidate protein ligands. Although TAMs have been associated with tumor promotion in many previous studies, to our knowledge, the current study provides the first direct evidence that a specific scavenger receptor, SR-A, is implicated in trophic tumor–host interactions. SR-A is necessary and sufficient to promote tumor invasiveness, and it does not seem to require additional signaling via TLR pathways. Our experiments indicate that communication between TAMs and tumor cells requires SR-A at an early time point, and results in chimeric mice establish that SR-A is only needed on hematopoietic cells, consistent with its expression on macrophages. Importantly, loss of SR-A affected tumor metastasis, which was delayed and not prevented, rather than tumor growth at the initial inoculation site.
The small molecule SR-A inhibitor 4F prevents tumor invasiveness in vitro and in vivo. (A) Dose response curve. Macrophages and ID8 tumor cells were cocultured in the presence of 4F, an SR-A ligand. Scrambled 4F (scr4F), which contains the same amino acids as 4F but in random order to prevent amphipathic helix formation, served as a negative control. (B) Invasion assay of macrophages with either ID8 or Panc02 cells in the presence of 100 μg/ml scr4F or 4F. Data in (A) and (B) are mean ± SEM (n = 6). Representative data are shown from at least three independent experiments. (C) 4F prevents tumor growth in vivo. Animals were treated with 4F s.c., and tumor burden was assessed using IVIS imaging system. Loss of SR-A does not add to the effect of 4F on tumor cell invasion.

Two cancer-derived cell lines of ovarian and pancreatic origin, but not diploid embryonic fibroblasts, gave consistent results in migration assays of macrophage cocultures. SR-A was the only pattern recognition receptor tested that showed a phenotype in our in vitro assay; in particular excluding another scavenger receptor, CD36, which is expressed in primary BMMΦs. MARCO is not expressed in bone marrow cultures as used in this study, but it did not mimic the role of SR-A in vivo. On the contrary, loss of MARCO seemed to enhance, rather than reduce, tumor multiplicity in vivo. Although these two class A receptors share many common structural and functional features, they differ subtly on several points. SR-A and MARCO have distinct ligand-binding domains and share overlapping, but distinct, ligand repertoires. SR-A is expressed broadly on most tissue-resident macrophages, whereas MARCO is restricted to subsets of macrophages. However, MARCO can be readily induced on most macrophage populations after inflammatory challenge (40, 41). Further studies are needed to establish the basis of their contrasting behavior in host-tumor interactions.

The in vitro model did not require contact between tumor cells and macrophages, indicating that secretion products from either cell contributed to their interactions but only when cultured together. The cytokine TNF-α is one such coculture-induced molecule that has been implicated in tumor invasiveness (19, 25). Although TNF-α was induced in an SR-A–dependent fashion (Supplemental Fig. 1D) and might partially explain the loss of invasiveness in SR-A−/− cocultures, we hypothesized that there were additional factors present that acted as ligands for SR-A; consistent with this was our ability to detect ligand activity in a specific ELISA used previously to characterize SR-A ligands. Therefore, the tumor-promoting effect of SR-A could be attributed to ligand-induced receptor signaling, switching TAMs toward a tumorigenic phenotype, or receptor-mediated clearance of a tumor-inhibiting ligand. Ligands detected by ELISA were present at comparable levels in cocultures of tumor cells with both wt and SR-A−/− macrophages, but they could be readily scavenged from coculture supernatants by incubation on BMMΦ monolayers, tentatively favoring the latter hypothesis.

We used a previously validated protocol, SDS-PAGE and Far Western blotting, to identify ligand-containing bands on gels, which we subsequently analyzed by mass spectrometry (Supplemental Tables I, II). Candidate protein ligands identified in our study are significantly enriched in ECM components, and SR-A, like many other scavenger receptors, was shown to bind a variety of these proteins (7). Remarkably, an expression-profiling study of TAMs comigrating with metastasizing tumor cells in vivo showed a specific enrichment in tissue- and organ-development genes, suggesting that TAMs help to shape the environment for the spreading tumor cells (42). This is in agreement with the enrichment of ECM components found in our SR-A candidate list. Indeed, another scavenger receptor, stabilin-2, exhibits protumor activity through its ability to scavenge hyaluronic acid, an abundant ECM component. Loss of stabilin-2 increased circulating levels of hyaluronic acid and led to reduced tumor invasiveness in the lungs (43). It is conceivable that SR-A might similarly scavenge ECM breakdown products from the tumor microenvironment, thereby clearing the road for tumor cells to emigrate and metastasize to distant sites. Although we did not observe significantly enhanced ligand activity in SR-A−/− macrophages, which would have reflected a clearance role for SR-A, the ELISA conditions may not be quantitative enough to detect subtle differences in ligand availability.

Another aspect of SR-A function in relation to tumor biology is its role in the clearance of apoptotic cells, which downregulates macrophage activation by production of TGF-β and PGE. The natural ligand for SR-A in apoptotic cells has not been defined, but it may be related to phosphatidylserine and oxidized lipoprotein, as is the case with other scavenger receptors. Such an additional pathway may reinforce and amplify the benefit to the tumor while sparing inflammatory injury. Given the molecular nature of known SR-A ligands, and based on our observation that lipid depletion from coculture supernatants reduces its ligand content, it is also possible that the coculture-induced ligand is of lipid origin. Bioactive lipids are known to contribute to tumor progression, and SR-A promotes uptake of a range of oxidized lipoproteins and lipids, including lysophosphatidic acid, the precursor for tumor-promoting lysophosphatidic acid (44). It is noteworthy that we did not detect any lipoprotein-related candidate SR-A ligand, includ-
ing apolipoprotein E, which has been implicated in cancer. Further studies are needed to identify the SR-A natural ligand(s) in tumor-macrophage interactions.

Although identification of tumor environment–derived SR-A ligands is highly desirable for our understanding of the mechanism underlying TAM-dependent promotion of metastasis, our results using an SR-A inhibitor may provide a more direct therapeutic handle.

The ability of 4F peptide, an apolipoprotein A1 mimic, to inhibit the interaction of tumor cells and macrophages in vitro and to slow tumor burden in vivo provides support for SR-A involvement. Although 4F peptide is not a specific inhibitor for SR-A, we previously demonstrated its efficacy in inhibiting SR-A–mediated adhesion and endocytosis (20). 4F is easier to deliver than are SR-A Abs or large polymeric ligands, and it successfully passed safety trials for clinical use in humans (45), arguments in favor of its potential therapeutic use in blocking SR-A–enhanced tumor invasiveness. Two recent studies reported promising antimtumor effects of 4F in an ovarian cancer model in vitro and in vivo (ID8 cells). Subcutaneous or orally administered 4F was able to decrease ID-8 cell tumor burden in mice, a mechanism attributed to its ability to scavenge pro-oncogenic lysosphatidic acid from the serum of tumor-bearing mice (39). In the same model, 4F inhibited viability and proliferation of tumors in vitro and in vivo through targeted upregulation of Mn-superoxide dismutase in tumor cells, improving the antioxidant profile of ID-8 cells (37). It could be hypothesized that a reduction in oxidative stress in the tumor milieu would also decrease the bioavailability of oxidized scavenger receptor ligands. Unfortunately, we were unable to detect lysosphatidic acid in our coculture assay and, therefore, cannot make conclusions about its function in our model. Importantly, in our model, 4F treatment and loss of SR-A did not show cumulative effects on tumor invasiveness in vivo, suggesting that 4F and SR-A act in the same pathway.

Association studies present only weak evidence for a causative role of SR-A in the development of human cancer. Germline mutations in the human MSR1 gene have been associated with hereditary prostate cancer and were found to be enriched in individuals with nonhereditary prostate cancer (46). Importantly, among the nonsynonymous changes, several affect highly conserved amino acids in both the cytoplasmic-signaling domain, as well as in the extracellular ligand-binding domain, potentially altering SR-A function. However, a large-scale meta-analysis did not find any significant risk associated with the most prevalent of these mutations: a truncation of the ligand-binding domain (47). Likewise, when specific mutations were assessed for association with metastatic progression of prostate tumors, no significant association was found (48). Results from the Framingham cohort also found no significant genome-wide association of single-nucleotide polymorphisms with either breast cancer or prostate cancer (49). More recently, germline mutations in MSR1 were associated with the incidence of Barrett’s esophagus, a relatively common condition (1–10% of general population) that may evolve into esophageal adenocarcinoma (50). In summary, further studies are required, based on defined molecules involved in SR-A function and regulation in tumor-associated macrophages. One possibility is that soluble SR-A, shed by macrophages (C. Neyen, S. Mukhopadhyay, and S. Gordon, unpublished observations), may provide a useful diagnostic and prognostic biomarker.

In conclusion, we described previously unexplored pathways by which tumors can subvert macrophages in an unholy alliance. Further studies in mouse and humans will be necessary to provide further details on novel drug targets to intervene with this receptor therapeutically.

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### Disclosures

The authors have no financial conflicts of interest.

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