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Review

Histidine Rich Glycoprotein and Cancer: A Multi-faceted Relationship

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Abstract. *A better understanding of the interplay between the tumor environment, the immune system, and hemostatic apparatus is essential to effectively improve cancer treatment. Histidine-rich glycoprotein (HRG) is an abundant plasma protein with a wide array of functions. HRG has the ability to bind multiple ligands thereby modulating immunity, cell adhesion, angiogenesis, and thrombosis. Many of these functions are involved in tumor progression and antitumor response. We outline current data on HRG as an important potential player and as a potential future target for cancer therapy.*

The interactions of the tumor environment, the hemostatic apparatus, and anticancer immune control are of paramount importance in cancer development and outcome (1-4). Histidine-rich glycoprotein (HRG) is a relatively abundant plasma protein with two cystatin-like domains and a wide spectrum of targets and functions (5, 6). First described in 1972, the role of HRG was originally identified as regulating clotting and fibrinolysis due to the association of venous thrombosis with elevated levels of HRG (7). As an adaptor protein, HRG is able to modulate multiple interactions and many other roles have since been described.

One of the more recent discoveries has been the role of HRG in pathogen control, which has provided additional insight into the function of HRG in disease states. HRG has anti-inflammatory effects as demonstrated in *HRG*-deficient mice that had higher bacterial burden and higher levels of the pro-inflammatory cytokine interleukin-6 than wild-type mice after *Streptococcus pyogenes* challenge (8). The anti-fungal properties of HRG, particularly at low pH, were demonstrated in a mouse model of candidiasis in which HRG-deficient mice were susceptible to infection and wild-type mice were resistant (9).

While the full spectrum of physiological roles of HRG is not yet fully described, its importance in hemostasis and angiogenesis, influence on tumor progression and metastasis formation, and the antitumor immune response are gradually coming to light.

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Structure and Isolation of HRG

Structure of HRG. The human *HRG* gene consists of seven exons and six introns totaling approximately 12 kb with the C-terminal half of HRG encoded in exon 7 (5). Located on human chromosome 3q27, the *HRG* gene is located adjacent to the other cystatin type 3 proteins: fetuin-A, fetuin-B, and kininogen (5).

The protein has two cystatin-like domains, a histidine-rich region (HRR) that is surrounded by two proline rich regions, and a C-terminal domain (Figure 1). Multiple tandem repeats of the motif GHHPH in the *HRR* contribute to the high histidine content (10). In humans, two polymorphisms of HRG have been identified; both 77-kDa and 75-kDa proteins have been isolated from human blood, which differ by a serine or proline at position 186, respectively (5). The serine allows for a *N*-glycosylation site, increasing the molecular weight by the addition of carbohydrates (11). There are five disulfide bonds which protect the protein from proteolytic cleavage (Figure 1) (12).

Purification of HRG from plasma. HRG was originally identified in serum as a protein that had a high affinity for

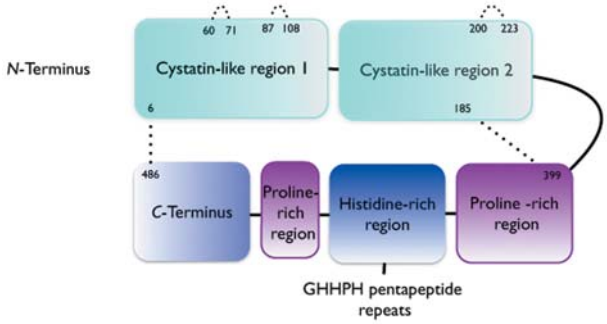

Figure 1. *Schematic of histidine-rich glycoprotein domains. There are two cystatin-like domains at the N-terminus followed by two proline-rich regions that surround the histidine-rich region. The C-terminus is linked to the N-terminus cystatin-like region by a disulfide bond. There are internal disulfide bonds in both cystatin-like regions. The second cystatin-like region is also linked to the first proline-rich region by a disulfide bond. Dotted lines indicate disulfide bonds.*

carboxymethylcellulose (13, 14). The most common method for purifying HRG has been with phosphocellulose and nitrilotriacetic acid chromatography, and these preparations have been used to determine the biological activities of HRG *in vitro* (15, 16). However, isolation of HRG using these methods can result in retention of HRG-binding partners, including immunoglobulin, plasminogen, and fibrinogen (16). For example, HRG purified from plasma may contain plasminogen contaminants, which may stimulate angiogenesis (16-18). Additionally, these purification methods often require further downstream steps that reduce protein yields and create a mixture of proteolytically-cleaved HRG species. These cleavage species may have biologically different effects those of the parent protein.

Patel *et al*. have recently addressed this issue by purifying HRG using a two-step process combining metal ion affinity and ion exchange chromatography that enables further study of HRG without contaminating proteins or cleavage products (16). The metal ion affinity step using cobalt sepharose beads takes advantage of the histidine-rich sequences within HRG that efficiently bind immobilized metal ions. Combined with ion exchange chromatography, Patel *et al*. were able to generate intact HRG without detectable cleavage products (16). Subsequent studies of the purified protein suggest that some previously-reported functions of HRG, such as the binding of heparin and phosphatidic acid are consistent with previous reports (16). However, other previously reported functions, such as mediating phagocytosis of necrotic and apoptotic cells, may be due to contaminating plasma proteins (6, 10, 13, 16, 19-22). *In vitro* studies to fully-elucidate the interactions of HRG will require for contaminant-free purified protein.

HRG Deficiency

Hrg-deficient mice. In the steady state, Hrg-deficient mice have no gross abnormalities and are viable and able to reproduce (23). HRG deficiency is gene-dosage dependent with $HRG^{+/-}$ mice having half the serum concentration of Hrg as $Hrg^{+/+}$ mice (23, 24). Targeted deletion of *Hrg* in mice is associated with increased anti-thrombin activity, increased fibrinogen, and dysregulation of platelet function, leading to shorter bleeding time that is more remarkable in females, indicating that HRG functions both as an anti-coagulant and anti-fibrinolytic *in vivo* (23). Mice deficient in HRG have fewer platelets in the peripheral blood, which is also indicative of increased platelet activation (25).

HRG deficiency in humans. Congenital deficiencies in HRG have been reported in several families (26, 27). The Tokushima allele is a single missense mutation in exon 3 which generates a glycine amino acid instead of glutamic acid in the first cystatin-like domain, leading to a reduction in circulating HRG of 20% that of normal levels and is associated with thrombophilia (26, 28).

Disease states also alter HRG levels. HRG levels have been measured in patients with sepsis, heart disease, liver cirrhosis, and in the acute-phase response (22, 29, 30). During pregnancy, and as a result of low dose oral contraception HRG levels are also lowered (31). In women with pre-eclampsia, reduced levels of HRG and increased levels of fibrinogen have been detected in the placenta suggesting that both factors are involved in the angiogenic alterations observed during pregnancy (32).

Binding Partners of HRG. The HRR within HRG is able to bind several hemostatic factors that include heparan sulfate, heparin, tropomyosin, and heme reviewed in Poon *et al*. (6). HRG also binds divalent metal ions including Cd^{2+}, Ni^{2+}, and Cu^{2+}, and many of its known interactions require or are enhanced by the presence of Zn^{2+}, including binding to heparan sulfate (21, 33). *In vitro* experiments showed that in the presence of Zn^{2+}, HRG prevents binding of a murine T-cell line to lamin, collagen, or fibronectin-coated tissue culture dishes (34). Furthermore, cell surface binding of HRG is mediated through glycosaminoglycans (GAG) as demonstrated using GAG-sufficient and -insufficient Chinese hamster ovary cell lines (21). These results indicate that HRG modulates adhesion of cells both to each other and with components of the extracellular matrix.

HRG may interfere with heparan sulfate degradation by heparanase through the occlusion of cleavage sites. Importantly for tumor control, HRG binding may also prevent heparanase-mediated fibroblast growth factor release from the extracellular matrix (12).

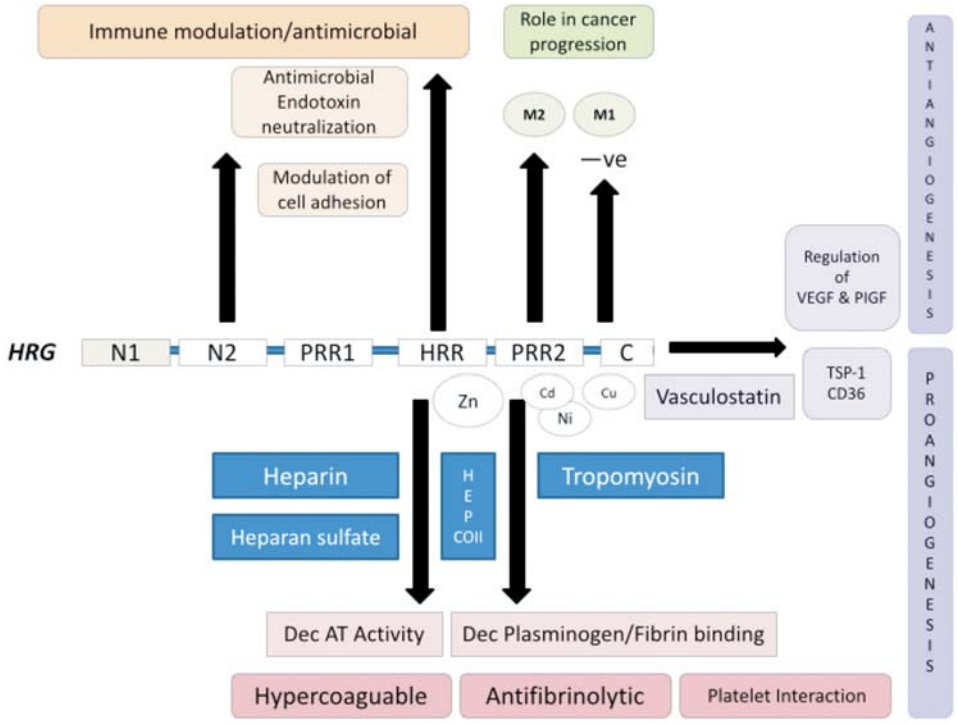

Figure 2. *Multifaceted effects of histidine-rich glycoprotein. HRG modulates hemostasis, immunity, and tumor control through its interactions with multiple binding partners and divalent cations, particularly* Zn^{2+}. *Dependent on context, HRG can also be pro- or anti-angiogenic in the tumor environment. In the tumor microenvironment, HRG promotes tumor-associated macrophages towards an antitumor M1 phenotype.*

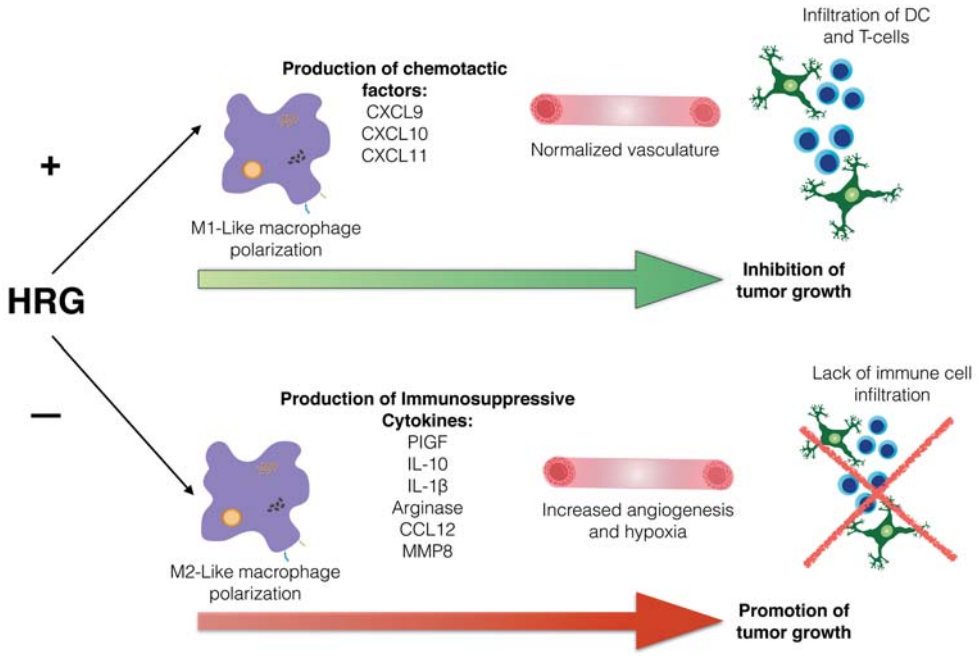

Figure 3. *Histidine-rich glycoprotein has distinct effects on the tumor microenvironment. HRG is associated with increased accumulation of tumor-associated macrophages that have an anti-tumor M1 phenotype and importantly the down-regulation of PIGF. The presence of HRG leads to the down regulation of pro-tumorigenic M2 macrophage associated cytokines. Vasculature is normalized in the presence of HRG, including pericyte coverage. The presence of chemokines CXCL9, CXCL10 and CXCL11 in the tumor environment leads to increased numbers of tumor infiltrating lymphocytes including cytotoxic T-lymphocytes, natural killer, and* $CD11c^{+}$ *dendritic cells.*

Heparin binding to HRG is zinc-dependent and is enhanced at low pH levels, which is of particular importance in wound healing, infection, and the low pH environment of the extracellular space in tumors (35). There is a high degree of sequence homology between HRG and antithrombin at the putative N-terminal heparin binding site, which allows HRG to effectively limit the anti-coagulant activity of antithrombin by binding heparin (22).

HRG is able to bind tropomyosin under very specific conditions, such as platelet activation during coagulation. Zn^{2+} is released by α-granules and the local concentration of zinc increases to sufficient levels to support HRG binding to tropomyosin (12). This also has implications for tumor control as HRG binding of tropomyosin may be anti-angiogenic (12).

Functionally, HRG was initially thought primarily to inhibit fibrinolysis and have pro-thrombotic potential (22, 29, 30, 36). Fibrinogen-plasminogen binding is reduced in the presence of HRG, leading to a reduction in available plasminogen that can be converted to plasmin and degrade fibrin clots (29, 37). This enables HRG to act as an anti-fibrinolytic (29). HRG has also been thought of as an anti-thrombotic, as it can replace fibrin-bound thrombin in fibrin clots, in a Zn^{2+}, dependent manner (18, 38). In the presence of Zn^{2+}, HRG has a high affinity for factor XIIa and its addition increases clotting times, but not FIXa or FXIa suggesting that the interaction is specific to the intrinsic coagulation pathway (39).

Effects of HRG on Angiogenesis: Pro- and Anti-angiogenesis

Angiogenesis is essential for tumor growth and metastasis. During angiogenesis, blood vessels are destabilized when pericytes detach from endothelial cells forming the vessel walls. New vessels are formed by endothelial cell sprouting and pericytes then re-attach to form mature vessels. Proper contact between endothelial cells and pericytes ensures vessel integrity and limits metastasis (40). The angiogenic switch occurs when pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), out-weigh anti-angiogenesis and tumor progression occurs. HRG is reported to have both pro- and antiangiogenic properties, owing to its multi-domain structure and the activities of its proteolytically-released fragments, notably the histidine/proline rich region (5, 15, 17, 41).

Potential pro-angiogenic activity. HRG localizes in the stroma of some tumors upon release from platelets and may block interaction of anti-angiogenic proteins such as thrombospondins (42). In a study utilizing the thrombospondin (TSP-1) receptor CD36 and *Hrg*-deficient animals, it was hypothesized that HRG interacts with TSP-1 and promotes angiogenesis by blocking binding to CD36 *via* thrombospondin type-I repeat domains (TSR) (43). CD36 is expressed on a variety of cell types, including macrophages, monocytes, platelets, and some endothelial cells, and upon binding of TSP-1, counteracts angiogenic signals mediated through fibroblast growth factor receptors. $Hrg^{-/-}$ mice did not control TSR-transfected B16F1 tumors as well as did wild-type control mice (43).

Klenotic *et al.* showed that HRG can also have pro-angiogenic properties in a brain cancer model by acting as a decoy receptor for CD36 (44). HRG was expressed primarily in the basement membrane in 83% of grade IV human glioblastomas (44). In the absence of HRG, the angiostatic protein vasculostatin, produced by glial cells, binds CD36 on endothelial cells resulting in apoptosis. In the setting of vascular inflammation, HRG from plasma or platelets binds vasculostatin preventing caspase- mediated apoptosis and encouraging angiogenesis through endothelial cell proliferation. HRG was found to co-localize with TSP-1 in the tumor matrix of human breast cancer and masked the anti-angiogenic epitope of TSP-1, inhibiting its anti-angiogenic properties (42).

Anti-angiogenic activity. The quality of tumor vasculature is impaired in $Hrg^{-/-}$ mice. In contrast to the reported pro-angiogenic activity of HRG, $Hrg^{-/-}$ mice bearing T241 fibrosarcomas had tumor vessels with increased area as well as decreased coverage and vessel perfusion (45). T241 fibrosarcomas were transduced with *HRG* to force stromal deposition of HRG and evaluate overexpression of HRG on tumor growth. *HRG*-transduced tumors showed improved tumor vasculature including increased vessel perfusion and percentage of pericyte covered vessels (46). A lack of pericyte coverage on tumor vessels has been associated with increased hypoxia, epithelial to mesenchymal transition, and reduced overall survival (47). Increased hypoxic regions and hemorrhage were associated with lack of HRG (46). About 60% of *HRG*-transduced Panc02 tumors had normal vasculature in comparison to nearly all control tumors, which exhibited multiple layers of disconnected endothelial cells and luminal protrusions. Accordingly, the vessel area was increased and vessels had less coverage and perfusion (45). These reports are also consistent with the orthotopic RipTag2, model where HRG deficiency led to decreased pericyte coverage and vessel perfusion (48).

$Hrg^{-/-}$ mice also had increased production of VEGF and placental growth factor (PIGF) (45). HRG inhibited both VEGF and FGF-mediated angiogenesis, and inhibited chemotaxis of endothelial cells *in vitro* (41). The minimal active domain of the HRG (amino acids 330-364) was identified from the histidine/proline rich region and has potent anti-angiogenic activity through inhibition of endothelial cell chemotaxis (15).

Table I. *Histidine rich glycoprotein and angiogenesis*.

Effect	Experimental evidence	Reference
Pro-angiogenic	HRG acts as a decoy receptor for CD36 preventing antiangiogenic activity	
	Hrg-deficient mice injected with TSP-1-secreting Lewis lung carcinoma cells had smaller tumors, however this effect was not observed in TSP-1-negative B16F1 cells	43
	Vessel density of Lewis lung carcinomas was decreased in *Hrg*-deficient mice	43
	Tumor growth was suppressed in *Hrg*-deficient mice when B16F1 was transfected with TSR	43
	Vasculostatin binding to CD36 endothelial cells was prevented by HRG	44
Anti-angiogenic	HRG inhibits angiogenesis	
	Reduced expression of antiangiogenic chemokines CXCL10 and CXCL11 in *Hrg*-deficient mice	45
	Increased vessel area with decreased vessel coverage and perfusion in *HRG*-deficient mice	45, 46
	Increased tumor hypoxia in T241 tumors in Hrg-deficient mice	46
	Inhibition of tumor vascularization in HRG-treated mice bearing fibrosarcomas	41
	Decreased angiogenesis in mice treated with a minimal peptide from the His-Pro rich domain of HRG	41
	Increased numbers of angiogenic islets in Rip1-Tag2/$Hrg^{-/-}$ mice	24, 25

CD36: Thrombospondin receptor; TSP-1: thrombospondin-1; TSR: thrombospondin type 1 repeat; CXCL10: chemokine (C-X-C motif) ligand 10, interferon gamma induced protein; CXCL11 chemokine (C-X-C motif) ligand 11, interferon-inducible T-cell alpha chemoattractant; Rip1-Tag2: Rat insulin promoter 1- SV40 large T antigen.

Table II. *Histidine rich glycoprotein and platelet function*.

Effect	Experimental evidence	Reference
Platelet function	HRG regulates platelet function	
	Activated platelets promoted the binding of the His/Pro-rich domain of HRG to endothelial cells in the presence of Zn^{2+}	24
	Increased platelet aggregation in tumors of $Hrg^{-/-}$ mice	25, 48
	HRG regulates platelet activity, reducing EMT expression and metastasis	48
	Platelet removal decreases the number of angiogenic islets, restores E-cadherin expression and up regulation of Akt2 that is present in $Hrg^{-/-}$ mice	26

EMT: Epithelial to mesenchymal transition; Akt2: v-akt murine thymoma viral oncogene homolog 2.

Sub-cutaneous administration of HRG to mice with established T241 fibrosarcomas resulted in reduced vascularization and improved tumor control (41). Additionally, the presence of angiogenic islets is increased in insulinomas in $Hrg^{-/-}$ mice with increased tumor proliferation and a corresponding two-fold increase in tumor volume (48). The development of gliomas was delayed and reduced in severity in HRG-treated mice, which coincided with improved tumor vasculature (49). These data suggest that HRG partially mediates tumor control through normalization of blood vessels.

The different results between the studies that deemed HRG to be pro-angiogenic and those that found it to have antiangiogenic properties may be explained by the absence of CD36 on larger vessels, or in that the HRR domain of HRG may have qualities that are independent of the parent protein. Whether or not HRG is pro-angiogenic or anti-angiogenic may be highly contextual depending on the components of the tumor environment, or dependent on proteolytic cleavage *in vivo* (Table II) (41, 50).

Effects Due to Interactions with Platelets and Other Coagulation Factors

The expression of tissue factor by tumor cells promotes platelet activation and coagulation. Tumor-derived tissue factor also supports the metastatic potential of tumors (2, 51). Platelet activation and coagulation play an important role in tumor metastasis, possibly by shielding circulating tumor cells from immune surveillance and reducing endothelial barrier function (52). Increased platelet counts are associated with tumor metastasis and poor prognosis. Platelet-stored HRG is released upon activation (24, 36) increasing local concentrations at selected sites, such as sites of infection or tumors.

The histidine/proline-rich fragment of HRG was detected in human kidney cancer sections and co-localized with platelets, identified by CD41 and CD42b (24). This was associated with anti-angiogenic properties, indicating that platelets could be a major source of HRG for tumors (24). In the absence of HRG, platelet activation becomes enhanced and the angiogenic switch was accelerated in an orthotopic pancreatic cancer mouse model described by Ringvall *et al*. (25).

Cedervall and colleagues recently investigated the role of platelet-released HRG in epithelial-to-mesenchymal transition in a mouse model of pancreatic cancer (48). They demonstrated that HRG is a potent tumor suppressor by regulating platelet signaling. In $Hrg^{-/-}$ mice, platelet depletion, early during tumor development suppressed the enhanced angiogenic switch associated with *Hrg*-deficiency (48). When platelets were depleted in more advanced tumors there was also improved tumor control in $Hrg^{-/-}$ mice (48). Taken together, these data suggest that HRG counteracts proangiogenic factors associated with platelet activity (Table II). Platelet depletion also restored levels of tissue factor regulated E-cadherin (48). Loss of the cell-cell adhesion molecule E-cadherin is associated with the promotion of metastasis, and its restoration offers an explanation for the decreased numbers of metastases in platelet depleted $Hrg^{-/-}$ mice (51).

Impact on Other Hemostatic Factors

Fibrinolysis, coagulation, and angiogenesis are modified by the interaction of HRG with multiple targets including thrombospondins, heparin, and complement. Either directly or indirectly, the regulation of these processes can all have an effect on tumor control (5, 6, 17, 45, 46, 53).

HRG is able to limit the anti-coagulant activity of anti-thrombin by binding to heparin at the same sites as anti-thrombin (54). In circulation, HRG is bound to plasminogen, reducing plasminogen available to bind fibrin (53). HRG further functions as an anti-coagulant by incorporating into fibrin clots, mediated by the HRR, which is consistent with the shorter bleeding times observed in *Hrg*-deficient mice (18, 23).

HRG and the Modulation of Antitumor Immune Responses

Modulation of tumor associated macrophages (TAMs). Myeloid- derived TAMs are a key component of tumor stroma and depending on the tumor microenvironment may possess anti-tumor (M1) or pro-tumor (M2) characteristics (55-57). TAMs are involved in regulating the angiogenic switch (58) and their presence in tumors are associated with poor outcomes (59-62). The classically-activated M1 TAMs are stimulated by lipopolysaccharide and interferon gamma (IFN-γ) to produce chemokines such as chemokine (C-X-C motif) ligand 9, chemokine (C-X-C motif) ligand 10, and high levels of interleukins IL-12 and IL-23. These cytokines then promote Th1 T-cell mediated anti- tumor responses *via* STAT4 signaling in naïve and memory $CD4^+$ T-cells (63). In contrast, M2 TAMs, which can express programmed death ligand 1 (PD-L1) and produce chemokine (C-C motif) ligand 17, chemokine (C-C motif) ligand 22, and high levels of IL-10 and IL-1RA, are implicated in tumor angiogenesis, metastasis and immune suppression through promotion of Th2 T-cell responses and inhibition of Th1 $CD4^+$ T-cells (55, 56). As macrophage polarization is plastic and can shift based on environmental cues in different tissues (64, 65), there has been considerable interest in therapeutic interventions that promote skewing towards an M1 phenotype (57, 63, 64, 66-68). A recent study in a mouse model of established gliomas indicated that inhibition of colony- stimulating factor 1 receptor resulted in re-polarizing TAMs from an M1 to a M2 phenotype, and in improved tumor control and increased survival (69).

In *Hrg*-transduced tumors, TAMs were skewed towards an M1 phenotype, including lower levels of IL-10 production, which permitted increased accumulation of DC, natural killer (NK), and $CD8^+$ T-cells (46). Highlighting the importance of HRG on TAM skewing, clodronate depletion of macrophages resulted in equivalent tumor control in *HRG*-transduced tumors and control tumors (46).

The ability of HRG to modulate TAMs may be due to the regulation of the VEGF family member PlGF produced by TAMs. HRG specifically down regulates PIGF in TAMs, leading to reduced production of IL-10 and the observed skewing towards an M1 phenotype (46). *In vivo* studies with *PIGF*-deficient animals indicated that the improved tumor control observed in *Hrg*-transduced tumors was a direct cause of PIGF down-regulation, including tumor vessel normalization, as discussed previously (46). Using pimonidazole to detect hypoxic regions, Rolny *et al*. were able to detect a significant reduction in hypoxia in *Hrg*-transduced tumors (46). As macrophages are more likely to skew towards an M2 phenotype under hypoxic conditions, HRG may exert its effects partially through affecting changes in oxygenation of the tumor environment. Expression of genes associated with hypoxia, including *Arg1, Mmp8,* and *Ccl12* were increased in TAMs from $Hrg^{-/-}$ mice (45).

These results suggest an important role for HRG in programming TAMs (Table III). The signature of HRG-influenced TAMs appears to be specific for inhibition of tumor vessel growth and metastasis, with reductions in pro-angiogenic cytokines such as IL-1β and of TNF-α (46). Targeting of TAM polarization to improve tumor control through modulation of the tumor microenvironment is of great interest (63, 68, 70). Tumors that are particularly affected by TAMs, such as pancreatic cancer, may be especially sensitive to TAM-targeted therapies. HRG may prove to be a valuable tool for modulating TAMs towards an antitumor phenotype (67, 71).

Table III. *Histidine rich glycoprotein and anticancer immune reaction.*

Effect	Experimental Evidence	Reference
TAM skewing	*Hrg*-transduced tumors have increased TAMs, but decreased M2 accumulation	
	Decreased *Arg1*, *IL-10*, and *IL-1β* transcripts from TAM in Hrg-transduced tumors	46
	Decreased PIGF in TAM from HRG transduced tumors	45
	$Hrg^{-/-}$ mice have increased M2 macrophages	
	Decreased transcripts of Cxcl10, Cxcl11, Cd80 in $Hrg^{-/-}$ mice	45
Lymphocytes	HRG promotes infiltration of lymphocytes into the tumor	
	Decreased infiltration of $CD8^+$ T cells in T241 tumors in Hrg-deficient mice	45
	Increased infiltration of $NK1.1^+$ cells into T241 tumors transduced with HRG	46

TAMs: Tumor-associated macrophages; Arg1: arginase 1; IL-10: interleukin-10; IL-1β: interleukin-1 beta, catabolin; PIGF: placenta growth factor; Cd80: cluster of differentiation 80, B7-1; CD8: cluster of differentiation 8; NK1.1: natural killer cell-associated marker 1.1.

Tumor infiltrating lymphocytes affected by HRG. Analysis of tumor immune infiltrates indicated that HRG deficiency resulted in less infiltration of cells able to mount an anti-tumor response, and reduced levels of angiostatic and T-cell attractant factors, explaining the reduced number of tumor infiltrating $CD8^+$ T cells (45). Expression of genes associated with DC maturation and T-cell priming are specifically up-regulated in TAMs upon HRG stimulation (45). Correspondingly, *HRG*-transduced tumors showed increased infiltration of antitumor immune cells (46). Increased accumulation of activated DC were observed in *HRG*-transduced tumors, which corresponded with increased accumulation of $CD8^+$ T-cells (46). NK cells were also increased in *HRG*-transduced tumors (46). Figure 2 illustrates the multi-faceted effects of HRG.

Does HRG Have Prognostic Value for Patients with Cancer?

HRG is easily detectable in the steady-state at high concentrations in plasma and lowered levels are detected in certain disease conditions, including some types of cancers in comparison to healthy tissue (6, 46). Proteomics approaches have been employed to determine if HRG could be useful as a prognostic tool in several types of cancers. In a study to identify potential glycoprotein biomarkers in ovarian cancer, lower levels of HRG compared to healthy donor controls were associated with stage I/II ovarian cancer (72). Lectin-specific glycosylated proteins in the serum of patients with ovarian cancer and normal donor controls were isolated using a lectin array. The proteins were further identified by liquid chromatrography and tandem mass spectrometry (LC-MS/MS) and confirmed by enzyme-linked immunosorbent assay (ELISA). Fucosylated HRG levels were significantly higher in patients with stage III ovarian cancer compared to normal and benign donors, but was not significantly higher in patients with stage I/II disease (72). Overall, levels of HRG were lower in patients with ovarian cancer. The combination of HRG and corticosteroid-binding globulin, also identified by lectin array, differentiated between normal donors and patients with early-stage ovarian cancer. These glycosylated proteins could potentially be used in conjunction with CA125 screening to detect ovarian cancer.

A Phase III trial (NCT01664169) that compared patients with pancreatic cancer receiving gemcitabine with or without bevacizumab, anti-VEGF monoclonal antibody, identified HRG as a weakly-positive prognostic biomarker (73). LC-MS/MS on patient serum samples initially identified HRG as a predictive protein along with complement factor H. Further screening by ELISA on these samples suggested that HRG was weakly-associated with improved overall survival, but not affected by bevacizumab treatment (73). This effect may be due to the anti-angiogenic properties of HRG.

A proteomics study using isobaric tagging and LC-MS/MS analysis of 20 patients with endometrial disease found that those with atypical hyperplasia had significantly lower levels of HRG in comparison to normal donor controls, potentially indicating increased likelihood of progression (74). In a matrix-assisted laser desorption/ionization time-of-flight spectrometry analysis of preoperative biopsies of breast cancer, HRG was elevated in samples from obese patients, potentially identifying an inflammatory response (75). Given the difficulty of early identification of both ovarian and pancreatic cancer, future studies of HRG as a biomarker are warranted.

HRG as Adjuvant Therapy

In pre-clinical settings, administration of HRG synthetic peptides have demonstrated anti-angiogenic activity (41). This suggests that HRG supplementation is a potential therapeutic and has the added benefit of positively modulating the immune system in the tumor microenvironment (15, 25, 41, 45, 76).

Table IV. *In vivo studies demonstrating the role of histidine rich glycoprotein in cancer control.*

Effect	Experimental evidence	Reference
Tumor growth and metastasis	HRG deficiency increases tumor growth	
	Tumor growth decreased in tumors transduced with *HRG*	46
	HRG controls tumor metastasis in mouse models of insulinoma, ductal adenocarcinoma and fibrosarcoma	45, 46, 48
	HRG mediates decreased proliferation and increased apoptosis in tumors	25, 45, 46
	HRG prevented development of malignant glioma	49
	HRG-transduced tumors are more sensitive to chemotherapy	46
	E-cadherin expression is reduced in tumors of $Hrg^{-/-}$ mice	48

Tumors transfected with HRG had improved responses to sub-therapeutic doses of chemotherapy, likely through the normalization of tumor blood vessels. Adjuvant HRG could improve responses to chemotherapy or make it possible to titrate chemotherapy doses in order to limit toxicities (46). The normalization of tumor blood vessels also makes HRG supplementation attractive as part of an adoptive cell transfer strategies and antibody therapies. Normalized tumor vasculature makes it possible for immune cells to infiltrate the tumor and exert and an antitumor effect, as well as allowing efficient delivery of therapeutic agents to the tumor site (77). In *HRG*-transduced tumors, numbers of activated DC, NK, and CTL were increased, suggesting that HRG therapy may help facilitate trafficking of both endogenous and adoptively transferred cells.

As HRG modulates both angiogenesis and antitumor immunity (Figure 3), further study targeting HRG, or downstream targets, such as PIGF, is warranted. Indeed, $Pigf^{-/-}$ mice are not affected by HRG supplementation, indicating an important link between HRG and PIGF (46).

Concluding Remarks

Modulation of the tumor microenvironment as a part of cancer treatment has great potential and there are a multitude of targets that can affect tumor immunity, angiogenesis, and coagulation. Studies have demonstrated roles for HRG in hemostasis, angiogenesis, and immunity, which in turn can greatly affect tumor control and metastasis (Table IV). *In vivo*, HRG has significant effects on tumor growth and metastasis through modulation of TAMs towards an M1 phenotype, activation of platelets, and normalization of angiogenesis. Few studies have tested for HRG as a prognostic factor, and the results have not been definitive. However, tumor biopsies have shown that HRG deposits are present in the tumor stroma, indicating that the effects are likely dependent on their concentration in the tumor and type of tumor (24).

The importance of HRG in pre-clinical tumor models, through the use of genetically deficient mice, has been clearly demonstrated. However, the significance of HRG has yet to be validated in clinical settings. The role of HRG may be highly dependent on both the tumor type and factors, such as pH, within the tumor. This may explain why some groups have observed that *HRG*-deficiency promotes tumor growth (25, 45, 46) and others have seen the opposite effect (42, 43). Proteolytic cleavage of HRG may alter activity dependent on the tumor microenvironment (41-43, 50). Based on the elegant studies by Rolny *et al.* and Tugues *et al.*, a key element of HRG in tumor control is its ability to modulate the tumor microenvironment through polarization of TAMs (45, 46). Optimal strategies to utilize a protein that can both create an immunostimulatory environment through polarization of macrophages towards an antitumor phenotype as well as modulate blood vessel normalization, should be explored.

References

1 Borsig L: Antimetastatic activities of heparins and modified heparins. Experimental evidence. Thrombosis research *125(Suppl 2)*: S66-71, 2010.

2 Degen JL and Palumbo JS: Hemostatic factors, innate immunity and malignancy. Thrombosis research *129(Suppl 1)*: S1-5, 2012.

3 Palumbo JS, Talmage KE, Massari JV, La Jeunesse CM, Flick MJ, Kombrinck KW, Jirouskova M and Degen JL: Platelets and fibrin(ogen) increase metastatic potential by impeding natural killer cell-mediated elimination of tumor cells. Blood *105*: 178-185, 2005.

4 van Sluis GL, Bruggemann LW, Esmon CT, Kamphuisen PW, Richel DJ, Buller HR, van Noorden CJ and Spek CA: Endogenous activated protein C is essential for immune-mediated cancer cell elimination from the circulation. Cancer letters *306*: 106-110, 2011.

5 Lee C, Bongcam-Rudloff E, Sollner C, Jahnen-Dechent W and Claesson-Welsh L: Type 3 cystatins; fetuins, kininogen and histidine-rich glycoprotein. Frontiers in bioscience *14*: 2911-2922, 2009.

6 Poon IK, Patel KK, Davis DS, Parish CR and Hulett MD: Histidine-rich glycoprotein: the Swiss Army knife of mammalian plasma. Blood *117*: 2093-2101, 2011.

7 Engesser L, Kluft C, Briet E and Brommer EJ: Familial elevation of plasma histidine-rich glycoprotein in a family with thrombophilia. British J Haematology *67*: 355-358, 1987.

8 Shannon O, Rydengard V, Schmidtchen A, Morgelin M, Alm P, Sorensen OE and Bjorck L: Histidine-rich glycoprotein promotes bacterial entrapment in clots and decreases mortality in a mouse model of sepsis. Blood *116*: 2365-2372, 2010.

9 Rydengard V, Shannon O, Lundqvist K, Kacprzyk L, Chalupka A, Olsson AK, Morgelin M, Jahnen-Dechent W, Malmsten M and Schmidtchen A: Histidine-rich glycoprotein protects from systemic Candida infection. PLoS pathogens *4*: e1000116, 2008.

10 Koide T, Foster D, Yoshitake S and Davie EW: Amino acid sequence of human histidine-rich glycoprotein derived from the nucleotide sequence of its cDNA. Biochemistry *25*: 2220-2225, 1986.

11 Hennis BC, van Boheemen PA, Wakabayashi S, Koide T, Hoffmann JJ, Kievit P, Dooijewaard G, Jansen JG and Kluft C: Identification and genetic analysis of a common molecular variant of histidine-rich glycoprotein with a difference of 2kD in apparent molecular weight. Thrombosis and haemostasis *74*: 1491-1496, 1995.

12 Jones AL, Hulett MD and Parish CR: Histidine-rich glycoprotein: A novel adaptor protein in plasma that modulates the immune, vascular and coagulation systems. Immunology and cell biology *83*: 106-118, 2005.

13 Poon IK, Hulett MD, and Parish CR: Histidine-rich glycoprotein is a novel plasma pattern recognition molecule that recruits IgG to facilitate necrotic cell clearance *via* FcgammaRI on phagocytes. Blood *115*: 2473-2482, 2010.

14 Wakabayashi S: New insights into the functions of histidine-rich glycoprotein. International review of cell and molecular biology *304*: 467-493, 2013.

15 Dixelius J, Olsson AK, Thulin A, Lee C, Johansson I and Claesson-Welsh L: Minimal active domain and mechanism of action of the angiogenesis inhibitor histidine-rich glycoprotein. Cancer research *66*: 2089-2097, 2006.

16 Patel KK, Poon IK, Talbo GH, Perugini MA, Taylor NL, Ralph TJ, Hoogenraad NJ and Hulett MD: New method for purifying histidine-rich glycoprotein from human plasma redefines its functional properties. IUBMB life *65*: 550-563, 2013.

17 Juarez JC, Guan X, Shipulina NV, Plunkett ML, Parry GC, Shaw DE, Zhang JC, Rabbani SA, McCrae KR, Mazar AP, Morgan WT and Donate F: Histidine-proline-rich glycoprotein has potent antiangiogenic activity mediated through the histidine-proline-rich domain. Cancer research *62*: 5344-5350, 2002.

18 Leung LL: Interaction of histidine-rich glycoprotein with fibrinogen and fibrin. The Journal of clinical investigation *77*: 1305-1311, 1986.

19 Gorgani NN, Smith BA, Kono DH and Theofilopoulos AN: Histidine-rich glycoprotein binds to DNA and Fc gamma RI and potentiates the ingestion of apoptotic cells by macrophages. Journal of immunology *169*: 4745-4751, 2002.

20 Hulett MD and Parish CR: Murine histidine-rich glycoprotein: cloning, characterization and cellular origin. Immunology and cell biology *78*: 280-287, 2000.

21 Jones AL, Hulett MD and Parish CR: Histidine-rich glycoprotein binds to cell-surface heparan sulfate *via* its N-terminal domain following Zn^{2+} chelation. The J Biological Chemistry *279*: 30114-30122, 2004.

22 Wakabayashi S and Koide T: Histidine-rich glycoprotein: a possible modulator of coagulation and fibrinolysis. Seminars in thrombosis and hemostasis *37*: 389-394, 2011.

23 Tsuchida-Straeten N, Ensslen S, Schafer C, Woltje M, Denecke B, Moser M, Graber S, Wakabayashi S, Koide T and Jahnen-Dechent W: Enhanced blood coagulation and fibrinolysis in mice lacking histidine-rich glycoprotein (HRG). J Thrombosis Haemostasis: JTH *3*: 865-872, 2005.

24 Thulin A, Ringvall M, Dimberg A, Karehed K, Vaisanen T, Vaisanen MR, Hamad O, Wang J, Bjerkvig R, Nilsson B, Pihlajaniemi T, Akerud H, Pietras K, Jahnen-Dechent W, Siegbahn A and Olsson AK: Activated platelets provide a functional microenvironment for the antiangiogenic fragment of histidine-rich glycoprotein. Molecular Cancer Res: MCR *7*: 1792-1802, 2009.

25 Ringvall M, Thulin A, Zhang L, Cedervall J, Tsuchida-Straeten N, Jahnen-Dechent W, Siegbahn A and Olsson AK: Enhanced platelet activation mediates the accelerated angiogenic switch in mice lacking histidine-rich glycoprotein. PloS one *6*: e14526, 2011.

26 Shigekiyo T, Yoshida H, Kanagawa Y, Satoh K, Wakabayashi S, Matsumoto T and Koide T: Histidine-rich glycoprotein (HRG) Tokushima 2: novel HRG deficiency, molecular and cellular characterization. Thrombosis Haemostasis *84*: 675-679, 2000.

27 Souto JC, Gari M, Falkon L and Fontcuberta J: A new case of hereditary histidine-rich glycoprotein deficiency with familial thrombophilia. Thrombosis and haemostasis *75*: 374-375, 1996.

28 Shigekiyo T, Yoshida H, Matsumoto K, Azuma H, Wakabayashi S, Saito S, Fujikawa K and Koide T: HRG Tokushima: molecular and cellular characterization of histidine-rich glycoprotein (HRG) deficiency. Blood *91*: 128-133, 1998.

29 Lijnen HR, Jacobs G and Collen D: Histidine-rich glycoprotein in a normal and a clinical population. Thrombosis Res *22*: 519-523, 1981.

30 Saito H, Goodnough LT, Boyle JM and Heimburger N: Reduced histidine-rich glycoprotein levels in plasma of patients with advanced liver cirrhosis. Possible implications for enhanced fibrinolysis. The American J Med *73*: 179-182, 1982.

31 Haukkamaa M, Morgan WT and Koskelo P: Serum histidine-rich glycoprotein during pregnancy and hormone treatment. Scandinavian journal of clinical and laboratory investigation *43*: 591-595, 1983.

32 Karehed K, Wikstrom AK, Olsson AK, Larsson A, Olovsson M and Akerud H: Fibrinogen and histidine-rich glycoprotein in early-onset preeclampsia. Acta obstetricia et gynecologica Scandinavica *89*: 131-139, 2010.

33 Wang F, Chmil C, Pierce F, Ganapathy K, Gump BB, MacKenzie JA, Metchref Y and Bendinskas K: Immobilized metal affinity chromatography and human serum proteomics. Journal of chromatography B, Analytical technologies in the biomedical and life sciences *934*: 26-33, 2013.

34 Olsen HM, Parish CR and Altin JG: Histidine-rich glycoprotein binding to T-cell lines and its effect on T-cell substratum adhesion is strongly potentiated by zinc. Immunology *88*: 198-206, 1996.

35 Fukamachi T, Chiba Y, Wang X, Saito H, Tagawa M and Kobayashi H: Tumor specific low pH environments enhance the cytotoxicity of lovastatin and cantharidin. Cancer letters *297*: 182-189, 2010.

36 Leung LL, Harpel PC, Nachman RL and Rabellino EM: Histidine-rich glycoprotein is present in human platelets and is released following thrombin stimulation. Blood *62*: 1016-1021, 1983.

37 Borza DB and Morgan WT: Histidine-proline-rich glycoprotein as a plasma pH sensor. Modulation of its interaction with glycosaminoglycans by ph and metals. The Journal of biological chemistry *273*: 5493-5499, 1998.

38 Vu TT, Stafford AR, Leslie BA, Kim PY, Fredenburgh JC and Weitz JI: Histidine-rich glycoprotein binds fibrin(ogen) with high affinity and competes with thrombin for binding to the gamma'-chain. The Journal of biological chemistry *286*: 30314-30323, 2011.

39 MacQuarrie JL, Stafford AR, Yau JW, Leslie BA, Vu TT, Fredenburgh JC and Weitz JI: Histidine-rich glycoprotein binds factor XIIa with high affinity and inhibits contact-initiated coagulation. Blood *117*: 4134-4141, 2011.

40 Xian X, Hakansson J, Stahlberg A, Lindblom P, Betsholtz C, Gerhardt H and Semb H: Pericytes limit tumor cell metastasis. The J Clinical Investigation *116*: 642-651, 2006.

41 Olsson AK, Larsson H, Dixelius J, Johansson I, Lee C, Oellig C, Bjork I and Claesson-Welsh L: A fragment of histidine-rich glycoprotein is a potent inhibitor of tumor vascularization. Cancer Res *64*: 599-605, 2004.

42 Simantov R, Febbraio M, Crombie R, Asch AS, Nachman RL and Silverstein RL: Histidine-rich glycoprotein inhibits the antiangiogenic effect of thrombospondin-1. The J Clinical Investigation *107*: 45-52, 2001.

43 Hale JS, Li M, Sinyuk M, Jahnen-Dechent W, Lathia JD and Silverstein RL: Context dependent role of the CD36--thrombospondin – histidine-rich glycoprotein axis in tumor angiogenesis and growth. PloS one *7*: e40033, 2012.

44 Klenotic PA, Huang P, Palomo J, Kaur B, Van Meir EG, Vogelbaum MA, Febbraio M, Gladson CL and Silverstein RL: Histidine-rich glycoprotein modulates the anti-angiogenic effects of vasculostatin. The American journal of pathology *176*: 2039-2050, 2010.

45 Tugues S, Honjo S, Konig C, Noguer O, Hedlund M, Botling J, Deschoemaeker S, Wenes M, Rolny C, Jahnen-Dechent W, Mazzone M and Claesson-Welsh L: Genetic deficiency in plasma protein HRG enhances tumor growth and metastasis by exacerbating immune escape and vessel abnormalization. Cancer research *72*: 1953-1963, 2012.

46 Rolny C, Mazzone M, Tugues S, Laoui D, Johansson I, Coulon C, Squadrito ML, Segura I, Li X, Knevels E, Costa S, Vinckier S, Dresselaer T, Akerud P, De Mol M, Salomaki H, Phillipson M, Wyns S, Larsson E, Buysschaert I, Botling J, Himmelreich U, Van Ginderachter JA, De Palma M, Dewerchin M, Claesson-Welsh L and Carmeliet P: HRG inhibits tumor growth and metastasis by inducing macrophage polarization and vessel normalization through down-regulation of PlGF. Cancer Cell *19*: 31-44, 2011.

47 Cooke VG, LeBleu VS, Keskin D, Khan Z, O'Connell JT, Teng Y, Duncan MB, Xie L, Maeda G, Vong S, Sugimoto H, Rocha RM, Damascena A, Brentani RR and Kalluri R: Pericyte depletion results in hypoxia-associated epithelial-to-mesenchymal transition and metastasis mediated by met signaling pathway. Cancer Cell *21*: 66-81, 2012.

48 Cedervall J, Zhang Y, Ringvall M, Thulin A, Moustakas A, Jahnen-Dechent W, Siegbahn A and Olsson AK: HRG regulates tumor progression, epithelial to mesenchymal transition and metastasis *via* platelet-induced signaling in the pre-tumorigenic microenvironment. Angiogenesis *16*: 889-902, 2013.

49 Karrlander M, Lindberg N, Olofsson T, Kastemar M, Olsson AK and Uhrbom L: Histidine-rich glycoprotein can prevent development of mouse experimental glioblastoma. PloS one *4*: e8536, 2009.

50 Poon IK, Olsson AK, Hulett MD and Parish CR: Regulation of histidine-rich glycoprotein (HRG) function *via* plasmin-mediated proteolytic cleavage. The Biochemical Journal *424*: 27-37, 2009.

51 Onder TT, Gupta PB, Mani SA, Yang J, Lander ES, and Weinberg RA: Loss of E-cadherin promotes metastasis *via* multiple downstream transcriptional pathways. Cancer research *68*: 3645-3654, 2008.

52 Schumacher D, Strilic B, Sivaraj KK, Wettschureck N and Offermanns S: Platelet-derived nucleotides promote tumor-cell transendothelial migration and metastasis *via* P2Y2 receptor. Cancer cell *24*: 130-137, 2013.

53 Blank M and Shoenfeld Y: Histidine-rich glycoprotein modulation of immune/autoimmune, vascular, and coagulation systems. Clinical reviews in allergy & immunology *34*: 307-312, 2008.

54 Koide T, Odani S and Ono T: The N-terminal sequence of human plasma histidine-rich glycoprotein homologous to antithrombin with high affinity for heparin. FEBS letters *141*: 222-224, 1982.

55 Bonde AK, Tischler V, Kumar S, Soltermann A and Schwendener RA: Intratumoral macrophages contribute to epithelial-mesenchymal transition in solid tumors. BMC Cancer *12*: 35, 2012.

56 Qian BZ and Pollard JW: Macrophage diversity enhances tumor progression and metastasis. Cell *141*: 39-51, 2010.

57 Ruffell B, Affara NI and Coussens LM: Differential macrophage programming in the tumor microenvironment. Trends in immunology *33*: 119-126, 2012.

58 Lin EY, Li JF, Gnatovskiy L, Deng Y, Zhu L, Grzesik DA, Qian H, Xue XN and Pollard JW: Macrophages regulate the angiogenic switch in a mouse model of breast cancer. Cancer research *66*: 11238-11246, 2006.

59 Allavena P and Mantovani A: Immunology in the clinic review series; focus on cancer: tumour-associated macrophages: undisputed stars of the inflammatory tumour microenvironment. Clinical and experimental immunology *167*: 195-205, 2012.

60 Ino Y, Yamazaki-Itoh R, Shimada K, Iwasaki M, Kosuge T, Kanai Y and Hiraoka N: Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. British journal of cancer *108*: 914-923, 2013.

61 Nonomura N, Takayama H, Nakayama M, Nakai Y, Kawashima A, Mukai M, Nagahara A, Aozasa K and Tsujimura A: Infiltration of tumour-associated macrophages in prostate biopsy specimens is predictive of disease progression after hormonal therapy for prostate cancer. BJU international *107*: 1918-1922, 2011.

62 Wynn TA, Chawla A and Pollard JW: Macrophage biology in development, homeostasis and disease. Nature *496*: 445-455, 2013.

63 Ong SM, Tan YC, Beretta O, Jiang D, Yeap WH, Tai JJ, Wong WC, Yang H, Schwarz H, Lim KH, Koh PK, Ling KL and Wong SC: Macrophages in human colorectal cancer are pro-inflammatory and prime T cells towards an anti-tumour type-1 inflammatory response. European J Immunology *42*: 89-100, 2012.

64 Heusinkveld M, de Vos van Steenwijk PJ, Goedemans R, Ramwadhdoebe TH, Gorter A, Welters MJ, van Hall T and van der Burg SH: M2 macrophages induced by prostaglandin E2 and IL-6 from cervical carcinoma are switched to activated M1 macrophages by $CD4^+$ Th1 cells. Journal of immunology *187*: 1157-1165, 2011.

65 Saccani A, Schioppa T, Porta C, Biswas SK, Nebuloni M, Vago L, Bottazzi B, Colombo MP, Mantovani A and Sica A: p50 nuclear factor-kappaB overexpression in tumor-associated macrophages inhibits M1 inflammatory responses and antitumor resistance. Cancer research *66*: 11432-11440, 2006.
66 Biswas SK and Mantovani A: Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nature Immunology *11*: 889-896, 2010.
67 Mitchem JB, Brennan DJ, Knolhoff BL, Belt BA, Zhu Y, Sanford DE, Belaygorod L, Carpenter D, Collins L, Piwnica-Worms D, Hewitt S, Udupi GM, Gallagher WM, Wegner C, West BL, Wang-Gillam A, Goedegebuure P, Linehan DC and DeNardo DG: Targeting tumor-infiltrating macrophages decreases tumor-initiating cells, relieves immunosuppression, and improves chemotherapeutic responses. Cancer Res *73*: 1128-1141, 2013.
68 Shiao SL, Ganesan AP, Rugo HS, and Coussens LM: Immune microenvironments in solid tumors: new targets for therapy. Genes & development 25: 2559-2572, 2011.
69 Pyonteck SM, Akkari L, Schuhmacher AJ, Bowman RL, Sevenich L, Quail DF, Olson OC, Quick ML, Huse JT, Teijeiro V, Setty M, Leslie CS, Oei Y, Pedraza A, Zhang J, Brennan CW, Sutton JC, Holland EC, Daniel D and Joyce JA: CSF-1R inhibition alters macrophage polarization and blocks glioma progression. Nature medicine *19*: 1264-1272, 2013.
70 Heusinkveld M and van der Burg SH: Identification and manipulation of tumor associated macrophages in human cancers. Journal of translational medicine *9*: 216, 2011.
71 Shojaei F: Anti-angiogenesis therapy in cancer: current challenges and future perspectives. Cancer letters *320*: 130-137, 2012.
72 Wu J, Xie X, Liu Y, He J, Benitez R, Buckanovich RJ and Lubman DM: Identification and confirmation of differentially expressed fucosylated glycoproteins in the serum of ovarian cancer patients using a lectin array and LC-MS/MS. J Proteome Res *11*: 4541-4552, 2012.
73 Roberts AS, Campa MJ, Gottlin EB, Jiang C, Owzar K, Kindler HL, Venook AP, Goldberg RM, O'Reilly EM and Patz EF Jr.: Identification of potential prognostic biomarkers in patients with untreated, advanced pancreatic cancer from a phase 3 trial (Cancer and Leukemia Group B 80303). Cancer *118*: 571-578, 2012.
74 Wang YS, Cao R, Jin H, Huang YP, Zhang XY, Cong Q, He YF and Xu CJ: Altered protein expression in serum from endometrial hyperplasia and carcinoma patients. J Hematology & Oncology *4*: 15, 2011.
75 Schaub NP, Jones KJ, Nyalwidhe JO, Cazares LH, Karbassi ID, Semmes OJ, Feliberti EC, Perry RR and Drake RR: Serum proteomic biomarker discovery reflective of stage and obesity in breast cancer patients. Journal of the American College of Surgeons *208*: 970-978; discussion 978-980, 2009.
76 Gabrilovich DI, Ostrand-Rosenberg S and Bronte V: Coordinated regulation of myeloid cells by tumours. Nature reviews Immunology *12*: 253-268, 2012.
77 Huang Y, Goel S, Duda DG, Fukumura D and Jain RK: Vascular normalization as an emerging strategy to enhance cancer immunotherapy. Cancer research *73*: 2943-2948, 2013.

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