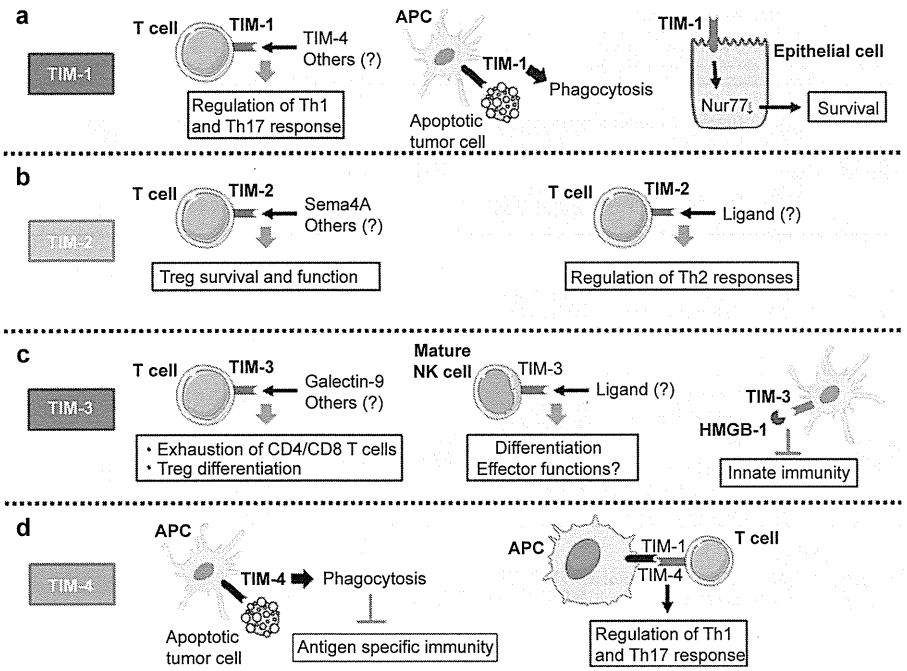

Figure 2 The role of the TIM gene family in antitumor immune responses. TIM molecules regulate multiple immune pathways. (**a**) TIM-1 expression on T cells regulates the differentiation of T helper subsets,[32,33] whereas TIM-1 expressed on APCs serves as a phagocytosis receptor that facilitates the removal of apoptotic cells.[34] TIM-1 is also expressed on kidney epithelial cells and promotes cell survival through the degradation of nuclear factor NUR77.[35] (**b**) TIM-2 promotes the differentiation of Th2 cells and regulates T-cell survival and activation upon interaction with Sema4A on myeloid cells.[38,40] (**c**) TIM-3 on DC suppresses innate immune signals mediated by nucleic acids or DAMPs,[27,28] whereas TIM-3 expressing T cells display exhausted phenotypes and trigger apoptosis by interacting with galectin-9.[46] TIM-3 also regulates NK cell differentiation and function.[55] (**d**) TIM-4 expression is largely restricted to APCs, and it serves as a phosphatidylserine receptor that regulates the engulfment of apoptotic cells; it interacts with TIM-1 on T cells to regulate the differentiation of Th1 and Th17 cells.[34,57,59] APC, antigen-presenting cell; DAMP, damage-associated molecular pattern; DC, dendritic cell; NK, natural killer; Sema4A, Semaphorin 4A; TIM, T-cell immunoglobulin mucin.

and the production of Th2 cytokines such as IL-4 and IL-10, thus demonstrating a critical role for TIM-2 in the regulation of Th2-mediated immunity.[38,39]

TIM-2 has also been identified as a potential ligand for the semaphorin family member Semaphorin 4A (Sema4A).[40] Sema4A is expressed on antigen presenting cells such as dendritic cells and activated B cells, and it plays an important role in supporting the effector function and survival of Tregs.[41] Because Tregs are a major population that inhibit intratumoral effector responses and tumor infiltration of Treg may be associated with poor prognosis in cancer patients,[42] the interaction between TIM-2 and Sema4A may act as a barrier against efficient antitumor immunity by activating Treg-dependent mechanisms (Figure 2). The relevance of TIM-2-mediated regulation in Th2 and/or Treg function remains largely unknown. However, it is plausible that TIM-2 may contribute to an immunosuppressive environment that favors tumor cell survival at early stages of immunosurveillance; TIM-2 might additionally have a dual role in inflammation-driven tumorigenesis by regulating protumorigenic Th2 and anti-inflammatory Treg responses [43,44] (Figure 3).

TIM-3

TIM-3 negatively regulates Th1 cell responses by binding with galectin-9, and under normal conditions, TIM-3-mediated immune regulation is fine-tuned through negative regulation by HLA-B-associated transcript-3.[45,46] Consistent with its immunoregulatory actions, several studies have revealed that the interaction between TIM-3 and galectin-9 causes exhaustion and apoptosis of antigen-specific CTLs in chronic viral infection and cancer.[47–49] It has been demonstrated that the TIM-3/galectin-9 pathway is responsible for repressing intratumoral immune responses in patients with hepatitis B virus-associated hepatocellular carcinomas.[50] Moreover, the exhaustion phenotype characterized by the coexpression of TIM-3 and programmed death 1 (PD-1) is frequently detected on $CD8^+$ T cells in tumor-bearing hosts and correlates with impaired antitumor immune responses in murine acute myelogenous leukemia models and in melanoma patients.[51,52] TIM-3 expression has also been identified on $Foxp3^+$ Tregs, although the functional relevance of this population compared to other subsets should be explored in future studies.[53]

In addition, recent studies have revealed that TIM-3 regulates innate immune responses. The upregulation of TIM-3 on DC in the tumor microenvironment negatively regulates innate responses to nucleic acids released from apoptotic tumor cells through its interaction with HMGB1. The interaction between TIM-3 and HMGB1 interferes with the endocytosis of nucleic

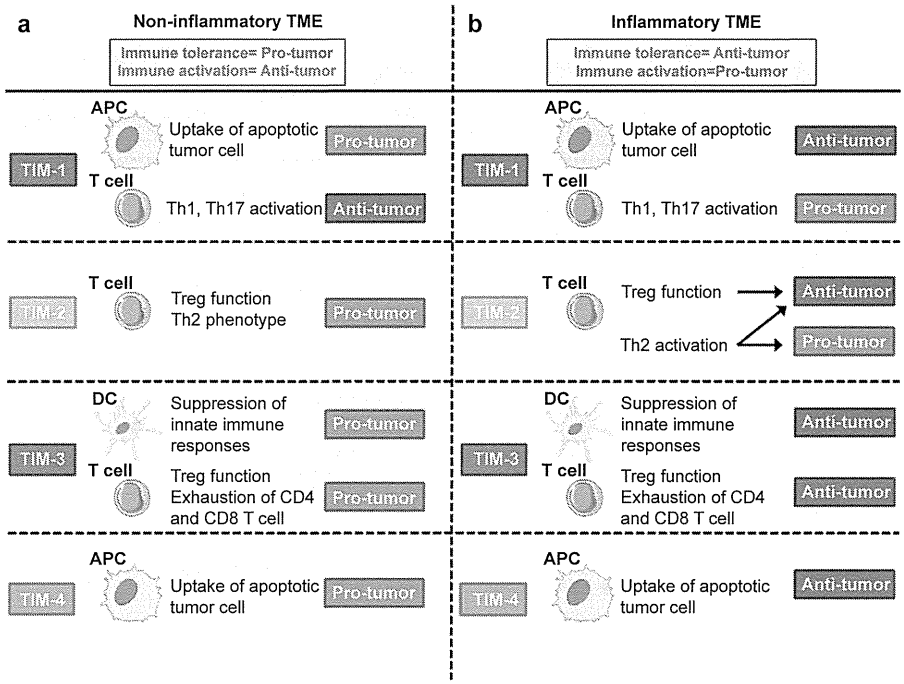

Figure 3 The dual role of TIM family members in sterile or inflammatory tumor microenvironments. TIM members may serve as dual regulators of antitumor immune responses depending on the quality of the tumor microenvironment. (**a**) In sterile, non-inflammatory TMEs, TIM-1 and TIM-4 on APCs suppress antigen-specific immune responses by facilitating tolerogenic phagocytosis. Moreover, TIM-2 may create tolerogenic environments by activating Treg populations, whereas TIM-3 negatively regulates DAMP-mediated innate immune signals and compromises tumor-specific CTL responses. (**b**) In contrast, immunoregulatory activities mediated by TIM-3 and TIM-4 may have a beneficial role in preventing protumorigenic inflammation, while TIM-1 and TIM-2 have dual roles in tumorigenesis by regulating T helper cell differentiation. DAMP, damage-associated molecular pattern; TIM, T-cell immunoglobulin mucin; Treg, regulatory T cell.

acids into DC endosomes, which blocks innate immune signaling pathways upstream of PRR-sensing systems.[27,28,54] Moreover, TIM-3 expression has been demonstrated on a specific subset of natural killer cells, which might regulate cytokine profiles and cytotoxic activities by distinct mechanisms[55] (Figure 2).

TIM-3 serves as a negative regulator of both innate and adaptive immunity in the tumor microenvironment. We hypothesize that in an early phase of cancer immunoediting, TIM-3 could reduce antitumor immunosurveillance through coordinated and distinct suppressive actions on innate and adaptive antitumor immune responses. However, TIM-3 might dampen protumorigenic inflammation by negatively regulating inflammation in the tumor microenvironment. Moreover, TIM-3-mediated suppression of innate immune signals in inflammation-associated tumor microenvironments may modulate the tumorigenic activities of myeloid cells and attenuate tumor progression, metastasis and resistance to anticancer therapies[56] (Figure 3). Although further studies are needed to clarify the role of TIM-3 during immunoediting, our hypothesis further highlights the complex features of immunoregulatory molecules in the regulation of cancer immunosurveillance and immune subversion.

TIM-4

TIM-4 is another member of the TIM family that mainly functions as a phosphatidylserine receptor to enhance the engulfment of apoptotic cells.[34,57] In contrast to TIM-3, which is expressed on multiple immune and non-immune cells, TIM-4 expression is restricted to APCs such as DCs and macrophages.[58] Recent analysis of TIM-4-deficient mice has shown that TIM-4 dampens inflammatory responses and maintains immune tolerance, implying that TIM-4 may have a potential role in the regulation of immune responses in the tumor microenvironment[59,60] (Figure 2).

The role of TIM-4 in tumor immunosurveillance, and in particular the regulation of antitumor immune responses and inflammation-associated carcinogenesis, remains obscure. It is tempting to speculate that TIM-4 plays an important role in controlling tumor-specific responses by regulating the processing and presentation of antigens from phagocytosed apoptotic tumor cells. Consistent with this hypothesis, phagocytic systems for apoptotic cells such as MFG-E8/integrin-$\alpha v\beta 3$ and Gas-6/TAM (Mer/Axn/tyro-3) create an immunosuppressive milieu that contributes to the maintenance of immune homeostasis and impaired tumor immunosurveillance.[61,62]

Consistent with this hypothesis, we recently found that vaccination with irradiated B16 melanoma cells expressing Flt3 ligand, combined with antibody blockade of TIM-4, elicited potent antitumor responses against B16-OVA melanoma tumors by activating the antitumor effector responses of intratumoral $CD8^+$ T cells.[63]

Thus, TIM-4 suppresses antigen-specific responses by repressing the presentation of immunogenic tumor-associated antigens and by establishing tolerized tumor environments. In contrast, tumor-associated APCs may utilize TIM-4 to counteract tumorigenic inflammation through apoptotic cell phagocytosis and the induction of immunosuppressive lymphocytes. Consistent with this idea, the presence of dying tumors due to impaired phagocytic systems provides potential sources for sterile inflammation and immune responses, which should create favorable circumstances for inflammation-driven tumorigenesis.[21,64] Thus, depending on the inflammatory milieu within the tumor microenvironment, it is critical to evaluate whether TIM-4-mediated immune regulation is beneficial or detrimental (Figure 3).

THERAPEUTIC POTENTIAL OF TARGETING TIM MOLECULES AGAINST TUMORS

Accumulating evidence has demonstrated the therapeutic potential of targeting TIM-3 to activate the antitumor immune responses of T cells infiltrating human tumors. For example, combined blockade of TIM-3 and PD-1 reversed the exhaustion phenotype and enhanced tumor antigen-specific CTL activities.[65] Moreover, recent studies unveiled the therapeutic potential of targeting TIM-3 and TIM-4 in preclinical murine tumor models. For example, treatment with anti-TIM-3 monoclonal antibody (mAb) alone or in combination with immunotherapy or chemotherapy augmented antitumor responses against established subcutaneous tumor models.[51,53,66] In addition, treatment with anti-TIM-4 mAb augmented antitumor responses against B16 melanoma models.[63] More importantly, combined treatment with anti-TIM-3 and anti-TIM-4 mAb further maximized the efficacy of cancer vaccines by increasing the numbers and effector functions of tumor-infiltrating natural killer and $CD8^+$ T cells compared to either mAb alone.[63] Taken together, this experimental evidence has supported the therapeutic potential of targeting TIM-3 and TIM-4 to stimulate antitumor immune responses and improve the clinical responses of conventional anticancer regimens. Interestingly, a recent study suggested that an siRNA that disrupted the interaction between TIM-4 on DCs and TIM-1 on T cells by targeting the FG–CC′ loop enhanced the therapeutic efficacy of DC vaccines against gastric cancer; this demonstrated that the FG–CC′ loop in TIM-1/4 may be a suitable target to develop novel immunotherapeutics.[67]

In contrast, anticancer strategies to suppress TIM-3 and TIM-4 activities might be detrimental in controlling tumorigenesis during sterile inflammation; further sterile inflammation caused by the blockade of TIM-3 and/or TIM-4 might accelerate protumorigenic inflammation. In the condition above, pharmacological inhibition of TIM-1 and TIM-2 in T cells might be useful for attenuating tumor progression by targeting protumorigenic inflammation and suppressing tumorigenic microenvironments.

Finally, we speculate that the therapeutic potential of TIM inhibitors that has been demonstrated in animal tumor models does not necessarily translate into clinical therapies for treating human cancer patients because exogenous implantation of established tumor cell lines does not reflect the natural course of immunosurveillance and immunoevasion in tumor microenvironments. Therefore, it is critical to evaluate the clinical implications of immune regulatory drugs such as TIM inhibitors by utilizing genetically modified animal models to evaluate and compare the pro- and anti-tumorigenic actions of host immunity on the temporal and spatial processes of tumorigenesis.[68,69]

CONCLUSIONS

We describe the potential role of the TIM gene family in the regulation of anti- and pro-tumorigenic responses during cancer immunosurveillance and evasion. The role of TIM family members in the temporal dynamics of immunosurveillance should be broadly applicable to other molecules that have immune modulatory and antitumor activities. For example, therapeutic strategies for targeting immunosuppressive factors (CTLA-4, PD-1, LAG3, IDO, *etc.*) or boosting immunostimulatory pathways (CD28, CD137, OX40, *etc.*) may be promising therapeutic strategies to stimulate antitumor immunity and trigger tumor regression during the early phase of tumor immunosurveillance. In addition, immunomodulation may be useful for treating advanced stage cancer patients in whom tumor-mediated immune regulation does not result in immunoevasion and pro-tumor inflammation. In contrast, targeting immunoregulatory pathways such as TIM, CTLA-4 and PD-1 might be detrimental for controlling tumorigenic activities during the advanced stages of carcinogenesis; restoring innate and antigen-specific immune responses might further amplify protumorigenic inflammation and promote tumor progression.

Recent advances to develop immunoregulatory drugs targeting immune checkpoints such as anti-CTLA-4 mAb and anti-PD-1 mAb elicited potent antitumor responses in patients with advanced solid cancers.[70,71] Moreover, accumulating evidence has revealed that multiple subsets of immunoregulatory populations, including Treg, MDSC, B7-1 and B7-4-expressing macrophages, contribute to immune suppression and are suitable therapeutic targets in combination with immune checkpoint molecules such as TIM family members.[17] However, it remains largely unknown whether particular immune cell subtypes exist that elicit antitumor responses and promote tumorigenic inflammation by these immune modulatory drugs. Thus, a deep understanding of the spatial and temporal regulatory mechanisms of immunosurveillance and immunoevasion during tumorigenesis should help to correctly regulate the homeostatic and inflammatory balance between host immunity and tumorigenicity in clinical settings.

In conclusion, we discuss the potential impact of TIM family members on tumor initiation and prognosis and their utility as therapeutic targets for treating patients with cancer. The development and elucidation of drugs targeting the TIM gene family should advance our understanding of the balance between beneficial antitumor immunity and harmful immune-mediated adverse inflammation and create ideal combinatorial

strategies to minimize side effects and maximize clinical responses in the future.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ACKNOWLEDGEMENTS

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Yin and yang of tumor inflammation: How innate immune suppressors shape the tumor microenvironments

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Mini Review

Pattern recognition-mediated sensing systems direct host immunity towards either antitumor immunosurveillance or protumorigenic inflammation. These activities imply dual and conflicting roles in the regulation of tumor-associated inflammation. On the one hand, recent evidence has revealed that several signaling components and cell-surface receptors suppress innate immune signals and constitute a negative feedback machinery preventing excess and continuous inflammation within tumor microenvironments. On the other hand, these same components also negatively regulate intrinsic tumorigenic activities by targeting nuclear factor-kappaB (NF-κB)-mediated antiapoptotic and inflammatory signals. Furthermore, the activation status of innate immune suppressors may reflect the functional plasticity of interactions between tumor cells and innate immune cells and determine whether tumor inflammation supports anti- or pro-tumorigenic responses. Thus, innate immune suppressors may provide valuable information about the immunogenic or tumorigenic status of tumor-associated inflammation thereby serving as potential biomarkers that predict tumor progression. Comprehensive analysis for identifying general and unique features of each innate immune suppressor in the regulation of tumor inflammation should explore the development of new biomarkers for improving future therapeutic strategies.

Pattern Recognition-Mediated Innate Immune Sensing Systems in Tumors: A General Overview

Innate immunity is a first line of detection system consisting of pattern recognition receptors (PRRs) expressed on most microbes and stressed host cells. This system orchestrates effector responses against microbes and endogenous tissue insults and regulates tissue homeostasis.[1,2] Accumulating evidence has positioned PRR-sensing mechanisms as indispensable regulators of tumor immunosurveillance.[3] Moreover, innate immune signals create microenvironments hostile for tumorigenicity in conjunction with tumor suppressor systems including oncogene-induced senescence and DNA damage signals.[4,5] These findings imply that molecular links exist between intrinsic and immune-mediated surveillance in controlling antitumor immunity and tumorigenicity. On the other hand, emerging evidence has unveiled protumorigenic roles for PRR-mediated innate immune responses in fostering multiple arrays of tumorigenic cascades such as tumor angiogenesis, matrix remodeling and stromal responses.[6,7] In particular, tumor-associated inflammation is a major cross-road linking innate immune systems with pro-tumorigenic inflammation.[8] Furthermore, the pro-inflammatory milieu in tumors promotes the recruitment of immunosuppressive myeloid cells such as M2-type macrophages and myeloid-derived suppressor cells, thereby creating tolerogenic tumor microenvironments (TMEs).[9,10] These findings suggest that the regulation of TMEs by inflammatory signals may determine the direction of tumor-host immune cell cross-talk throughout the different stages of carcinogenesis. In this review, we provide overviews about the role of innate immune suppressors in the regulation of tumorigenic activities. We also provide perspectives as to how innate immune suppressors contribute to prognosis as well as responses to anticancer regimens in the context of tumor immunosurveillance and immunoevasion.

Key words: innate immunity, tumor immunosurveillance, tumor inflammation, tumor microenvironments, biomarker

Abbreviations: CYLD: cylindromatosis; DAMPs: damage-associated molecular patterns; DUBs: deubiquitylating enzymes; HMGB1: high mobility group box-1; MDA-5: melanoma differentiation-associated protein-5; NLRP: nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing; PRRs: pattern-recognition receptors; RIG-I: retinoic acid-inducible gene-1; SOCS: suppressor of cytokine signaling; TAM: tyro-3/axl/Mer; TIM-3: T cell immunoglobulin-mucin domain protein-3; TLRs: toll-like receptors; TMEs: tumor microenvironments; TRAF: tumor necrosis factor receptor-associated factor

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Pattern Recognition Systems have a Dual Role in the Regulation of Therapeutic Responses to Anticancer Drugs

Damage-associated molecular patterns as a major sensor to tumor-associated innate immune signals

The temporal and spatial processes of transformation are comprised of intrinsic genetic and epigenetic mutations as well as extrinsic signals delivered from TMEs. In particular, inflammatory signals derived from tumor cells, stromal cells and inflammatory cells serve as critical sensors to determine how TMEs regulate qualitative and quantitative levels of pro-tumor and antitumor signals.[6–8] Among the inflammatory signals produced within TMEs, non-infectious or sterile inflammation is mainly regulated by the interaction between PRRs and damage-associated molecular patterns (DAMPs), and plays a critical role in controlling antitumor immunosurveillance and protumorigenic inflammation in TMEs.[11,12] These findings raise the possibility that the molecules and pathways that regulate innate immune signals by tumor-derived DAMPs may represent novel biomarkers suitable for evaluating tumor progression by distinguishing anti- and pro-tumor inflammation.

Antitumor functions of innate immune signals

Recent studies have verified the essential role of Toll-like receptor (TLRs)-mediated innate signals as sensitive parameters that predict clinical responses to various anticancer regimens. For example, dendritic cells sense high mobility group box1 (HMGB1) release from TMEs to stimulate innate signals. These signals subsequently result in cross-presentation of immunogenic antigens to antitumor CTLs, and the TLR4-HMGB1-mediated pathways are correlated with potent antitumor responses to chemotherapy and radiotherapy.[13,14] In accordance with these observations, the loss-of-function allele of TLR4 serves as a sensitive marker and predicts poor clinical responses to chemotherapy in breast and colon cancer patients,[13,14] However, this marker is ineffective in the prognosis of non-small cell lung cancer patients.[15] Thus, the molecules stimulated by TLR-4 pathways may serve as biomarker candidates to measure antitumor immunosurveillance in cancer patients.

Nucleic acid-sensing TLRs (TLR3, 7, 8 and 9) also stimulate innate signals that are associated with antitumor adjuvant effects.[16,17] The nucleic acid-sensing TLRs play an important function in restraining tumorigenicity induced by endogenous retroviruses, suggesting that TLR-mediated regulation of tumorigenicity relies mainly on the quality and extent of inflammation within tumor microenvironments.[18]

Cytosolic sensors comprised of DNA (cyclic GMP-AMP synthase) or RNA detection systems (RIG-I, MDA5, LGP2) represent additional components of the innate immune system that are utilized to detect intracellular invasive bacteria or viruses.[19] Although the contribution to the regulation of tumor immunosurveillance by innate immune signals delivered by cytosolic sensors remains largely unclear, recent reports have shown that treatment with the RIG-I agonist, triphosphate-RNAs, elicits a strong antitumor response in murine melanoma models.[20] Moreover, it is highly likely that cytosolic DNA elicit antitumor innate immune responses by sensing STING-dependent activation of type I IFN signals.[21,22] Thus, the activities of RIG-I/MDA-5 or STING-dependent pathways may be well correlated with antitumor immunogenic status in TME.

Moreover, RIG-I and MDA-5 protect tumor cells from chemotherapy-induced apoptosis by interacting with Bcl2 in the mitochondria. In this regard, it is likely that cytosolic sensors counteract cell death signals induced by anticancer drugs, which is a unique feature that distinguishes them from TLR-mediated signals.[23] Thus, it is plausible that identifying molecules derived from RIG-I/MDA-5-dependent, but TLR-independent, pathways may be useful to further develop new classes of biomarkers useful for the diagnosis and treatment of malignant diseases.

The inflammasome-mediated IL-1β producing systems are mainly regulated through the recognition of endogenous DAMPs by myeloid cells.[2] In particular, DAMPs released by dying or stressed tumors critically control inflammasome pathways and thus have a tremendous impact on tumorigenicity and clinical responses to anticancer chemotherapy.[11,12] Similar to TLR signals, inflammasome pathways also function as antitumorigenic mediators. For example, IL-1β generated from $Ly6C^{high}CD11b^{+}$ inflammatory DCs promotes antigen cross-presentation and priming of antitumor CTLs, augmenting the antitumor efficacy of cytotoxic therapies.[24,25] Furthermore, immunogenic cell death triggered by oxaliplatin or anthracyclin contributes to the generation of antitumorigenic IL-1β from DCs, enhancing antitumor CTL responses.

Chemotherapy-mediated ER stress in tumor cells responses trigger activation of autophagy and inflammasome signals, which are associated with antitumor immune responses and better responses to cytotoxic therapies.[26] In addition, inflammasomes have a protective role in the regulation of the natural course of tumorigenesis. NLRP-3 has an antitumorigenic role in colitis-driven inflammatory colon tumorigenesis through the promotion of tissue repair responses and maintenance of tissue homeostasis.[27,28]

Pro-tumor function of innate immune signals

On the other hand, TLR4-mediated innate signals contribute to progression in tumorigenic inflammation as shown in several chemical carcinogenesis and genetically-modified murine tumor models.[29,30] Moreover, tumor-derived inflammatory components confer myeloid cells with the capability of supporting the proangiogenic and metastatic potential of tumors through TLR2-NF-κB-dependent inflammatory cascades.[31,32] Thus, the molecules stimulated by TLR-2/4 pathways may serve as biomarker candidates reflecting pro-tumorigenic inflammation in cancer patients. Moreover, the activation of nucleic acids-sensing TLRs by endogenous inflammatory

signals may contribute to the pro-invasive and metastatic phenotypes of tumors and are correlated with poor prognosis in patients with non-small cell lung and prostate cancer.[33–35]

Cytosolic DNA sensing pathways via STING may induce type I IFN and IDO from $CD11b^+$DCs, and create tolerogenic environments by activating Treg and suppressing Th1 cells.[36] Thus, innate immune sensing systems mediated by STING also have a dual role in controlling anti- and pro-tumor immunity in TMEs.

Similar to TLR signals, inflammasome pathways also function as tumor-promoting mediators, which are mainly dependent on the cell types and immunogenicity of dying tumor cells. For example, MDSC-derived IL-1β creates pro-tumorigenic inflammation and a chemoresistant niche by facilitating the differentiation of proangiogenic IL-17-producing $CD4^+$ T cells, and the tolerogenic cell death induced by gemcitabine or 5-fluorouracil supports the production of pro-tumorigenic IL-1β from MDCSs.[37,38] Autophagy-mediated regulation of inflammasomes also underscores the protumorigenic role of the inflammasome on antitumor responses to chemotherapy. ATG16L1-dependent autophagic pathways negatively regulate inflammasome activation and IL-1β production in macrophages in response to LPS, explaining the tumor suppressor functions of autophagic pathways by restraining protumorigenic inflammation.[26] In addition, inflammasomes have a protumorigenic role in the regulation of the natural course of tumorigenesis. NLRP-3 and/or NLRP-6-mediated inflammasomes make a significant contribution towards creating protumorigenic inflammation and impairing antitumor immune responses.[38–40]

Taken together, these findings highlight the multifaceted regulatory pathways of PRR-mediated innate immune sensing systems in the regulation of antitumorigenic and pro-tumorigenic inflammation. Such pathways may depend on the different immunogenicity and/or the altered inflammatory milieu exerted by distinct environmental stimuli (microbial *vs.* chemical stress, etc.). Further elucidation should verify the utility of PRR pathways as predictive biomarkers for tumor initiation and the therapeutic responses to anticancer regimens in clinical settings (Fig. 1).

Negative Regulators of Innate Immune Signals in the Regulation of Tumor Immunity

Recent studies have identified multiple molecules and pathways that contribute to the suppression of innate immune responses by interfering with signaling cascades. These negative regulatory systems suppress harmful and excessive inflammatory insults, which may lead to autoimmune responses and lethal hyper-inflammation upon microbial infection.[41,42] Recent studies have revealed that several sets of negative innate immune regulators suppress inflammation-driven tumorigenicity and tumor resistance to anticancer therapies. Given this, such regulators may have an enormous impact on the innate regulation of inflammation in TME.

Various sets of negative regulators target signal components that specifically act on distinct innate sensing pathways. For example, TLR pathways are subjected to negative regulation by dissociation of adaptor molecules (TIPE2, IRAK-M, NLRX1, NLRC5, MSK1, etc.)[43–47] and modification of transcriptional regulation (p50, Nurr1, Zc3h12a, etc.)[48–50] in innate immune cells. Although most have been identified as regulators of innate immune responses mediated by microbe-associated PRRs, several of them are known to regulate inflammation and oncogenesis. For example, TIPE2, which has been identified as a member of TNFα-induced protein 8 (TNFAIP8) that regulates apoptotic pathways and oncogenesis, triggers anti-inflammatory programs in myeloid cells by inactivating caspase-8 and NF-kappaB signals. TIPE2 also serves as a suppressor of oncogenic RAS signaling. These activities imply a possible role for TIPE2 as a suppressor of tumor inflammation.[43,51] Moreover, MSK1/2, which serves as a repressor of TLR pathways, serves as a signal component that regulates MAPK oncogenic pathways, suggesting it links immune regulation with oncogenesis.[52] Together, these findings imply that innate immune suppressors terminate innate immune responses mediated by tumor-associated DAMPs and modulate tumor-associated inflammation. It remains unknown which types of immune suppressors regulate pro-tumor or antitumor inflammation and further investigations are required to clarify this important issue.

The ubiquitin-mediated degradation systems, which include various regulators such as E3-ubiquitin ligases and deubiquitination enzymes (DUBs), also play a critical role in regulating biological functions of both immune cells and tumor cells. Several DUBs, including A20 and CYLD, function as negative regulators of innate immune signals by removing ubiquitin chains from intermediate TLR adaptors (mostly of the TRAF gene family) and degrading them.[53–55] Interestingly, DUBs-mediated polyubiquitination and degradation of TRAF family proteins has a negative effect on NF-kappaB signals activated in transformed cells, thereby compromising tumor cell survival and suppressing feed-forward loops of pro-tumorigenic inflammation.[56,57]

The SOCS gene family members are well-known E3-ubiquitin ligases whose deregulation is closely associated with tumor-associated inflammation.[58] In particular, SOCS3, which serves as a repressor of Jak2-Stat3 pathways, has emerged as a critical checkpoint mediator to survey the inflammatory status mediated by myeloid cells and tumors.[59,60] SOCS3 is a major downstream molecule of the TAM receptor that attenuates TLR-mediated signals by degrading TIRAP/MyD88-adaptor-like (MAL) and TRAF proteins.[61] In addition, loss of SOCS3 triggers hyper-activation of gp130-mediated signals, which constitute downstream pathways of protumorigenic cytokines including IL-6 and IL-11.[62] Thus, SOCS3 potentially has a translational use in predicting prognosis and therapeutic responses of tumors.

A20 (TNFAIP3) is an ubiquitin-editing enzyme expressed in myeloid cells. A20 targets multiple molecules such as

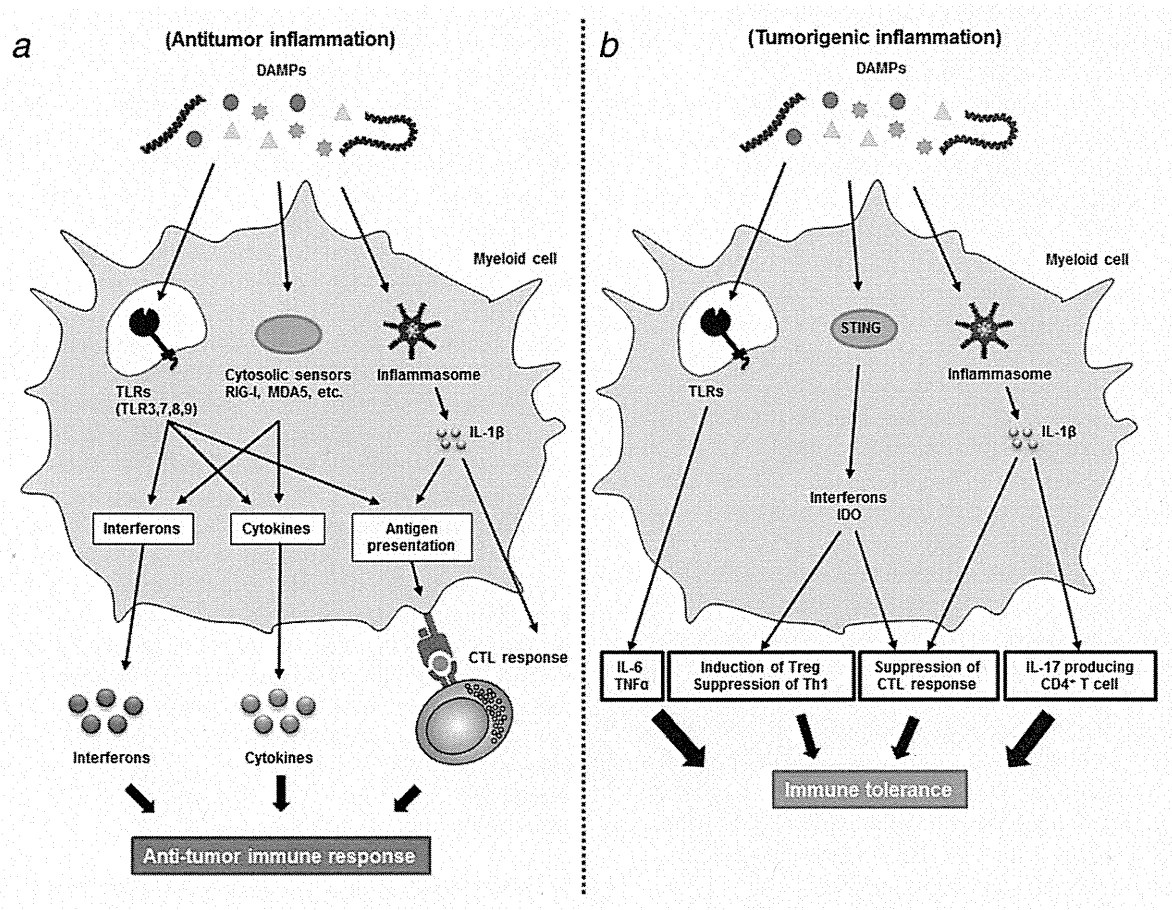

Figure 1. Dual role of pattern-recognition-mediated innate immune signals in the regulation of antitumor drug responses. (*a*) Antitumor functions of innate immune systems. TLR-mediated innate sensing of APCs has a positive effect on presenting immunogenic antigens while producing various cytokines and chemokines which are crucial to activate innate and adaptive effector cells, making a contribution to generate effective antitumor immunity. Moreover, RHL or STING-mediated cytosolic pathways and NLRP3-dependent inflammasome signals are also involved in the activation of antitumor innate and adaptive immunity through production of type IIFNs, immunogenic cytokines and increased cross-priming activities of APCs. (*b*) Pro-tumor functions of innate immune systems. TLR and inflammasome-mediated innate signals plays a critical role in tumorigenic activities by generating protumorigenic inflammation through carious cytokines such as IL-6, TNF-α and IL-1β from tumor-associated myeloid cells. STING-dependent type IIFN and ISO induced by the cytosolic DNA sensing signals contribute to tolerogenic environments by activating Treg and suppressing Th1 cells. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

RIPK1, MALT1, TRAF6 or NEMO, for degradation by removing K63-linked polyubiquitin chains. This leads to suppression of TLR-Myd88 and RIG-I-mediated signaling pathways and inflammatory responses.[54] Moreover, A20 functions as a tumor suppressor that prevents lymphomas from arising by suppressing cell-intrinsic NF-kappaB signal components and pro-carcinogenic inflammation mediated by tumor-associated myeloid cells.[57,63,64]

A tumor suppressor DUB, cylindromatosis (CYLD), also serves as a DUB that removes K63-linked polyubiquitin chains from TRAF6/7 for degradation and suppresses TLR2-mediated immune signals in macrophages.[55,65,66] Recent studies have revealed that CYLD has a predominant function in driving antitumorigenic programs by suppressing NF-kappaB-dependent antiapoptotic and inflammatory programs.[67,68] USP4 and DUBA mediate degradation of TRAF6 and TRAF3 *via* formation of K63-linked polyubiquitin chains, leading to the down-regulation of NF-kappaB and IFN signaling, respectively.[69,70] Moreover USP4 activation is regulated by PI3K-Akt signals and activates TGF-β-mediated oncogenic and invasive tumorigenic programs by deubiquitylating the TGF-β receptor,[71] suggesting an anti-inflammatory and oncogenic role.

Although there is still little evidence that DUBs regulate tumor-associated inflammation by cell-intrinsic and immune-mediated mechanisms, tumor cells may have evolved strategies to evade pro-apoptotic signals by repressing DUBs. At the same time, tumor-driven inflammation deregulates PRR-mediated signal activation of both transformed and innate immune cells, thus overcoming DUB-mediated negative feedback machineries and accelerating tumorigenic inflammation.

Negative Immune Regulators as Cell-Surface Receptors: Potential Biomarkers for Predicting the Quality of Tumor Inflammation

Advances have recently been made in identifying several negative regulators of innate immune signals. However, most of these molecules are intracellular signaling kinases and ubiquitin-regulating enzymes expressed broadly in many cell types under physiological conditions. Moreover, tremendous hurdles exist in quantifying intracellular signals in a cell-type specific fashion, although some negative regulators, such as A20 and CYLD, have distinct functions in lymphocytes and transformed cells as described above. Thus, it would be valuable to identify soluble or membrane-bound molecules as these allow for discriminating different cells and for detecting them in patient samples to develop clinically-available modalities. In this context, T cell immunoglobulin mucin domain protein-3 (TIM-3) may be a suitable candidate for developing a new biomarker for cancer. TIM-3 induction is frequently detected on exhausted T lymphocytes and myeloid cells during chronic viral infection or malignancy.[72] In particular, DC-expressed TIM-3 suppresses innate immune responses by interfering with HMGB1-mediated endocytosis of nucleic acid-sensing TLRs and RHLs.[73,74] Consistent with the role of HMGB1 in stimulating antitumor immunogenicity of DCs subsequent upon treatment with chemotherapy,[13] TIM-3 negatively regulates antitumor responses to chemotherapy by suppressing HMGB-1-mediated innate signals of DCs triggered by tumor-derived nucleic acids.[73,74] These findings imply that TIM-3 may serve as a promising candidate to predict clinical responses to anticancer therapies.

TAM receptor tyrosine kinases (Tyro3, Axl, Mer) are mainly expressed on myeloid cells and serve as phosphatidylserine (PS) receptors that promote phagocytosis of apoptotic cells via recognition of Gas-6.[75] Recent studies have unveiled unique functions of DC-expressed TAM receptors in attenuating tissue inflammation and antiviral defense through suppression of TLR-mediated innate signals.[61,76] On the other hand, TAM receptors are upregulated on tumor cells where they positively regulate tumor growth and increase the metastatic activities of tumor cells.[77,78] Thus, the measurement of TAM receptors in tumor tissues may serve as a useful surrogate marker to simultaneously understand both the activation status of innate immunity and tumorigenicity.

The interaction between CD24 and Siglec-G reduces DAMPs-mediated innate immune responses and attenuates excessive inflammation that can lead to septic shock and autoimmunity.[79] Siglec-G belongs to a family of immunoglobulin-like lectins that contain ITIM motifs and associate with SHP2 and transduces signals to inhibit innate and B cell-dependent immune responses.[80,81] Moreover, Siglec-G expression is upregulated on macrophages by RNA virus-dependent NF-kappaB signals. This results in recruitment of SHP2 and the E3 ubiquitin ligase, c-Cbl, which leads to degradation of RIG-I by K48-linked ubiquitylation and suppression of innate host defenses against RNA viruses.[82] These findings demonstrate that Siglec-G serves as a negative regulator of sterile and RNA virus-specific inflammatory responses. It remains unclear why RNA viruses, but not other microbes such as DNA viruses and bacteria, induce Siglec-G on macrophages, and whether tumor-derived inflammation could modulate expression and function of Siglec-G. Thus, further evaluation of the relevance of the Siglec-G-CD24 interaction as a potential biomarker for tumor prognosis and treatment responses is necessary.

CD11b (Integrin-αM) has classically been utilized as a surface marker that is preferentially expressed on myeloid cells, but recent reports suggest that CD11b has an unexpected role in the regulation of TLR-mediated immune signals. TLR-mediated activation of PI3K pathways stimulates phosphorylation of Myd88 and TRIF by Syk tyrosine kinase via CD11b. The phosphorylation of Myd88 and TRIF promotes recruitment of the E3 ubiquitin ligase Cbl-b resulting in their degradation. This serves as a negative feedback machinery to prevent excessive inflammation.[83] These findings also suggest the possibility that CD11b might be a potential candidate with which to measure the activation status of tumor innate immunity.

Altogether, in the future it will be of great importance to evaluate how innate immune suppressors, whether soluble molecules or membrane-bound receptors, impact the regulation of tumor immunogenicity and protumorigenic inflammation, and whether their activity levels are correlated with prognosis and responses to anticancer regimens in cancer patients (Fig. 2).

Dynamic Function of Innate Immune Suppressors in the Regulation of Tumor Inflammation: Mechanistic Insights

Tumor immunosurveillance makes a substantial contribution to the detection and elimination of tumorigenic cells through the coordinated actions of innate and adaptive immune systems. However, accumulating evidence has unveiled additional genetic and epigenetic alterations by which tumor cells reprogram themselves to utilize immune systems for creating pro-tumorigenic inflammatory environments.[6,84] In addition, tumor-associated inflammation creates favorable conditions for the generation of tumorigenic and immunosuppressive myeloid cells and lymphocytes by continuously receiving a distinct milieu of cytokines, chemokines and growth factors from TMEs.[8,9] Thus, the inflammatory TMEs regulate the plasticity of cross-talk between tumor cells and tumor-associated immune cells in a reciprocal manner, and render the immune cells to switch their primary functions from "trusted supervisors to survey against tumors" to "notorious betrayers to support tumor growth."

The conflicting effects of TMEs on innate immune systems imply that the functional status of innate immune suppressors may have a great impact on the tumorigenic or immunogenic qualities of tumor-associated inflammation. For example, it is notable that DUBs such as A20 and CYLD serve as negative feedback machineries to restrain

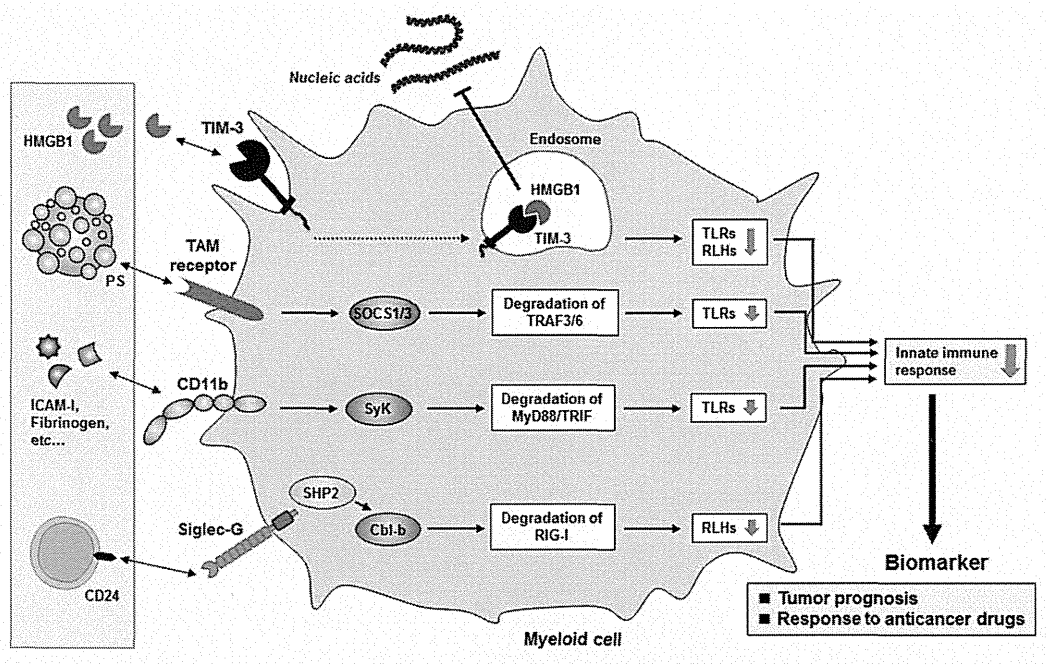

Figure 2. Potential impact of receptor innate immune suppressors as tumor biomarkers. Several innate immune suppressors are expressed as cell-surface receptors and serve as potential markers for predicting tumor prognosis and anticancer drug responses. Receptor innate immune suppressors, such as TIM-3, TAM receptors, CD11b and Siglec-G, etc., have unique functions in the regulation of innate immune signals in myeloid cells. TIM-3 expressed on DCs represses TLR and RLH signals by interfering with endocytosis of PRRs and contributes to the suppression of antitumor immunity induced by cytotoxic chemotherapy. On the other hand, TAM receptors and CD11b trigger the interaction of TLRs or their adaptor protein Syk with E3-ubiquitin ligases (SOCS1/3 and Cbl-b), thus subjecting the signaling components (TRAF3/6 and Myd88/TRIF, etc.) to degradation by ubiquitylation. The interaction of Siglec-G and CD24 promotes the recruitment of SHP2 and c-Cbl, which leads to the degradation of RIG-I by K48-linked ubiquitylation. The suppressive actions of innate immune suppressors have a potential role in regulating anti- and pro-tumorigenic inflammation in TMEs. Thus, the receptor innate immune suppressors may serve as functional markers for tumor progression by quantifying the functional status of tumor-associated inflammation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

excess immune responses activated by innate immune signals in myeloid cells.[51,52] Moreover, DUBs also function as tumor suppressors in transformed cells by degrading NF-kappaB-related antiapoptotic and proinflammatory molecules. Thus, it is tempting to speculate that certain subsets of innate immune suppressors function as dual regulators of tumor cells and innate immune cells; they suppress tumor growth in a cell-intrinsic fashion and fine-tune innate immune responses of myeloid cells. The regulation by innate immune suppressors to achieve transient immune activation in TMEs may be beneficial for shielding antitumor surveillance systems from continuous inflammatory insults, which may disrupt their antitumor effector activities and re-educate them to be pro-tumorigenic inflammatory cells. Thus, the delicate equilibrium of pro- and anti-inflammatory signals regulated by innate immune suppressors has an indispensable role in establishing the appropriate homeostasis of TMEs that may be critical for keeping antitumor innate immune systems normally responsive to transformed cells (Fig. 3).

In marked contrast, inactive or loss-of-variant mutations of innate immune suppressors such as A20 are frequently observed in transformed cells with extensive genetic and epigenetic mutations. Such changes cause continuous activation of NF-kappaB-mediated inflammatory signals in TMEs.[63,64] These changes, followed by deregulation of innate immune suppressor functions in tumor cells, result in a continuous supply of inflammatory mediators, including cytokines, chemokines and growth factors, leading to an excess supply of inflammatory signals to innate immune cells. This so-called "unbroken" autocrine inflammatory loop may disrupt or desensitize negative-feedback systems operated by intact innate suppressors, thus raising the potential of transforming antitumor guardians into pro-tumorigenic supporters. Thus, genetic and epigenetic alteration of innate immune suppressors in tumor cells contributes to tumorigenic activities by stimulating both tumor- and myeloid cell-derived inflammatory signals (Fig. 3).

From these perspectives, we propose the hypothetical framework whereby the expression levels and activation status of innate immune suppressors might serve as surrogate markers to aid in understanding whether tumor-associated immune systems execute antitumor surveillance or support pro-tumorigenic inflammation. Thus, innate immune suppressors have tremendous potential as biomarker candidates

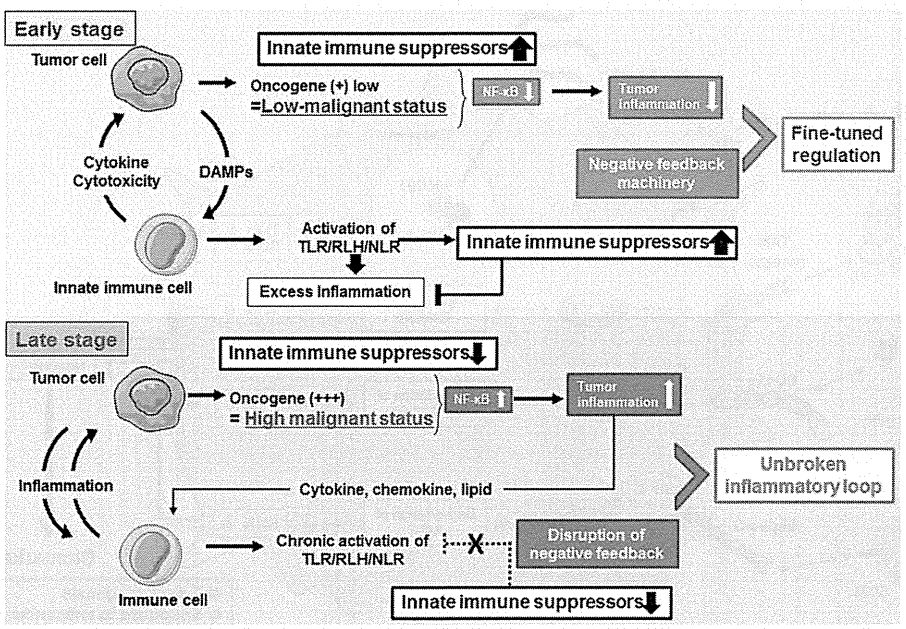

Figure 3. Role of innate immune suppressors in the regulation of antitumor immunity and tumorigenic inflammation. Innate immune suppressors negatively regulate intrinsic tumorigenic programs through suppression of NF-kappaB-dependent antiapoptotic and inflammatory signals. In addition, innate immune suppressors control the integrity of tumor inflammation by transiently stimulating antitumor immune signals and restraining excess and harmful inflammatory insults in TMEs. In contrast, additional transforming processes exploit tumor cells to lose the function of innate immune suppressors and chronically activate NF-kappaB-mediated inflammatory signals in TMEs. The tumor-driven inflammation may desensitize negative-feedback systems operated by innate immune cells, thereby increasing the potential of anti-tumor immune cells to be pro-tumorigenic activators. Thus, genetic and epigenetic alteration of innate immune suppressor genes in tumor cells contributes to tumorigenic activities by creating "unbroken" autocrine inflammatory loops in TMEs.

that accurately predict tumor prognosis and treatment responses to anticancer modalities by measuring the immunogenic status of tumor microenvironments. However, careful assessment will be required to determine the utility of biomarkers because their values may differ according to the inflammatory status of tumor microenvironments, tumor stages and/or tumor types.

Concluding Remarks

We have presented a comprehensive overview and perspective as to how tumors modulate antitumor immunity and tumorigenic inflammation by manipulating negative regulators of innate immune signals. Recent advances in identifying various negative regulators of innate signals reaffirm the importance of blocking harmful inflammatory insults in order to tightly regulate cellular integrity and tissue homeostasis. However, the roles of most innate immune regulators in the regulation of tumor-associated inflammatory responses remain largely unclear at present. Thus, more detailed analysis is required to comprehensively elucidate how TMEs regulate the expression and function of innate immune suppressors thereby contributing to the creation of privileged microenvironments favoring or antagonizing tumor progression.

Although the functional status of A20 and CYLD are associated with clinical prognosis and pathogenesis of patients with some malignant diseases,[63,64,66] it remains largely unknown whether other negative regulators of innate immune signals influences clinical and pathological features of human tumors. In this regard, it is necessary to develop the innovative strategies that accurately quantify the levels of innate immune suppressors, since the variability of measurements, staining and scorings among different institutions has been a major problem to standardize the currently utilized biomarkers in a routine diagnostic setting. Moreover, a deep understanding of cancer immunoediting processes regulated by innate immune suppressors should allow the development of novel biomarkers, which may be valuable in accurately measuring the threshold whereby tumor inflammation acts as a tumor-promoting or tumor-suppressing mediator.

Finally, discrimination between antitumor immunity and protumorigenic inflammation by these biomarkers will enable us to develop therapeutic strategies that stimulate antitumor innate immunity while avoiding tumor-promoting inflammation. Moreover, the innate immune suppressors might be a good therapeutic target for augmenting endogenous immune responses and improving the responses to other anticancer regimens, when tumor immunosurveillance is not overwhelmed by protumorigenic inflammation.

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Review Article

Clinical significance of macrophage heterogeneity in human malignant tumors

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The fact that various immune cells, including macrophages, can be found in tumor tissue has long been known. With the recent introduction of the novel concept of macrophage differentiation into a classically activated phenotype (M1) and an alternatively activated phenotype (M2), the role of tumor-associated macrophages (TAMs) is gradually beginning to be elucidated. Specifically, in human malignant tumors, TAMs that have differentiated into M2 macrophages act as "protumoral macrophages" and contribute to the progression of disease. Based on recent basic and preclinical research, TAMs that have differentiated into protumoral or M2 macrophages are believed to be intimately involved in the angiogenesis, immunosuppression, and activation of tumor cells. In this paper, we specifically discuss both the role of TAMs in human malignant tumors and the cell–cell interactions between TAMs and tumor cells.

It has long been known that many leukocytes including macrophages are present in tumor tissues and that these cells, together with fibroblasts and vascular endothelial cells, form the tumor microenvironment (Fig. 1).[1–4] Previously, activated macrophages were believed to exhibit antitumor activity by directly attacking tumor cells in the tumor microenvironment.[5] However, many recent studies have indicated the protumoral functions of tumor-associated macrophages (TAMs), and thus, TAMs are believed to directly or indirectly promote tumor progression.[6–8] Great advances have been made in TAM research over the past dozen years or so, with one of the most significant breakthroughs being the development of immunohistochemical methods for identifying TAMs in tumor tissue. Numerous studies using human samples have been carried out using CD68 as a macrophage marker, whereas CD163 and CD204 have been used as markers of M2 macrophages in recent studies.[9,10] Although variability is observed according to tumor tissue type and location, over 80% of immunohistochemical studies using various human tumor tissues have shown that higher numbers of TAMs are associated with worse clinical prognosis.[9] Supporting these clinical observations, *in vitro* experiments using human tumor cells and experiments using animal models indicate that TAMs promote tumor cell growth by suppressing antitumor immunity and inducing angiogenesis.[11,12]

As the relationship between TAMs and malignant tumors becomes clearer, TAMs have begun to be seen as the target of new cancer treatments. Clarification of how TAMs are involved in tumor progression and metastasis is anticipated to lead to the development of novel treatments and drugs.

Intratumoral infiltration of TAMs

Intratumoral infiltration of monocytes/macrophages is induced by various chemokines including chemokine (C-C motif) ligand (CCL)2, CCL5, CCL7, and chemokine (C-X3-C motif) ligand (CX3CL)1, as well as cytokines such as macrophage colony-stimulating factor (M-CSF), granulocyte-macrophage colony-stimulating factor, and vascular endothelial growth factor (VEGF), which are produced by tumor cells.[13–15] Subsequent differentiation into TAMs is induced by various factors produced by tumor cells. While the tumor size is small, macrophages from the surrounding tissue accumulate in and around the tumor by tumor cell-derived chemotactic molecules described above, and TAMs derived from the surrounding tissue macrophages account for the majority of TAMs.[4,16] As the tumor subsequently increases in size and an intratumoral vascular network forms, monocyte-derived TAMs become the dominant source of TAMs.[4,16]

Although many macrophage chemotactic factors are secreted by tumor cells, CCL2 and M-CSF are considered to be impor-

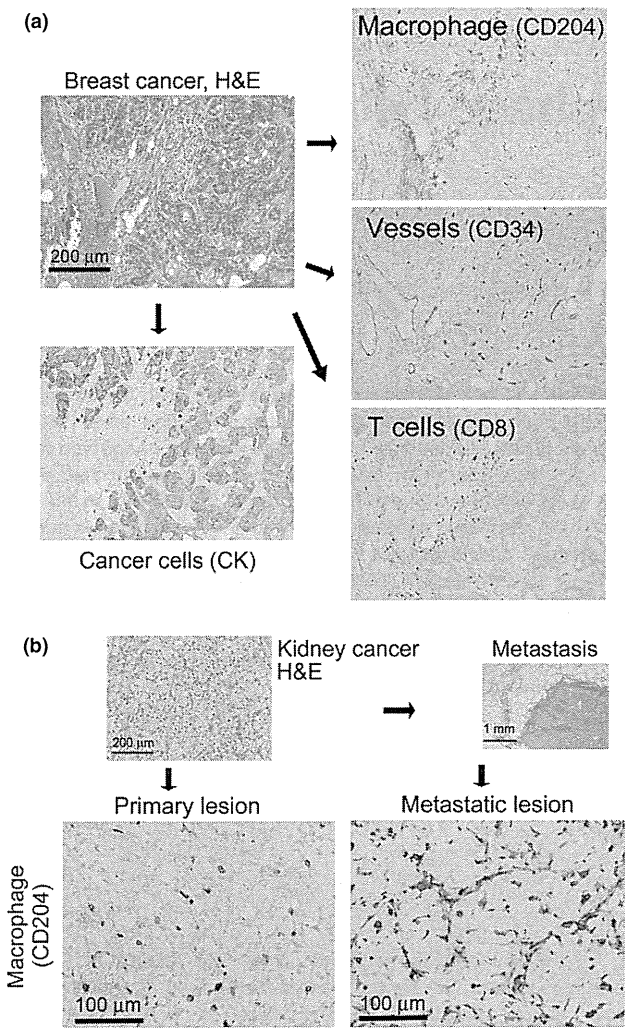

Fig. 1. Tumor microenvironment. (a) Tumor tissue contains not only tumor cells, but also large numbers of normal cells, including tumor-associated macrophages, lymphocytes, blood vessels, and fibroblasts, that affect tumor development in various ways. The photographs show an example of a clinical case of human breast cancer (invasive ductal carcinoma). The relative distributions of the above-mentioned cell types differ by organ and tissue type as well as individual case. CK, cytokeratin. (b) Metastatic tumors contain a larger number of tumor-associated macrophages. The photographs show an example of a clinical case of human kidney cancer (clear cell renal cell carcinoma). The primary tumor tissues and the metastatic (lung) tumors are shown.

tant molecules involved in macrophage infiltration. CCL2 is expressed in a wide variety of tumor cells, including gliomas, squamous cell carcinoma, ovarian cancer, prostate cancer, lung cancer, cervical cancer, and undifferentiated sarcoma, CCL2 also plays an important role in the intratumoral infiltration of monocytes.[13,17] In addition to inducing monocyte infiltration, M-CSF plays a critical role in the differentiation of monocytes into macrophages and, in particular, into M2 macrophages.[18–20]

Role of TAMs in tumor progression

Based on numerous studies using murine tumor models, activated TAMs were found to produce a variety of angiogenic, immunosuppressive, and growth-related factors.[7,8] However, few studies have been carried out using human materials, and thus the detailed mechanisms and molecular characterization of TAMs in human tumors have yet to be described. One method for studying the relationship between TAMs and tumor development is to carry out statistical analysis using clinical data related to survival rates or survival times. Studies comparing TAM infiltration into diseased tissue, using CD68 as a macrophage marker, are summarized in Table 1. The majority of studies in human malignant tumors have found that a higher level of TAM infiltration is associated with lower survival rates, and these observations indicate that TAMs may enhance tumor progression. However, other reports in certain types of cancer such as gastric, colon, and prostate cancer, have shown that a higher number of TAM infiltration results in a better outcome.

For a localized tumor a few millimeters in size to grow larger, intratumoral angiogenesis must occur. Genetic analysis has revealed that TAMs produce VEGF, interleukin (IL)-8 (CXCL8), basic fibroblast growth factor, thymidine phosphorylase, MMP, and other molecules that are involved in angiogenesis, indicating that TAMs promote the formation of intratumoral blood vessels. Furthermore, TAMs produce immunosuppressive factors, including prostaglandin E2 (PGE_2), indoleamine 2,3-dioxygenase, and IL-10, and thus contribute to the immunosuppressed state of cancer patients.[5–7] In fact, in studies using human tissue samples, the number of intratumoral TAM infiltration is positively correlated with formation of blood vessels and the number of regulatory T cells. Tumor-associated macrophage-derived PGE_2, indoleamine 2,3-dioxygenase, and IL-10 play important roles for induction of regulatory T cells and TAM-derived CCL17, CCL18, CCL22 are chemotactic factors for regulatory T cells.[5–7] These results indicate that TAMs create environments conducive to tumor progression through their effect on angiogenesis and immunosuppression. In addition, growth factors produced by TAMs, including basic fibroblast growth factor, hepatocyte growth factor, epidermal growth factor, platelet-derived growth factor, and transforming growth factor-β (TGF-β), are considered to directly promote tumor cell growth.[5–7]

Of further interest is the suggestion, based on the results of animal model analysis, that TAMs may play a role in forming premetastatic niches in organs to which the tumor will eventually metastasize.[21–23] Specifically, tumor necrosis factor-α, VEGF, and TGF-β (VEGF and TGF-β are also produced by cancer cells), which are secreted by TAMs in cancer tissues, are believed to be transported through the bloodstream to destination organs such as the lung, where they induce macrophages to produce S100A8 and serum amyloid A3.[23] Both S100A8 and serum amyloid A3 recruit macrophages and tumor cells to these organs and promote the formation of metastatic foci.[24,25] Thus, TAMs are believed to not only influence their local environment, but also to impact macrophages throughout the body and contribute to disease progression.

CD163 and CD204 as markers for protumoral or M2 macrophages

The heterogeneity of macrophage functions was suggested as early as the late 1990s.[26,27] Macrophage activation can be broadly divided into the following two types: classically activated macrophages (M1), which promote inflammation, and alternatively activated macrophages (M2), which inhibit

Table 1. High numbers of CD68+ tumor-associated macrophages are correlated with clinical prognosis in human malignant tumors

Tumor type	Favorable prognosis	Poor prognosis
Epithelial	Gastric cancer (adenocarcinoma)[68] Colorectal cancer (adenocarcinoma)[71] Prostate cancer (adenocarcinoma)[73]	Uterine cancer (endometrioid adenocarcinoma)[69,70] Esophageal cancer (squamous cell carcinoma)[72] Liver cancer (hepatocellular carcinoma)[74] Breast cancer (invasive ductal carcinoma)[75,76] Thyroid cancer (poorly differentiated)[77] Gastric cancer (adenocarcinoma, intestinal type)[78] Bladder cancer (urothelial carcinoma)[79]
Non-epithelial		Malignant mesothelioma (sarcomatous)[80] Malignant melanoma[81] Neuroblastoma[82] Ewing's sarcoma[83]
Hematopoietic		Hodgkin's lymphoma[84] Follicular lymphoma[85]

inflammation.[27,28] Those TAMs demonstrating enhanced expression of CD163 (hemoglobin scavenger receptor), CD204 (class A macrophage scavenger receptor), CD206 (mannose receptor, C type 1), stabilin-1, arginase-1, and accelerated production of IL-10, VEGF, PGE_2, and MMP9, generally show characteristics of M2 macrophages.[6–8] The proangiogenic and immunosuppressive activity in the tumor microenvironment mediated by TAMs can also be considered the result of M2 macrophage function.[6–8] Because CD163 and CD204 are specifically expressed on macrophages, and antibodies to these antigens that are suitable for immunohistochemical analysis are commercially available,[10,29,30] many researchers have used these molecules as markers of the M2 phenotype in both *in vitro* and *in vivo* studies. The details of the functions of these molecules remain unclear; however, a few studies have indicated that these molecules are involved either in regulating the inflammatory responses or in maintaining the protumoral functions of macrophages.[31–33] The clinicopathological studies using anti-CD163 or anti-CD204 antibodies are summarized in Table 2. In malignant lymphoma, glioma, and kidney cancer, higher CD163 expression on TAMs is associated with worse clinical prognosis, but no correlation exists between clinical prognosis and the number of CD204-expressing TAMs.[10,34–36] In esophageal cancer, a higher number of CD204-expressing TAMs is associated with poor clinical outcome, but the number of CD163-positive TAMs is not.[37] These observations suggest that CD163 and CD204 are not expressed in completely identical macrophage populations. In addition, the functional significance of CD163- or CD204-positive TAMs might be different among sites and histological types of cancer. We suggest that both CD163 and CD204 should be analyzed to evaluate the polarization of TAMs and that CD163- and/or CD204-positive TAMs are considered as "protumoral" macrophages/TAMs.

In a recent review, based on their location and function, Qian and Pollard[38] classified TAMs into the following six types: angiogenic; immunosuppressive; invasive; metastasis-associated; perivascular; and activated macrophages. Not all of these macrophage types of TAMs show the phenotype of M2 macrophages. Tumor-associated macrophages with M1 characteristics have also been observed in animal models of glioma and human pancreatic cancer.[39,40] Although the concept of "M1/M2 macrophages" is a convenient hypothesis simply dividing TAMs into two populations, we should note that TAMs contain various macrophage populations with a wide range of polarization statuses stimulated by complex signals in tumor microenvironment.

Significance of direct cell–cell interactions between TAMs and tumor cells

As shown in Figure 1, TAMs and tumor cells often directly contact each other, indicating that intimate cell–cell interactions exist between them. During the initial stages of tumor progression, monocyte migration factors produced by tumor cells induce infiltration of monocytes/macrophages, as described above. The macrophages that have infiltrated the tumor are

Table 2. Correlation between CD163+ or CD204+ tumor-associated macrophages and clinical prognosis in human malignant tumors

Tumor type	Favorable prognosis	Poor prognosis
Epithelial	Colorectal cancer (adenocarcinoma)[86]	Kidney cancer (clear cell type)[34] Liver cancer (hepatocellular carcinoma)[87,88] Liver cancer (cholangiocellular carcinoma)[89] Pancreatic cancer (invasive ductal carcinoma)[90,91] Lung cancer (adenocarcinoma)[92,93] Lung cancer (squamous cell carcinoma)[92,94] Oral cancer (squamous cell carcinoma)[95] Ovarian cancer (serous adenocarcinoma)[96] Esophageal cancer (squamous cell carcinoma)[37]
Non-epithelial	Osteosarcoma[97]	Leiomyosarcoma[98] Brain tumor (high-grade glioma)[10,42] Malignant melanoma[99,100]
Hematopoietic		Diffuse large B-cell lymphoma[101] Hodgkin's lymphoma[101–104] Follicular lymphoma[105] Angioimmunoblastic T-cell lymphoma[35] Adult T-cell leukemia/lymphoma[36] Multiple myeloma[106]

activated by tumor cell-derived molecules, including IL-6, M-CSF, PGE_2, and heat shock protein-27, and differentiate into protumoral/M2 macrophages.[6,20] Protumoral/M2 TAMs produce a variety of angiogenic and immunosuppressive factors, as described above, and create a microenvironment conducive to tumor progression. Signal transducer and activator of transcription 3 (Stat3) has received recent attention as an important transcription factor that mediates the interaction between TAMs and tumor cells.[12] Many angiogenic and immunosuppressive factors are transcriptionally regulated by Stat3. Therefore, activation of Stat3 not only plays an important role in the differentiation of macrophages into protumoral /M2 macrophages, it is also involved in tumor cell growth, metastasis, epithelial–mesenchymal transition, and the acquisition of resistance to anticancer drugs and radiation therapies.[12,41] Direct coculture of tumor cells and macrophages shows that Stat3 in macrophages is activated and that various factors secreted by activated macrophages, including EGF, IL-6, and IL-10, activate Stat3 in tumor cells.[18,42] Activation of the M-CSF receptor (CD115) and sphingosine-1-phosphate receptor 1 (S1PR1) on the cell surface is believed to contribute to the cell–cell interaction mediated by Stat3.[42,43] Membrane-type M-CSF on the surface of tumor cells serves as a ligand for CD115, and sphingosine-1-phosphate derived from tumor cells serves as a ligand for S1PR1. Stimulation of these receptors activates a variety of signal transduction pathways, including that of Stat3, causing TAMs to differentiate into the protumoral/M2 phenotype.[44] The activation of Stat3 through cell–cell interactions between tumor cells and macrophages contributes to the formation of the microenvironment necessary for development of primary and metastatic lesions (Fig. 2).

Recent studies using a murine cancer model showed that Stat3 is also an important molecule in the maintenance and anticancer drug responses of cancer stem-like cells (CSCs).[45–47] The TAM-derived milk fat globule-EGF factor VIII, which is a glycoprotein belonging to an epidermal growth factor superfamily, contributes to Stat3 activation in cooperation with proinflammatory cytokines such as IL-6. And Stat3 activation is preferentially associated with tumorigenesis and drug resistance in CSCs.[46] In human colorectal cancer, overexpression of stem cell markers in tumor cells is reported to be associated with a high number of TAMs.[48] Further studies are expected to clarify the details of the relationships between TAMs and CSCs.

Tumor-associated macrophages and myeloid-derived suppressor cells

Regarding the functional analysis of TAMs, tumor xenograft mouse or rat models are more useful than human tumors. The majority of myeloid cells infiltrating tumor tissues are immature cells in some types of murine tumors.[49,50] A strong immunosuppressive response has long been known to be induced when cancer cells are transplanted into mice. In the 1980s, myeloid cells in the bone marrow of tumor-bearing mice were shown to inhibit the activation of lymphocytes.[51,52] Subsequently, the same types of cells were shown to exist in the spleen, and with the advancement of analysis resulting from the identification of myeloid markers CD11b and Gr1, these cells were also shown to exist in lymph nodes and tumor tissues.[51,52] Immature myeloid cells are derived from bone marrow myeloid cells and exhibit immunosuppressive activity; therefore, they are referred to as myeloid-derived suppressor cells (MDSCs).[51,52] Distinct from mature neutrophils and monocyte/macrophages, MDSCs have recently been divided into granulocytic MDSCs ($CD11b^+Ly6C^{int}Ly6G^{hi}$), showing characteristics similar to neutrophils, and monocytic MDSCs ($CD11b^+Ly6C^{hi}Ly6G^{neg}$), showing characteristics similar to monocytes/macrophages.[53] Despite the observed differences among tumor histopathological types, mature macrophages (TAMs, $Gr1^-$) and MDSCs ($Gr1^+$) appear to coexist in the tumor tissues of mice. As MDSCs from tumor tissues differentiate into mature macrophages in *ex vivo* assays, MDSCs are considered to be the immature phenotype of TAMs.[52,53] However, which cell type plays a greater role in angiogenesis and the activation of tumor cells remains unclear.

Systemic immunosuppression is also observed in human patients with advanced malignant tumors, suggesting the existence of cells similar in nature to the MDSCs that are found in mice. A significant increase in the number of $CD14^+HLA\text{-}DR^{low}$, $CD11b^+CD14^-CD15^+$, or $Lin^-HLA\text{-}DR^-CD33^+$ cells is observed in the peripheral blood of patients with malignant tumors.[49,53] In an *ex vivo* study using human blood or tumor samples of melanoma patients, MDSCs were shown to contribute more substantially to immunosuppression than TAMs.[54] Given that these cell types indicate immunosuppressive activity, they may correspond to the MDSCs that are found in mice. As differences in gene expression and cell markers exist between mice and humans, sufficient care must be taken when attempting to apply the results of mouse studies to humans.

Dendritic cells in human tumor tissues

Dendritic cells (DCs) serve as other myeloid lineage cells in the tumor microenvironment, and play a critical role in integrating both innate and adaptive arms of immune responses. Myeloid DCs (mDCs) and plasmacytoid DCs constitute two major subsets of the DC population, and are distinguished from macrophages according to their unique surface marker expressions. In human DCs, mDCs are further classified as blood dendritic cell antigen (BDCA)1$(CD1c)^+CD11b^+$ and BDCA3$(CD141)^+$ C-type lectin$(CLEC)9^+$ populations, which are equivalent to $CD11b^+CD4^{+/-}$ and $CD8\alpha^+$ or $CD103^+$ tissue-resident mDCs, respectively.[55,56] The $BDCA3^+$ mDCs are specialized for cross-presentation of antigens from necrotic cells, whereas $BDCA1^+$ mDCs have pleiotropic functions to prime diverse repertories of T cell subsets, in particular, dermal and mucosa-associated T cells.[56–58] Human plasmacytoid DCs are characterized for their expression of BDCA2(CD303) and CD123 (IL3Rα), and produce large amounts of type-I interferon in response to viral or self-nucleic acids.[54] As it is difficult to identify these molecules in paraffin-embedded pathological specimens, there are few articles describing DCs in human tumor samples. However, these phenotypic differences should help clarify the distinct functions and molecular pathways of TAMs and DCs in tumor tissues.

Targeting TAMs: a novel concept of anticancer therapy

As previously explained, TAMs promote tumor progression through induction of angiogenesis and suppression of antitumor immunity. In particular, in humans, protumoral TAMs are believed to exhibit characteristics similar to M2 macrophages, and are intimately involved in the progression of malignant tumors. As such, treatment strategies aimed at local inhibition of macrophage differentiation into the M2 phenotype are anticipated to be effective. Signal transduction path-

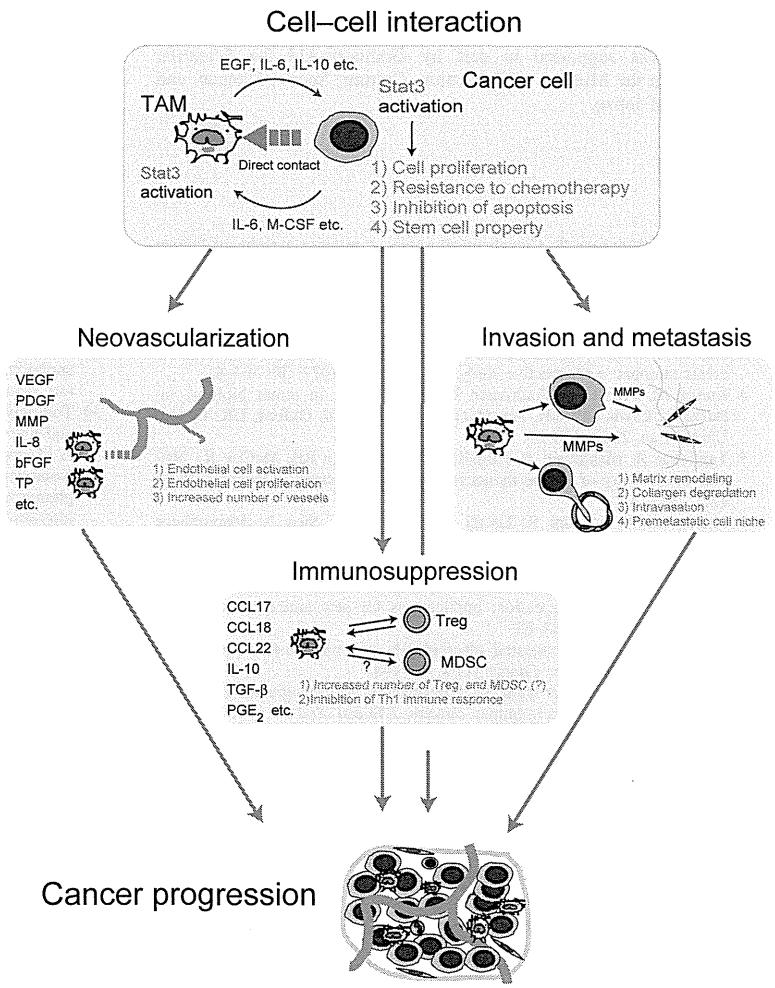

Fig. 2. Schema of the functional role of tumor-associated macrophages (TAMs). Tumor-associated macrophages are activated by macrophage colony-stimulating factor (M-CSF), interleukin (IL)-6, and other compounds secreted by tumor cells both to induce angiogenesis by producing angiogenic factors such as VEGF and platelet-derived growth factor, and to create immunosuppressive conditions by producing immunosuppressive factors such as IL-10 and prostaglandin E2 (PGE_2). At the same time, growth factors that are secreted by TAMs, such as epidermal growth factor (EGF), directly promote cancer cell growth, whereas MMP and other compounds responsible for stroma remodeling promote tumor cell infiltration and metastasis. Activation of tumor cells and TAMs induced by direct cell–cell interactions may represent an extremely important event in relation to the development of malignant tumors. bFGF, basic fibroblast growth factor; CCL, chemokine (C-C motif) ligand; MDSC, myeloid-derived suppressor cell; PDGF, platelet-derived growth factor; Stat3, signal transducer and activator of transcription 3; TGF-β, transforming growth factor-β; TP, thymidine phosphorylase; Treg, regulatory T cell; VEGF, vascular endothelial growth factor.

ways, including nuclear factor (NF)-κB, Stat3, Stat6, c-Myc, and interferon regulatory factor 4, are involved in differentiation into the M2 phenotype.[44,59–61] Nuclear factor-κB and Stat3 are also strongly involved in tumor cell growth, and drugs targeting these molecules are currently being developed. Among such molecule-specific drugs, synergistic efficacy due to direct effects on tumor cells, as well as inhibition of the differentiation of TAMs into the M2 phenotype, is expected. Among drugs currently in use, some are active against TAMs. Cyclosporin A and trabectedin not only directly inhibit tumor cell growth, they also suppress activation of TAMs.[16,62] Bisphosphonates not only suppress bone resorption by osteoclasts, they also inhibit the differentiation of TAMs into the M2 phenotype.[63] The angiogenic inhibitor bevacizumab (a VEGF-inhibiting antibody) has recently been used to treat solid tumors such as colorectal adenocarcinoma, and this drug also exhibits antitumor activity by suppressing TAM migration.[64,65]

We developed a screening system of chemical compounds that suppress macrophage polarization toward the M2 phenotype. By screening a library of naturally occurring compounds, we have identified several compounds, including corosolic acid, that suppress M2 polarization of macrophages.[66] These compounds suppress Stat3 activation and NF-κB activation both in macrophages and tumor cells *in vitro*.[66] However, as the blocking effect of these compounds on Stat3 and NF-κB was not adequate in tumor cells, the direct effect on tumor cells was weaker than that of other anticancer drugs.[66] In an *in vivo* study, corosolic acid appeared not to directly suppress tumor cells, but rather to stimulate the antitumor immunity of lymphocytes by inhibiting the activation of TAMs and MDSCs.[67] Corosolic acid was therefore considered to show antitumor activity by means of indirect effects to myeloid cells.

Conclusion

With the recent introduction of the concept of macrophage differentiation into M1 and M2 macrophages, and clarification of the function of each of these cell types, the role of TAMs in malignant tumors is gradually emerging. Specifically, in human tumors, TAMs that have differentiated into the M2 phenotype act as "protumoral macrophages" and contribute to the progression of disease. Based on current basic research, TAMs that have differentiated into the M2 phenotype are believed to be intimately involved in angiogenesis, immunosuppression, and activation of tumor cells. Clarification of the mechanisms of TAM activation and the process of differentiation into the protumoral/M2 phenotype is anticipated to lead to new strategies for treating malignant tumors.

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Disclosure Statement

The authors have no conflict of interest.

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