Review

Emerging Roles for Eosinophils in the Tumor Microenvironment

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Eosinophils are evolutionary conserved cells largely studied in the context of allergy. Although eosinophils were first described in tumors more than 120 years ago, their roles in cancer are often overlooked. This is puzzling given their potent immune modulatory, cytotoxic, and/or tissue repair capabilities, and recent studies demonstrating key roles for eosinophils in contexts far beyond their ‘classical’ field (e.g., metabolism, thermogenesis, and tissue regeneration). Recent data suggest that this frequently ignored cell is emerging as a potent immune effector and immune modulator in the tumor microenvironment. This review discusses the relevance of eosinophils to tumorigenesis and the potential to harness their function in cancer therapies.

Eosinophils: Forgotten Cells of the Tumor Microenvironment

The recognition of the tumor microenvironment as a critical factor in the biology of cancer is a central paradigm shift [1]. During the past 2 decades, the role of recruited or tissue-residing immune and nonimmune cells in tumor growth, metastasis, and resistance to therapy has been gradually elucidated and many of these cells (e.g., cytotoxic T cells, macrophages, cancer-associated fibroblasts) are now at the forefront of cancer research. Despite this, the role of other immune populations remains obscure. Among these are eosinophils, which are frequently observed in many solid tumors [2], and were noted to considerably increase in the peripheral blood of patients with cancer more than 120 years ago [3]. Nonetheless, eosinophils are largely overlooked in settings of cancer (Figure 1). The fact that the involvement of eosinophils in cancer has been greatly underinvestigated is highly puzzling for several reasons. First, studies of eosinophils in type 2 immune responses in ‘traditional’ diseases associated with eosinophilia (e.g., allergic inflammation and parasitic infections) [4] revealed that these cells display characteristic functions that may be extremely important in the tumor microenvironment, including cytotoxicity, promotion of angiogenesis, and tissue repair [4,5]. Second, recent studies highlight cardinal functions for eosinophils in settings that are far beyond classical type 2 immunity including glucose metabolism, thermogenesis, tissue regeneration, and development, as well as in innate and adaptive immunity [6–10]. Hence, eosinophils could potentially act as potent effectors and/or modulators of the tumor microenvironment. This review provides an overview about the current knowledge of the roles of eosinophils in the tumor microenvironment, and discusses outstanding questions and directions to be explored.

Interleukin-5 Is a Critical Factor in Eosinophil Biology

Eosinophils are produced in the bone marrow from distinct myeloid progenitor cells under the regulation of the transcription factors GATA-1 [11], PU.1, C/EBP, XBP-1 [12], and interferon consensus sequence binding protein (Icsbp) [13], and by the common β-chain signaling cytokines interleukin-3 (IL-3), IL-5, and granulocyte-macrophage colony-stimulating factor

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GM-CSF [14]. IL-5 is the most specific cytokine to the eosinophil lineage and is responsible for their selective expansion [15], release from the bone marrow into the peripheral circulation [16], and survival [17]. The critical regulatory role of IL-5 is clearly demonstrated in genetically manipulated mice, in which overproduction of IL-5 results in profound blood, spleen, and mucosal eosinophilia, whereas deletion of the Il5 gene causes a marked reduction of eosinophils in the blood, lungs, and the gastrointestinal (GI) tract following allergen challenge [18-21]. Based on the cardinal roles IL-5 has in eosinophil biology, therapies targeting the IL-5 pathway (i.e., mepolizumab and reslizumab) were recently approved by the Food and Drug Administration to target eosinophils for add-on maintenance treatment of severe asthmatic patients with eosinophilic phenotype [22,23]. However, targeting the IL-5 pathway does not fully ablate tissue eosinophilia, indicating a necessity to identify additional and combinatorial antieosinophil therapies, such as blockade of chemokines (primarily eotaxins) or of cell surface immunoreceptors [16,24-33].

**Eosinophils in the Tumor Microenvironment**

Tumor infiltrating eosinophils have been reported for a variety of solid tumors including gastric, colorectal, esophageal, head and neck squamous carcinomas, lung, breast, ovary, and uterine cancers, as well as in pleural effusions [2,34,35]. Eosinophilia is also seen in experimental mouse models of primary and metastatic tumors such as Lewis lung carcinoma, colorectal cancer, or metastatic melanoma [36,37]. Despite this, to date, the role of eosinophils in the clinical cancer setting is controversial since tissue eosinophilia (also termed tumor-associated tissue eosinophils) and peripheral blood eosinophilia have been associated with both good and poor prognoses (Table S1 in the supplemental material online). For instance, tumor-associated tissue eosinophils appear to be a good prognostic factor in GI and head and neck cancers, but poor prognostic factor in oral squamous cell carcinomas (SCCs) and Hodgkin’s lymphomas [38-40]. The divergent prognostic value might be explained by technical reasons such as variations in the criteria to enumerate tissue eosinophils and the lack of sufficient number of patients to power statistics [34,41]. Nonetheless, it may simply reflect the fact that tumor-associated eosinophils
can bear functional heterogeneity and plasticity in different cancer contexts. Likely, the tumor microenvironment shapes the phenotype and the genetic programs of eosinophils and promotes their antitumorigenic or protumorigenic roles. This notion that immune cells can have opposing functions as a consequence of activation state was demonstrated for additional myeloid cells such as macrophages, neutrophils, and dendritic cells. Thus, different tumor microenvironments may also polarize eosinophils into distinct populations with opposing effects on tumors.

**Recruitment of Eosinophils to the Tumor Microenvironment**

The GI tract is the largest eosinophil reservoir in the body under basal conditions but eosinophils also reside in fat pads, spleen, lymph nodes, and in the thymus. Eosinophil accumulation in the lungs and GI tract is a common feature of multiple diseases including parasite infections, asthma, immunoglobulin E-mediated food allergy, and eosinophilic esophagitis. The precise mechanisms that govern the recruitment of eosinophils to the tumor microenvironment and their local function are not clearly understood. Yet, various tumor cells have been shown to express eosinophil growth factors, such as IL-5, and several clinical studies have investigated and demonstrated the correlation between eosinophilia and eotaxin levels. In vivo, the accumulation of eosinophils in numerous tumors including lung, colorectal, and melanoma appears to be (at least partially) dependent on the IL-5 receptor–CCR3 signaling axis. However, eosinophils may be recruited to sites of tumors via additional pathways. For example, eosinophils infiltrate the lungs in metastatic models of melanoma and reside in necrotic areas of the metastatic tumor as well as in its fibrotic capsule. In this study, recruitment of eosinophils was an early and actively ongoing process that was independent of CCR3 ligands such as CCL11 and/or CCL24. Intriguingly, HMGB1 and IL-33 can mediate the recruitment of eosinophils to regions of dying cells as well. Indeed, eosinophils express the receptor for advanced glycation end products (RAGE) and ST2/IL-1RAcP, which mediate HMGB1 and IL-33-induced chemotaxis, respectively. Whether HMGB1 and/or IL-33 promote eosinophil recruitment in vivo remains to be investigated. Moreover, while eosinophils may be recruited to sites of hypoxia and tissue damage by danger signals, the cooperation of such signals with IL-5–CCR3-mediated signaling events remains to be defined.

![Figure 2. Mechanisms of Eosinophil Recruitment and Retention in the Tumor Microenvironment](image-url)

**Figure 2. Mechanisms of Eosinophil Recruitment and Retention in the Tumor Microenvironment.** Mobilization of eosinophils from the peripheral blood into sites of tumor growth and/or metastasis may be directed by various mechanisms including the presence of chemokines (CCR3 and CCR1 ligands). Th2 cell-derived cytokines (IL-5, IL-4), immunotherapy (GM-CSF, IL-4, and IL-2), as well as danger signals (HMGB1 and IL-33). Specifically, IL-5 potentiates CCR3-ligand-induced responses in eosinophils; thus stimulating their migration and retention. Additional factors regulating eosinophil migration and retention are commensal bacteria and IL-10. Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-, interleukin.
Additional pathways that were shown to regulate the infiltration of tumor-associated eosinophils are IL-10, microbiota, and CCR1. For instance, Apc<sup>−/−</sup>Dss mice, which spontaneously develop colorectal cancer, displayed increased intestinal eosinophilia that was increased in the absence of IL-10 and CD4<sup>+</sup> T cells [60]. Antibiotic treatment markedly decreased the eosinophilia and subsequent polyposis [60]. These observations suggest that commensal bacterial populations contribute to eosinophilic infiltration of polyps and to polyposis in the colon. Furthermore, eosinophilic infiltration in mouse models of colon cancer metastasis to the liver was regulated by CCR1 [61]. Migrating eosinophils expressed matrix metalloproteinase 9, but not matrix metalloproteinase 2, and worked in concert with monocytes, neutrophils, and fibrocytes to assist the colonization of metastatic cells (Figure 3, Key Figure). The recruitment of neutrophils and monocytes preceded that of eosinophils and fibrocytes raising the hypothesis that eosinophil recruitment is dependent on the accumulation of these cells in the tissue [61].
Taken together, chemotactic factors that guide the movement of eosinophils into the tumor microenvironment appear to depend on the anatomical location of the tumor and stage of disease.

The Potential Roles of Eosinophils in Cancer

Eosinophils store, synthesize, and release a large plethora of cytokines, chemokines, growth factors, enzymes, and lipid mediators [4,62], which could impact tumor cell growth and invasion directly or indirectly by modulating the properties of additional cells in the tumor microenvironment. Furthermore, they respond to a wide array of soluble mediators (e.g., cytokines, lipid mediators, complement) and express an extensive array of surface receptors [4,5]. In addition, eosinophils interact with numerous immune and nonimmune cells present in tumor microenvironment such as mast cells, macrophages, dendritic cells, T cells, B cells, fibroblasts, and endothelial cells [28]. Thus, the function of eosinophils may depend on the cellular composition of the local tumor microenvironment and the presence (or absence) of factors that may costimulate them.

Antitumorigenic Activities of Eosinophils

Eosinophils can exert potent cytotoxic activities (Figure 3) and subsequently mediate tissue damage through secretion of several unique proteins that are stored in their secondary granules, for example, major basic protein (MBP; type I and II), eosinophil peroxidase, eosinophil cationic protein, and eosinophil-derived neurotoxin [63]. By disrupting the integrity of lipid bilayers, MBP is cytotoxic to helminths, tumor cells, and mammalian cells (e.g., respiratory epithelial cells) [14,64–67]. Eosinophil peroxidase can catalyze the oxidation of nitric oxide and halides to promote oxidative stress (via reactive oxygen and nitrogen species), consequently leading to cell death [68]. Collectively, these data suggest that eosinophils may display tumoricidal activities. Indeed, co-culture of human eosinophils with colorectal cancer cells leads to the release of tumor necrosis factor–α (TNF–α), eosinophil cationic protein, eosinophil-derived neurotoxin, and granzyme A, and causes killing of tumor cells in vitro [69]. These antitumor effects are partially mediated by IL-18, which facilitates the contact of eosinophils and cancer cells by upregulating adhesion molecules such as LFA-1 and ICAM-1 [70]. Furthermore, we have recently shown that eosinophils express natural killer cell-associated killing receptors such as 2B4 (CD244), which mediate eosinophil antitumor activities toward malignant B cells [71].

Based on the potential of eosinophils to kill helminths and tumor cells in vitro, it has been proposed that eosinophils can display antitumorigenic activities in vivo. In support of this notion, various studies have shown that eosinophils are an important protective effector arm of the antitumor immunity orchestrated by T-helper type 2 cells and their respective cytokines (e.g., IL-4, IL-5). Injection of IL-4 secreting tumor cell lines into mice resulted in potent antitumor activity that was primarily mediated by infiltrating eosinophils. In this study, eosinophils and macrophages were found surrounding the tumor but nearly no CD8+ lymphocytes were observed [72,73]. The antitumor effect of transplanted IL-4-secreting tumor cells was associated with local production of eotaxin by structural cells such as endothelial cells [74], Figure 2). Furthermore, melanoma models of cytotoxic T cell-resistant lung and visceral metastases showed that clearance of metastasis was entirely dependent on the secretion of IL-5 from CD4+ Th2 cells, eotaxin expression, and to a lesser extent activation of STAT6 [75]. In this model, activated (i.e., degranulating) eosinophils observed in the tumors were involved in tumor regression [75]. Interestingly, although eosinophil lysates were found to be cytotoxic toward tumor cells, when resting and in vitro-stimulated eosinophils were incubated with tumor cells, no lysis of the tumor cells was observed in vitro. Thus, it appears that the tumor microenvironment provides additional factors that facilitate eosinophil degranulation and subsequent destruction of the tumor cells. Additional studies support an antitumorigenic function for eosinophils in cancer. For example, studies using Il5−/− mice (which display significant peripheral eosinophilia) [20], Cc11−/− mice
(which lack eotaxin) [76], Il5−/−/Ccl11−/− mice [77], and ΔdblGATA mice (which lack eosinophils) [78] revealed that eosinophils dramatically decreased the incidence of methylcholanthrene-induced fibrosarcoma in vivo and could kill fibrosarcoma cells in vitro [54]. Furthermore, studies using hepatocellular carcinoma cells overexpressing eotaxin showed eosinophil-mediated antitumor immunity in the presence of increased levels of IL-5 [79]. While tumor cell growth was similar in eotaxin overexpressing mice and in wild-type mice, growth was significantly suppressed in Il5−/− mice and enhanced following neutralization of IL-5Rα [79]. Importantly, while IL-5 in tumors can be traced to CD4+ T cells, evidence exists for innate lymphoid cell production of IL-5 [80]. Generation of IL-5 reporter mice revealed that lung innate lymphoid cells respond to IL-33, IL-25, and invading tumor cells to produce IL-5. In turn, IL-5 stimulation prolonged eosinophil migration and antitumor responses toward melanoma cells [80].

While eosinophils may kill certain tumor cells in vitro, evidence demonstrating direct eosinophil-mediated tumor extermination in vivo is largely missing. Furthermore, most of the antitumorigenic activities of eosinophils were demonstrated in experimental models that involve injections of genetically engineered cell lines, which secrete eosinophil homing and activating cytokines and chemokines (e.g., IL-4 and eotaxin). Thus, interpretation of these data needs to be done with caution and the activities of eosinophils should be assessed in additional and more ‘natural’-occurring cancer models including carcinogen-induced models and genetic models where engineered mice spontaneously develop tumors.

Recently, eosinophils have been shown to orchestrate antitumor immunity via indirect mechanisms. Using models of melanoma that were depleted of regulatory T cells (Treg), eosinophils promoted the recruitment of CD8+ T cells, improved vascular healing, and polarized macrophages into a proinflammatory phenotype, which is usually associated with antitumorigenic properties [81]. In this study, depletion of Treg cells in mice with melanoma resulted in accumulation of CD8+ T cells and eosinophils [82]. Eosinophil depletion using anti-Siglec-F antibodies (which induce eosinophil apoptosis in vivo) resulted in decreased accumulation of CD8+ T cells and subsequent reduced survival. Alternatively, co-transfer experiments of eosinophils and T cells were accompanied with altered numbers of activated CD8+ T cells; altered CCL5, CXCL9, and CXCL10 expression (all of which were highly expressed by eosinophils); altered vascular normalization; and the skewing of macrophages to a proinflammatory phenotype. Consistent with this, high expression of interferon-γ and TNF-α was observed in the tumor microenvironment [81], which decreased the expression of eosinophil angiogenic factors [e.g., vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), placental growth factor] and promoted activation of proinflammatory macrophages (Figure 3). The finding that eosinophils can promote CD8+ T-cell responses indirectly is not obvious since eosinophils have been previously shown to suppress CD4+ and CD8+ T-cell proliferation in graft versus host disease via direct cell–cell contact [83]. Taken together, these observations provide strong evidence to an indirect regulation of CD8+ T cells by eosinophils.

Clinically, a positive correlation exists between increased eosinophil levels and better immunotherapy treatment outcome. Tumor-associated eosinophilia has been described following various protocols of immunotherapy including treatment with IL-2, IL-4, GM-CSF (Figure 2) and more recently with anti-CTLA-4 (CD152, ipilimumab) [84–87]. This raises the hypothesis that eosinophils may mediate part of the response induced by these treatment regimens. Supporting this notion, degranulating eosinophils were observed in tumors following IL-2 treatment [88]. IL-4 treatment induced systemic eosinophilia accompanied by increased levels of serum and urine MBP, which are markers of eosinophil degranulation. Finally, a study aimed at defining whether inflammatory mediators and/or levels of myeloid cells could serve as a predictive factor for the response to ipilimumab showed that an early increase in eosinophil counts during the treatment was associated with improved clinical response [87]. These data were corroborated in an
independent study showing that increase in eosinophil counts of over 100/mm³ between the first and second ipilimumab infusions correlated with improved overall survival [89]. While these observations are interesting and suggest beneficial roles for eosinophils in tumor eradication, the involvement and contribution of human eosinophils primarily from a mechanistic standpoint remain to be clarified.

Protumorigenic Activities of Eosinophils

Eosinophils have been associated with multiple fibrotic conditions and can promote tissue healing and repair [90]. For instance, eosinophils store and release multiple growth factors and cytokines that can stimulate proliferation of fibroblasts and promote angiogenesis including tumor growth factor-β1 (TGF-β1), CCL18, FGFs, nerve growth factor, platelet-derived growth factor, IL-8, IL-6, and VEGF (Figure 3) [4,28,91]. Eosinophils are a chief source for TGF-β in the lungs and esophagus of asthmatic and eosinophilic esophagitis patients [92], and eosinophil-derived TGF-β is linked to epithelial growth, fibrosis, and tissue remodeling [93,94]. Furthermore, eosinophils contain granules with VEGF that is rapidly secreted upon cell activation with IL-5 [95]. VEGF and subcytotoxic concentrations of MBP act in concert to induce endothelial cell proliferation and potentiate the promitogenic effects of VEGF [96]. Finally, eosinophils were recently identified in white and brown adipose tissue at substantial levels, and compelling data establish the role of a key eosinophil–macrophage axis in weight gain, glucose metabolism, thermogenesis, and adipose tissue homeostasis. For example, under lean conditions, eosinophils in adipose tissue are a main cellular source of IL-4, which polarizes macrophages into an alternatively activated state (Arginase-1⁺, CD206⁺, CD301⁺ cells) that in turn improves control of glucose metabolism by secretion of insulin-sensitizing factors such as catecholamines and IL-10 [8,9,97]. Eosinophil-derived IL-4 also plays key functions in liver and muscle regeneration [7,98]. The finding that eosinophils store prominent amounts of IL-4/IL-13 that can direct macrophage polarization in vivo raises the possibility that eosinophils can shape tumor immunity by promoting the phenotype of immunosuppressive tumor-associated macrophages (Figure 3), which display, at least in part, some functional similarities with alternatively activated (e.g., IL-4/IL-13 activated) macrophages [44,99,100]. Indeed, eosinophil-derived IL-13 was shown to promote the suppressive phenotype of tumor-associated macrophages, a phenomenon that was suppressed by TNF receptor signaling in the tumor microenvironment [101]. Accumulation of eosinophils in Tnfr⁻//= mice orthotopically transplanted with EG7, LLC, and B16 cells correlated with IL-13 mRNA levels in the different models and with a suppressive tumor-associated macrophage gene signature (e.g., Retnla, Ccl24, Ccl17, Mgl2) [102–105].

IL-5 and eosinophils were identified in malignant pleural effusions (MPEs) of humans and mice [37]. MPE formation in response to lung and colon carcinoma cells was markedly decreased in Il5⁻//= mice [37]. Moreover, neutralization of IL-5 limited MPE formation in wild-type mice and exogenous IL-5 delivery promoted MPE formation. Subsequently, it was demonstrated that IL-5 enabled the formation of lung metastases via recruitment of eosinophils that secrete CCL22, which recruits Treg to the lungs [106]. These studies showed that eosinophils had no role in the formation and growth of primary tumors. Yet, they facilitated the colonization of the metastatic niche.

While eosinophils have been associated with conflicting prognostic values (i.e., both poor and better prognoses) in patients with SCC [107], (Table S1 in the supplemental material online), experimental models of SCC suggest that eosinophils have protumorigenic activities. Carcino-gen-induced SCC in wild-type mice and hamsters resulted in marked eosinophilic infiltrate. In hamsters, depletion of eosinophils using anti-IL-5 antibodies resulted in smaller tumor burden and lower tumor incidence following treatment with 7,12-dimethylbenz-(a)anthracene [108]. Similarly, 4-nitroquinoline-1-oxide-treated ΔdblGATA mice revealed decreased incidence of SCC, with lower cytological atypia [108]. Importantly, although these experimental models
support a protumorigenic function for eosinophils in SCC, several clinical studies linked the presence of eosinophils to worse prognosis, while others linked it to better disease prognosis.

Collectively, the aforementioned studies demonstrate the multifaceted roles eosinophils may have in the tumor microenvironment and suggest that in addition to their potential to display direct cytotoxic activities, activated eosinophils are capable of shaping the tumor microenvironment and can mediate tumor growth/rejection via indirect pathways. Whether eosinophils can influence tumor growth via interactions with additional cells (e.g., cancer-associated fibroblasts, myeloid derived suppressor cells, CD8+ T cells) remains to be defined (Figure 3).

Can Eosinophils Be Targeted for Cancer Therapy?
The importance of the immune system in the tumor microenvironment is best exemplified by current use of immune checkpoint blockers to reactivate antitumor activity [109]. The primary focus of these therapies are T cells, but myeloid cells (such as tumor-associated macrophages and myeloid-derived suppressor cells) have begun to draw considerable attention [99,110,111]. Thus, the ground is ripe for introducing new cellular targets, such as eosinophils, for immunotherapy. At present and given the limited data regarding eosinophils in cancer, this approach might seem premature. Therefore, an urgent need exists to define the roles and molecular regulators of eosinophils that will allow the setting of new pharmacological strategies to manipulate their activities in cancer. Specific emphasis should be put on expanding mechanistic studies in mice models and human cells, and integrating the results obtained from these studies with clinical data of sufficient statistical power. While extrapolation of mouse data into humans is not trivial, human and mouse eosinophils display significant functional similarities and overlaps [112]. Consequently, this provides a noteworthy opportunity to assess the roles of eosinophils in mice and translate the data in settings of clinical applications. Based on this, the road to target eosinophils in cancer may not be too long, especially in tumors where eosinophils display protumorigenic properties by supporting the colonization of metastasis, stimulating angiogenesis or proliferation of tumor cells. In these settings, eosinophil ablation can be done with recently FDA-approved anti-IL-5 drugs (i.e., mepolizumab and reslizumab) that show success in decreasing eosinophil levels in subsets of asthmatic and hyperesinophilic syndrome patients [113–115]. Strategies to suppress eosinophil accumulation, such as antieotaxin antibodies, can also be utilized. These strategies will be of value only when the IL-5–IL-5R or eotaxin–CCR3 axis is involved in eosinophil recruitment.

A more complicated challenge will be to design drugs that target eosinophils in tumors when these cells display tumoricidal activities. In contrast to T cells, which mediate antigen-driven and thus targeted cytotoxicity, global antigen-independent degranulation of eosinophils may cause collateral damage that is unwarranted. Data strengthening the hypothesis that pharmacologically targeted eosinophils would be ‘nonreactive’ against normal cells (or that normal cells are in some way refractory to the cytolytic effects of eosinophils) would be highly valuable. Conversely, deciphering the conditions that render tumor cells susceptible to the cytotoxic effects of activated eosinophils may open a therapeutic window for such an approach. Another possible route of intervention is targeting cell-surface receptors expressed by eosinophils that cause activation, degranulation, and release of an arsenal of mediators, among them cytotoxic substances [27].

Concluding Remarks
This is an exciting time for eosinophil research. The generation of new experimental tools enables the study of eosinophils in vitro and in vivo [5,42,116,117] and permits the gradual dissection of their involvement in multiple settings including cancer. These new tools should be utilized to address several fundamental questions regarding tumor-associated eosinophils (see Outstanding Questions). Given the paucity of data and the conflicting nature of existing data, the full spectrum of eosinophil involvement should be thoroughly studied. This spectrum involves the
underlying homing and survival signals in the tumorigenic tissue and the environmental triggers, which shape eosinophil responses in the primary tumor site, premetastatic niche, and during metastasis. One main research avenue should focus on the precise settings in which eosinophils display antitumorigenic functions and settings that elicit eosinophils to promote tumor growth. Directly related sequencing and proteomic studies aimed at illustrating the phenotypic landscape of tumor-associated eosinophils should be conducted. Generating these large genomic and proteomic data could be used as a reference point to compare eosinophil phenotypes in different tumors and further assess their phenotype with respect to additional myeloid cells in the microenvironment such as tumor-associated macrophages and neutrophils. Such phenotypic analyses could provide an excellent starting point to determine the molecular mechanisms that govern eosinophil activation in the tumor microenvironment.

Previous eosinophil-related studies revealed that eosinophils can intimately interact with numerous cells of both hematopoietic and nonhematopoietic origin [5,42]. The extent and magnitude of such interactions in the tumor microenvironment should be gradually assessed. Of specific interest are the interactions (direct and indirect) between eosinophils and tumor-associated macrophages, CD8+ T cells, and tumor cells. Attempts should be made to demonstrate in vivo evidence for eosinophil-mediated killing. These can and should be studied using available imaging techniques (e.g., intravitral and two-photon microscopy). Furthermore, outcomes of eosinophil degranulation and secretion of eosinophil granule proteins to tumor growth should be better studied in vivo using eosinophil granule protein-deficient mice [117]. Finally, models that assess the feasibility to therapeutically exploit the antitumorigenic activities of eosinophils should be developed. Of particular interest, models assessing the involvement of eosinophils in the antitumorigenic activities of CD8+ T cells following immunotherapy should be developed.

Addressing these questions will provide fundamental basic understanding of how eosinophils operate in the tumor microenvironment and will hopefully outline new modalities for immunotherapy, which can be combined with current treatment protocols or even alone.

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