

Potential Roles of Eosinophils in Cancer Therapy: Epidemiological Studies, Experimental Models, and Clinical Pathology

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Abstract: Eosinophils play important roles in allergic diseases as well as during helminth infection. As multifunctional leukocytes, eosinophils have also been indicated in anti-cancer immunity. Published studies have suggested an association between allergic conditions and a trend of decreased risk in numerous malignances. Moreover, eosinophil infiltration in tumor tissue is considered an independent prognostic factor. Eosinophils are often recruited to tumor sites, where eosinophil granule proteins and cytokines are released upon activation, which in turn damage and kill tumor cells. In the last decade, a number of patents based on potential cancer therapy using eosinophilic cytokines have been awarded. In this article, we review the current findings on epidemiology, experimental models, clinical pathology, and molecular mechanisms involved in the response of eosinophils towards cancer. Moreover, we discuss promising targeted therapies with eosinophilic cytokines as a novel perspective to combat cancer.

Keywords: Eosinophils, interleukin-5, tumor immunology, allergy, tumor-associated tissue eosinophilia, IgE.

INTRODUCTION

It has been a long while that eosinophils are being considered as granulocytes mainly involved in the pathogenesis of allergic diseases and helminth infections [1-3]. More recently, these cells are considered as multifunctional leukocytes playing important roles in inflammatory responses, tissue remodeling as well as in modulation of innate and adaptive immunity through expression of various cytokines [4]. Extensive studies have now provided evidences that eosinophils may prevent cancer development [5-8]. Numerous epidemiological studies have evaluated tumor-associated tissue eosinophilia (TATE) as a predictor of outcomes for cancer patients [9-12]. Although underlying detailed mechanisms of eosinophils function in antitumor responses still remain largely unclear, it has been widely studied in human and in experimental models [5, 6]. In this review, we provide a deep overview of the epidemiological and experimental evidence for the involvement of eosinophils in the anti-tumor response. Moreover, we also discuss targeted therapies with eosinophilic cytokines as a promising novel perspective to combat cancer.

THE BIOLOGY OF EOSINOPHILS

Eosinophils, multifunctional leukocytes, are developed in the bone marrow from haematopoietic stem cells. They are

released into the peripheral blood and then recruited into tissues in response to appropriate stimuli, most notably the cytokine interleukin-5 (IL-5) and eotaxin [3]. Besides being involved in allergic diseases and helminth infections, eosinophils also play important roles in inflammatory responses, tissue remodeling as well as in modulation of innate and adaptive immunity.

Mature human eosinophils contain numerous highly basic and cytotoxic granule proteins, such as major basic protein (MBP), eosinophil peroxidase (EPO), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN) [8, 13, 14]. These granules are frequently found in tissues of eosinophil-associated diseases, indicating roles in disease pathogenesis. In addition to these cationic proteins, eosinophil granules express an array of cytokines, chemokines, enzymes, and growth factors, including IL-2, -4, -5, -9, -10, -12, -13, and -25, RANTES (CCL5), eotaxin-1 (CCL11), transforming growth factors (TGF), vascular endothelial growth factor (VEGF), interferon gamma (INF- γ), tumor necrosis factor alpha (TNF- α), matrix metalloproteinase (MMP), and nerve growth factor (NGF) and so on [15-18]. Among the above mentioned factors, IL-5 is a major cytokine that is involved selectively in the maturation, activation, and proliferation of eosinophils. The type of cytotoxic molecules released by eosinophils depends on various types of stimulus as well as other microenvironmental status. In response to activation, eosinophils can also release some special mediators, for example IL-4, to stimulate adaptive immunity.

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ALLERGY AND ONCOGENESIS: IMPLICATIONS AND FACTS

Epidemiological Studies of IgE-mediated Allergy and Cancer

An inverse relationship between allergy-related diseases and cancers at many sites has been reported [19, 20]. Large prospective cohort studies involving 1,102,247 subjects found a significant inverse association between a history of either asthma or hay fever and overall cancer mortality (relative risk (RR) = 0.88, 95% confidence interval (CI): 0.83, 0.93) as well as colorectal cancer mortality (RR = 0.76, 95% CI: 0.64, 0.91), in comparison with persons with neither of these allergic conditions [21]. McDuffie *et al.* performed a skin test with seven allergens on 137 lung cancer patients and 137 age- and sex-matched control subjects [20], and found that 35.8% in patients groups and 58.4% in controls responded with one or more positive skin reactions, indicating that allergy may protect against the development of lung cancer [20].

So far, a number of well designed studies have identified an association between allergy and the trend of decreased cancer risk in many malignancies, including lung cancer, pancreatic cancer, colorectal cancer, prostate cancer, breast cancer and so on [19-26]. One possible explanation is that persons with a history of IgE-mediated allergy may bear an enhanced tumor immuno-surveillance [8].

Antihistamine and Cancer Risk

In 1989, a clinical study reported that ketotifen could prevent tumor growth in patients with neurofibromatosis [27]. Subsequently, a number of investigators have studied the possible association between use of antihistamine and the risk of cancer [28-31]. However, no statistically significant effect of antihistamines was evident for the inhibition of tumors. In experimental models, Mao *et al.* used an inhibitor of the H1 histamine and 5-HT serotonin receptors, cyproheptadine, to incubate with myeloma and leukemia cells [32]. Their findings showed that cyproheptadine preceded its cytotoxicity by reduction of Cyclin D expression and induced G0/G1 cell cycle arrest, limiting tumor cells proliferation [32].

The Influence of Environmental Exposures

It is well-known that cigarette smoking is an established risk factor for cancer of various origins. Patients with allergic diseases may be less likely to smoke due to an exacerbation of respiratory symptoms, which may modify or confound the association between allergy and cancer [33, 34]. Well-designed and adequately powered large-scale clinical studies are therefore needed to detect IgE-mediated allergy and its association with cancer incorporated with environmental exposures in the susceptibility of different types of cancer in the near future.

EOSINOPHIL INFILTRATION IN THE TUMOR TISSUE

Tumor-associated Tissue Eosinophilia (TATE)

In 1981, Lowe *et al.* first presented the conception of tumor-associated tissue eosinophilia (TATE) [35]. Lowe and his colleagues performed an assessment of cellular infiltration

of the tumor tissues from 460 subjects of cervical carcinoma in Malawi [36]. Of all tumors, 3% showed a massive eosinophil infiltrate and 37% with minor degrees of tissue eosinophilia [36]. They observed that in some areas the tumor cells appeared to have lost cohesion, with individual cells lying separately encircled by eosinophils [36]. Clinical investigations have shown that eosinophil infiltration in tumor tissue is common and occurs in several types of cancers, including hematological malignancies and solid tumors [37-46]. Furthermore, eosinophil recruitment and infiltration at the sites of solid tumors probably early events and were spatially restricted to specific regions within tumors [47].

Prognostic Value of TATE in Cancer Patients

TATE has been observed in numerous human cancers and in several animal tumor models [48, 49]. Since then, the possible prognostic value of eosinophils in cancer patients has attracted the interest of many investigators [50]. However, up to date, prognostic role of TATE in cancer patients remains conflicting rather than conclusive, given inconsistent results from cancer of different origins [8].

Thompson *et al.* studied eosinophil infiltration on the prognosis of laryngeal cancer in a series of 104 patients [51]. The original laryngeal biopsy histologic slides were examined and 31 patients had TATE in all [51]. After a minimum of 5 years follow-up, 90.3% patients with TATE were still alive, in contrast to 65.8 % of patients without TATE ($P = 0.009$) [51]. In another study, researchers also observed that the infiltration of tumors with eosinophils have better prognosis in patients with colon carcinoma [52]. Their data even showed that increased numbers of eosinophils were associated with significantly less incidence of colon carcinoma metastases (23.5 % versus 62.0 %) [52]. Collectively, TATE has been reported to be associated with a favorable prognosis for the patients with esophageal squamous cell carcinoma, prostate carcinoma, nasopharyngeal carcinoma, head and neck squamous cell carcinomas, and oral squamous cell carcinoma [11, 53-56].

However, increased levels of eosinophils are not always a consistent prognostic factor on cancer patient survival outcome. Sassler *et al.* conducted a prospective study to investigate prevalence and prognostic value of TATE in patients with stage III and IV laryngeal squamous cell carcinoma [57]. Among 248 patients, eosinophilia was found in 22.5% of tumor specimens by histopathological examination. However, this study showed that eosinophil infiltration was not related to tumor site, stage, patient age or sex, response to induction chemotherapy, or treatment modality [57]. After 48 months of follow-up, overall and disease-free survival rates did not differ significantly with respect to TATE [57]. Furthermore, histological analysis of 1511 biopsy specimens of patients with Hodgkin's lymphoma by the German Hodgkin's Lymphoma Study Group revealed prominent eosinophilia in 38% of cases, and the clinical significance of eosinophilia was different between histo-pathological categories [58]. Moreover, eosinophils proved to be the strongest unfavorable prognostic factor for survival and freedom from treatment failure in nodular sclerosis Hodgkin's disease [58].

However, one of the limitations of most published studies was that the researchers did not assess confounding factors, such as age, sex and environmental influences (smoker status)

at the patient level and incorporate them into the analysis. Efforts on future studies should solve those issues, and lead to a better understanding of eosinophils as a prognostic indicator for cancer patients.

THE MECHANISM OF ANTI-TUMOR EFFECTS OF EOSINOPHILS

Recruitment of Eosinophils into Tumor Tissues

Although infiltration of tumors by eosinophils is common and occurs in several types of cancers, there is no clear explanation of the underlying mechanism of the accumulation of eosinophils in tumor tissue. Cormier *et al.* injected B16-F10 melanoma cells subcutaneously above the right shoulder area of C57BL/6J female mice, a strain refractory to many types of tumours [47]. Ten days later, they found a strong eosinophil infiltration in the necrotic and capsule areas of the tumor, while only a few eosinophils were observed in the viable areas of the tumor [47]. In addition, they transferred fluorescent marker (CFDA)-labelled eosinophils into tumor bearing mice and resulted in the accumulation of labeled eosinophils in the necrotic areas of the tumor, which demonstrated that this recruitment was a dynamic event occurring in established tumors [47].

Cormier *et al.* also observed that eosinophil recruitment to tumor cells was independent of CD4⁺ T cells [47]. Accumulated evidences suggest that innate non-T interleukin [IL]-5-producing cells contribute to the maintenance of sufficient numbers of lung eosinophils and are therefore important for eosinophil recruitment mediated by IL-25 and IL-33. In contrast, deficiency of IL-5 signaling lead to a shortage of lung eosinophils and impaired eosinophil recruitment in response to invading tumors [59]. A higher incidence of methylcholanthrene (MCA)-induced fibrosarcomas was observed in CCL11 (eotaxin-1)-deficient mice compared to that of syngeneic wild type mouse [60]. In support of this finding, eotaxin was shown to be a critical eosinophil chemoattractant that mediates eosinophil tissue recruitment in tumors [61].

Increased number of eosinophils may result from either an enhanced recruitment mediated by chemotactic mediators or a decreased apoptosis of eosinophils in the local tissue, or both, which needs to be further addressed in future studies. However, little association was found between tissue eosinophilia and peripheral blood eosinophil counts, which suggested both local and systemic signaling pathways of recruitment of eosinophils in tumor tissue [58].

Eosinophils Establish Contact with Tumor Cells

Tumoricidal effect of eosinophils might depend on cell-cell contact with the target tumor cells and release of soluble factors locally. Based on this hypothesis, Legrand *et al.* performed a cell-cell adhesion assay to evaluate the role of cellular contacts in Colo-205 colorectal tumor cell apoptosis induced by eosinophils [62]. After 1 h of co-incubation, $26 \pm 3.8\%$ Colo-205 cells adhered to eosinophils. However, this binding was strongly inhibited when eosinophils were prefixed with 1% paraformaldehyde. Meanwhile, the level of Colo-205 cell death was significantly decreased [62]. In addition, they observed that attachment of eosinophils with Colo-205 cells was inhibited by anti-CD11a or anti-CD18 antibodies, which

significantly blocked eosinophil cytolytic activity [62]. These findings suggested that eosinophils may exhibit tumoricidal effects via CD11a/CD18-mediated contacts with tumor cells.

Eosinophils Secrete Granule Protein and Cytokines

Munitz *et al.* demonstrated that eosinophils express 2B4 (CD244) and cross-linking of 2B4 activated eosinophils displayed a cytotoxic effect towards mouse mastocytoma cells as well as B cell lymphoma by releasing EPO, INF- γ , and IL-4 [63]. These results indicated that eosinophils participate in both Th1- and Th2-like responses against tumor cells via 2B4 interactions. In another study, following co-incubation with different ratios of tumor cells, Legrand *et al.* detected ECP, EDN, and TNF- α in the culture supernatants of eosinophils, and observed that dose-dependent EDN release was correlated with the increasing ratios of eosinophil/tumor cell [62]. They demonstrated that release of ECP, EDN, and TNF- α by eosinophils was potentially involved in tumor cell death [62]. In addition, the investigators also suggested that eosinophils could induce tumor cell apoptosis by a granzyme-dependent mechanism [62, 64].

Eosinophils Interaction with Other Cells

Eosinophils interact and modulate the function of a number of cells such as leucocytes, mast cells, endothelial and epithelial cells [62]. A case of diffuse-type gastric carcinoma was reported, in which cell clusters contained one mast cell and one to three eosinophils in the tumour stroma [65]. In this case, evidence of focal polarized exocytosis of mast cell granules and signs of important in situ activation of eosinophils in the junction areas was observed [65]. This study provided morphological evidence of the cross-talk between activated mast cells and eosinophils, which might play an important role in the enhancement of host immunity against cancer cells [65]. Meanwhile, the investigators also observed a link between eosinophils and $\gamma\delta$ T lymphocytes [66]. The $\gamma\delta$ TCR/CD3 complex activated-eosinophils induced ROS production and the release of cytotoxic granule proteins, which contributed to innate responses against tumors [66].

The Anti-tumor Effect of Eosinophils in Experimental Models

Syngeneic BALB/c mice were injected subcutaneously with IL-4-producing plasmacytoma cells after treatment with RB6-8C5 or isotype control antibody [67]. Antibody mediated depletion of eosinophils and neutrophils by RB6-8C5 eliminate interactions with cells of the monocyte-macrophage or lymphocyte lineages. As a result, a higher rate of tumor was found in the RB6-8C5 treatment group than control group, with a significant reduction in the number of infiltrating eosinophils in tumor tissues [67]. This study implicated eosinophils in IL-4-mediated tumor cytotoxicity. Moreover, the incidence of MCA-induced fibrosarcomas was studied in IL-5 transgenic mice, which have greatly enhanced levels of circulating eosinophils, CCL11-deficient mice that lack a key chemokine that recruits eosinophils into tissues, and the eosinophil-deficient mouse strains, IL-5/CCL11^{-/-} and Δ dblGATA [60]. The results showed a higher tumor incidence in the IL-5/CCL11 and Δ dblGATA mouse strains than IL-5 transgenic mice, which again suggested a potential role for eosinophils in tumor immune surveillance.

Table 1. Patents on Eosinophils and Eosinophilic Cytokines in the Diagnosis and Treatment of Cancer.

#	No. of Patent	Publication Date	Claim	Reference
1	WO2004064783	Aug 5, 2004	Methods of identifying women at increased risk of having ovarian cancer. Identification is based upon the extent to which biological samples derived from the women exhibit elevated levels of eosinophil-derived neurotoxin (EDN)	[68]
2	WO2004045364	Nov 25, 2004	Methods for diagnosing ovarian neoplasia, monitoring ovarian cancer therapy, and monitoring progression of disease in subjects based on the level of the proform of major basic protein in biological samples from the subjects are described	[69]
3	US7335354	Feb 26, 2008	A method for reducing tumor size by inducing eosinophil infiltrates within said tumor by elevating eosinophil serum levels with helminth antigen and then later by injecting that same helminth antigen, extract or killed ova into said tumor	[70]
4	WO2002089829	May 30, 2003	The presence of a single nucleotide polymorphism of eosinophil cationic protein has been correlated to an advantageous phenotype being a reduced disposition to cancer or cancer-related disorders	[75]
5	EP2004691	Dec 24, 2008	An antibody or an antigen-binding fragment thereof with specific binding activity for human interleukin-4 for the prevention and/or treatment of cancer	[76]
6	WO2002004009	Aug 21, 2003	Treatment of cancer involve administering an interleukin-4 antagonist to a patient diagnosed with cancer	[77]
7	WO2013077534	May 30, 2013	Protein p14 exhibits anti-cancer and anti-allergy activity, and a pharmaceutical composition for the treatment, management, alleviation, improvement or prevention of cancer and/or allergic diseases, comprising the same	[78]
8	US8362207	Jan 29, 2013	A compound comprising, in combination: a cell surface binding ligand or internalizing factor; at least one effector molecule; a cytosol localization element covalently coupled between said binding ligand and said at least one effector molecule; and a subcellular compartment localization signal element covalently coupled between said binding ligand and said at least one effector molecule	[84]
9	WO2005062972	Oct 13, 2005	The treatment of IL13 dependent neoplastic disorders comprising the administration of novel anti-IL13 antibodies, includes diagnosing such tumors or cancer using the antibodies of the present invention to detect overexpression of IL13 in the patient.	[85]
10	WO2009018429	May 14, 2009	Compositions and methods for treating or preventing cancer involving the use of a TNF- α antagonist, an IL-13R α 2 antagonist, and/or a AP-1 antagonist	[86]
11	WO2008115750	Sep 25, 2008	Compositions and methods for the treatment, diagnosis and prognosis of cancers by detecting the level of IL-13 expression in cancerous cells in the tissue microenvironment as an indicator of controlling the type of immune response mounted against the cancer	[87]
12	EP1711528	Jun 30, 2011	Treatment of IL13 dependent neoplastic disorders comprising the administration of novel anti-IL13 antibodies. The invention also includes diagnosing such tumors or cancer using the antibodies of the present invention to detect overexpression of IL13 in the patient	[88]
13	US20110158936	Jul 18, 2012	The use of interleukin 17E to inhibit tumour growth in a subject is provided. Also provided is the use of interleukin 17E in combination with one or more anti-cancer therapeutics for inhibiting tumour growth in a subject	[89]
14	US20130052157	Feb 28, 2013	Treating cancer with cytotoxic agents. Preferably, the cytotoxic agents are selected from: IL-25, BMP10, FGF1 1, VDBP, ATIII and IL1-F7, and any combination thereof.	[90]
15	EP1725261	Jan 26, 2011	Provided are methods of modulating cytokine activity, e.g., for the purpose of treating immune and inflammatory disorders, including tumors and cancer	[91]
16	EP2283860	Jun 5, 2013	Provided are methods of modulating cytokine activity, e.g., for the purpose of treating immune and inflammatory disorders, including tumors and cancer.	[92]

RELEVANT PATENTS ON EOSINOPHILS IN CANCER DIAGNOSIS AND TREATMENT

A number of patents on eosinophils and eosinophilic cytokines for the diagnosis and treatment of cancer have been awarded in recent years (Table 1). TATE has been observed in numerous human cancers as described earlier, thus the usefulness of eosinophilic cytokines for differential diagnosis of cancer has drawn increasing attention. Two patents (WO2004064783; WO 2004045364) have described that the measurement level of MBP and EDN in biological samples might be helpful for differential diagnosis of ovarian cancer, monitoring cancer therapy, and monitoring progression of disease [68, 69].

Besides diagnosis, patent related to eosinophils and eosinophilic cytokines are more widely used in the treatment against cancer. An interesting patent (US7335354) demonstrated a method for reducing malignant tumor sizes by eliciting activated eosinophils to infiltrate the tumor using helminth excretory and secretory antigens, helminth ova, and helminth extracts [70]. Eosinophils, frequently elicited by helminth antigen, are strongly associated with the Th2 immune response. The helminth excretory and secretory antigens elicit such a pronounced Th2 immune response, which activated eosinophils in the mammal immunological response to the tumor as if it were a helminth [70].

It is well known that gene polymorphisms are associated with the risk of cancer through modulating its protein expression [71-73]. A well-designed study has demonstrated that ECP gene polymorphism affects the functional properties of native ECP [74]. WO 2002089829 was an invention used in the treatment of cancer relates to a single nucleotide polymorphism in the ECP gene [75]. This polymorphism was correlated to an advantageous phenotype promoting reduced disposition to cancer or cancer-related disorders [75].

It should be noted that there were some patents on eosinophilic cytokines having been invented to combat cancer directly. EP 2004691 is a recently developed monoclonal antibody or antibody fragment characterized by a dissociation constant equal to or less than 2×10^{10} M for human IL-4 [76]. This invention can be used for the prevention and/or treatment of cancer by specific binding activity to human IL-4. Another interesting patent (WO2002004009) was generated by procedures involving immunization of transgenic mice [77]. This is an interleukin-4 antagonist that was administered to a cancer patient and was considered to treat cancer. The IL-4 antagonists not only bind IL-4, but also bind IL-4 receptors (IL-4R), thus inhibiting IL-4-induced signal transduction, and downstream biological effects that result from the binding of IL-4 to a cell surface IL-4R. Moreover, a recent patent (WO2013077534) demonstrated that synthesized IFN- γ can regulate the production of IL-4, which hence exhibits anti-cancer and anti-allergy activity [78]. It was declared for the treatment, management, alleviation, improvement or prevention of cancer and/or allergic diseases [78].

IL-13 is a Th2-type cytokine, secreted from CD4⁺ T cells, mast cells, basophils, and eosinophils [79-81]. In par-

ticular, IL-13 plays an important role in the regulation of IgE synthesis and eosinophil infiltration [80, 82, 83]. Recently, a new US patent (US8362207) was awarded to a compound comprising of the combination of a cell surface binding ligand or internalizing factor; at least one effector molecule; a cytosol localization and a subcellular compartment localization signal element that is covalently coupled between the cell surface binding ligand and the effector molecule [84]. Besides this patent, other patents concerning IL-13 including WO2005062972, WO2009018429, WO 2008115750, and EP 1711528, have been invented for treating or preventing cancer [85-88].

Recent reports have described that IL-25 and IL-33 play important roles in eosinophil recruitment [59]. Moreover, there is evidence that IL-33 induces the degranulation of eosinophils and elicits eosinophilia in the lung [59]. Considering involvement of eosinophils in the anti-tumor response, new patents on eosinophil recruitment cytokines IL-25 (US 20110158936; US 20130052157) and IL-33 were also invented (EP 1725261 B1; EP 2283860) for cancer therapy [89-92].

It was reported that clonal eosinophilia is associated with hematologic malignancies [93]. Mepolizumab, a monoclonal antibody against IL-5, has been used in patients with eosinophil diseases [94,95]. In a large clinical trial, mepolizumab was administered in patients with hypereosinophilic syndrome and resulted in reduction of blood eosinophil count [96].

CURRENT & FUTURE DEVELOPMENTS

Considering that allergic inflammation enhances tumor immunosurveillance, it was biologically plausible that a history of allergy might reduce the risk of cancer development [97-99]. Several epidemiological studies were conducted to date and concluded an inverse relationship between allergy-related conditions and cancer. It is noteworthy that most of these studies did not assess confounding factors at the patient level and incorporate them into the analysis. Therefore, association between allergy diseases and cancer risk remains conflicting rather than conclusive.

Eosinophils are recruited to tumor tissues and interact with tumor cells, through secreting granule protein and cytokines, or by presenting tumor antigens to activate other types of immune cells, which might be part of the process of its anti-tumor mechanism Fig. (1). However, eosinophils are mostly located in the respiratory tract and gastrointestinal mucosal, so they probably have a local rather than systemic anti-tumor effect. In the past years, a number of valid patents on eosinophilic proteins or cytokines in the treatment of cancer have been filed or awarded. Nevertheless, there is still a long way to understand the exact mechanism of the anti-tumor effect of eosinophils and to improve potential therapeutic drugs. More studies are therefore needed to investigate the association between eosinophils and cancer, as well as potential mechanisms of the anti-tumor effect of eosinophils in the future.

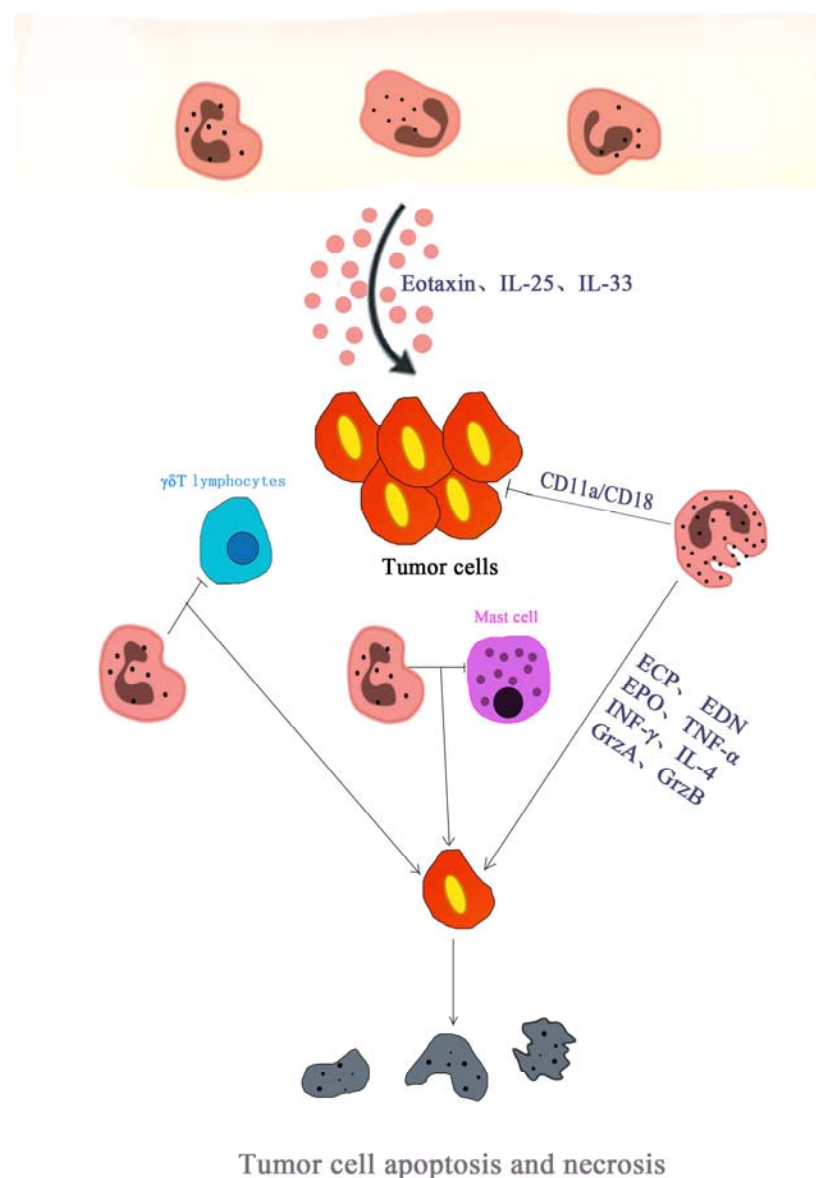


Fig. (1). The process of anti-tumor mechanism of eosinophils: eosinophils are recruited to tumor tissues and interact with tumor cells, through secreting granule protein and cytokines, or by presenting tumor antigens to activate other types of immune cells, thus induce tumor cell apoptosis. IL: interleukin; ECP: eosinophil cationic protein; EDN: eosinophil-derived neurotoxin; EPO: eosinophil peroxidase; TNF- α : tumor necrosis factor alpha; INF- γ : interferon gamma; GrzA: granzyme A; GrzB: granzyme B.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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