Review Article

Research progress on CD169-positive macrophages in tumors

Xianming Hou, Ge Chen, Yupei Zhao

Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China

Received December 9, 2019; Accepted April 25, 2020; Epub August 15, 2021; Published August 30, 2021

Abstract: CD169/Siglec1/sialoadhesin, a sialic acid-binding immunoglobulin-like lectin, is mainly expressed in metallophilic macrophages in the marginal zone of the spleen and in macrophages in the subcapsular sinus and medulla of lymph nodes. In addition to participating in anti-infectious immunity, recent studies have demonstrated that CD169+ macrophages are involved in tumor immunity and are associated with a favorable prognosis. The roles of CD169+ macrophages in tumors and the mechanisms of CD169+ macrophages and CD169 molecules involved in the tumor microenvironment and tumor immunity are described in this review.

Keywords: CD169, macrophages, tumors, immunity, tumor microenvironment

Background

Immune factors and immune cells play important roles at various stages in malignant tumor pathogenesis and progression. Lymphocytes and macrophages from different subpopulations play different roles in tumor progression. For example, cytotoxic T lymphocytes (CTLs), which primarily consist of CD8+ T-cells, inhibit tumors, while tumor-associated macrophages (TAMs) in the tumor stroma promote cancer. As an important inflammatory cell population in the tumor microenvironment, TAMs promote tumorigenesis, tumor growth, invasion and metastasis and affect tumor metabolism by various means [1] (Figure 1).

As a specific subpopulation of macrophages, CD169+ macrophages have been reported in recent studies and articles on malignant tumors. Most of the current research suggests that CD169+ macrophages inhibit tumors. This article reviews the recent research progress on CD169+ macrophages in malignant tumors and the specific mechanism of the CD169 protein.

Molecular biological characteristics of CD169

CD169, or siglec1, is a member of the sialic acid-binding immunoglobulin-like lectin family and belongs to the immunoglobulin superfamily. CD169 is a highly conserved protein in humans and mice. CD169 specifically recognizes NeuAcα2-3Gal at the N and C termini of the glycans or glycolipids on its ligands, but with low binding efficiency. In addition to sialylated pathogens, CD169 ligands include CD43 and MUC1. As a transmembrane protein, CD169 contains 17 immunoglobulin-like domains. Its extracellular segment is significantly longer than its intracellular segment, which does not contain tyrosine sequences, suggesting that CD169 cannot alter the cellular activation state. Current research has concluded that the main role of CD169 is to mediate the mutual adhesion and binding between cells to facilitate the phagocytosis of sialylated pathogens and structures [2-4].

CD169+ macrophages

Biological characteristics of CD169+ macrophages

CD169 is mainly expressed in metallophilic macrophages in the marginal zone of the spleen and in macrophages in the subcapsular sinus and medulla of lymph nodes Figure 2. Macrophages in bone, liver, lung, and small intestinal tissues also express CD169 at low
levels. When macrophages are exposed to transforming growth factor-β (TGF-β), they show dose-dependent downregulation of CD169. CD169 expression is remarkably upregulated when monocyte-derived macrophages are stimulated with type I or type II interferons or lipopolysaccharides. Mononuclear cell lines, primary monocytes, and human leukemic cell lines (THP-1) also express CD169 after exposure to interferon. The marginal cells adjacent to CD169+ macrophages in the lymph nodes can produce interferon-α (IFN-α).

CD169 mediates macrophage endocytosis on sialylated pathogens and bacteria in porcine and mouse models [2, 5, 6] and is also involved in hematopoiesis and B, T and NK cell activation [7]. CD169+ macrophages also possess T lymphocytes and antigen-presenting cell (APC) activities, when mice was exposed to dead tumor cells or bacterial glycolipids [8, 9]. In mouse models, CD169+ macrophages bind to gangliosides on the surface of the human immunodeficiency viral (HIV) and murine leukemia viral (MLV) envelopes in the draining lymph nodes.
CD169-positive macrophages in tumors

**Figure 2.** Quote from [8]. “The location of CD169-positive macrophages in lymph node (LN). The CD169+ macrophages are mainly located in the LN Sinus. CD11c, CD169 Double-Positive Macrophages are Located at the Boundary between the LN Sinus and the T Cell Zone or B Cell Follicle. LN were stained for CD169 (green), CD11c (red), and B220 (blue).”

**Table 1. Correlation of CD169+ macrophages with malignant tumors**

<table>
<thead>
<tr>
<th>Tumor types</th>
<th>Survival analysis</th>
<th>Disease Free survival (DFS)</th>
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<tr>
<td></td>
<td>Overall survival</td>
<td></td>
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<tr>
<td>High density of CD169+ macrophages in tDLNs</td>
<td>↑ (P=0.002)</td>
<td>-</td>
<td>Favorable TNM stage (P=0.049); Favorable TNM stage (P=0.029)</td>
<td>[32]</td>
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<tr>
<td>Prostate Cancer</td>
<td></td>
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<tr>
<td>Endometrial Carcinoma</td>
<td>↑ (P=0.0139)</td>
<td>-</td>
<td>Less LN metastasis (P=0.029); Less LN metastasis (P=0.03)</td>
<td>[15]</td>
</tr>
<tr>
<td>Colorectal Carcinoma</td>
<td>↑ (P=0.0092)</td>
<td>-</td>
<td>Low T classification (P=0.012); Less LN metastasis (P=0.03)</td>
<td>[14]</td>
</tr>
<tr>
<td>Breast Cancer</td>
<td>-</td>
<td>NS</td>
<td>Small tumor size (P=0.029); Less LN metastasis (P=0.001); Early clinical stage (P=0.009)</td>
<td>[17]</td>
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<tr>
<td>Liver cancer</td>
<td>↑ (P&lt;0.001)</td>
<td>-</td>
<td>Low AFP level (P=0.033); Favorable TNM stage (P=0.027)</td>
<td>[18, 19]</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>↑ (P=0.027)</td>
<td>NS</td>
<td>/NS</td>
<td>[19]</td>
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*↑" means survival rate improved. ‘-’ means no detailed information. ‘NS’ means No significant difference. AFP = α-fetoprotein. LN = lymph node.

nodes during viral infection, providing a route of viral capture [10-13].

**Correlation of CD169+ macrophages with malignant tumors**

Studies on the correlation between CD169+ macrophages and malignant tumors have demonstrated that CD169+ macrophages in lymph nodes and the tumor microenvironment mainly play various tumor-suppressing roles in malignant tumors (Table 1).

As a macrophage subpopulation primarily present in the subcapsular sinus of the lymph nodes and the marginal zone of the spleen, CD169+ macrophages have been studied in research on human tumors, primarily to investigate the interactions between regional lymph nodes and primary tumors. In regional lymph nodes, a high density of CD169+ macrophages is positively correlated with a good clinical prognosis for malignant tumors.

Some tumor patients show little but largely varied CD169 expression, which differs from that observed in murine tumor models [14]. High densities of CD169+ macrophages in regional lymph nodes were positively correlated with good clinical prognoses in colorectal
CD169-positive macrophages in tumors

cancer, endometrial cancer, and malignant melanoma. When CD169 expression in the subcapsular sinus macrophages in the regional lymph nodes was low or negative, colorectal cancer patients showed significantly reduced overall survival rates [14, 15].

In patients with endometrial cancer, the density of CD169⁺ macrophages in the subcapsular sinus in the lymph nodes was significantly correlated with overall survival, tumor grade, and lymph node metastasis. High CD169⁺ macrophage densities were significantly positively correlated with high overall survival rates in patients [15].

In patients with prostate cancer, high CD169 expression in their regional lymph nodes, prostate cancer led to significantly higher mortality rates in patients with low CD169 expression. Multivariate analysis showed that the death risk in patients was only related to CD169 expression in the lymph nodes and re-elevation of the prostate-specific antigen (PSA). Further studies showed that patients with re-elevated PSA and low CD169 expression in their regional lymph nodes exhibited drastically increased risks for death from prostate cancer than did those with high CD169 expression [16].

In breast cancer patients, high CD169⁺ macrophage densities in the sentinel lymph nodes were significantly associated with small tumor volumes, no lymph node metastasis, early clinical stages, and a low Ki-67 index. However, the CD169⁺ macrophage density was not significantly correlated with the clinical prognosis of the breast cancer. The density of the CD8⁺ lymphocyte infiltration in the tumor microenvironment was not significantly correlated with the density of CD169⁺ macrophages in the sentinel lymph nodes; however, the CD8⁺ lymphocyte density in the tumor tissues was also not significantly correlated with clinical pathological features such as tumor size [17].

A correlational study on tumors and CD169⁺ macrophages in the tumor microenvironment first showed that CD169 expression was lower in macrophages in tumor-affected tissues than in macrophages in normal tissues. This may be because tumor cells or macrophages that interact with tumor cells release TGF-β and anti-inflammatory cytokines.

In cancer tissues from patients with liver cancer, the macrophage density in the tumor tissues was significantly lower than that in the tumor-free regions, and the percentage of CD169⁺ macrophages was significantly decreased. However, high CD169⁺ macrophage density is associated with good overall survival in patients and is significantly correlated with a better clinical stage and low alpha-fetoprotein (AFP) levels. Multivariate analysis also suggested that the density of CD169⁺ macrophages in tumor tissues was an independent prognostic factor of overall survival of liver cancer patients [18, 19].

In contrast to liver cancer tissues, the number of macrophages in gastric cancer tissues was significantly greater than that in the tumor-free regions, but the percentage of CD169⁺ macrophages in the tumor tissues was also lower than that in the tumor-free tissues. CD204⁺ macrophages are associated with poor prognoses in liver and gastric cancer, while CD169⁺ macrophages are associated with good prognoses in both malignancies. Low CD204⁺ and high CD169⁺ macrophage densities are significantly correlated with longer overall survival rates in patients with these two malignancies [19].

**Mechanisms related to tumor immunity**

Research has shown that CD169⁺ macrophages mainly exert tumor suppressive effects in malignant tumors, and exploratory research on this mechanism has mainly focused on the following aspects.

Tumor cells can secrete extracellular vesicles. Extracellular vesicles include exosomes and other vesicles. Studies have shown that extracellular vesicles can create a premetastatic environment for healthy cells by transmitting oncogenic genes and nucleic acids. In tumor immunity, vesicles secreted by tumor cells can interact with immune cells through endocytosis, phagocytosis, and membrane fusion to degrade tumor proteins and present antigens [20, 21]. However, the specific roles of these interactions are unclear. Some extracellular vesicles can induce antitumor immunity after phagocytosis by dendritic cells (DCs), playing a role in inhibiting tumors, while some extracellular vesicles inhibit antitumor immunity.
Apoptosis causes cell fragmentation, which packs the cellular contents into exocrine secretory vesicles. Apoptotic vesicles produced from tumor cell apoptosis can both inhibit and induce immune responses to tumor antigens, and CD169 plays a key role in mediating this process (Figure 3). An in vivo study in CD169−/− mice showed significantly enhanced CTL responses to EL4-lymphoma-derived apoptotic vesicles (ApoVs) after sensitization with protein antigens, suggesting that CD169+ macrophages inhibit ApoV-associated antigens. This process is likely associated with inhibiting or modulating inappropriate immune responses.

Figure 3. CD169-Positive macrophages capture tumor derived extracellular vesicles. A. Quote from [31] “Genetically modified B16F10 melanoma tumor cells expressing a membrane-bound reporter, namely the vesicular membrane-associated protein CD63, fused with enhanced green fluorescence protein (CD63-eGFP). Multiphoton micrographs of tumor-draining lymph nodes (tdLNs) from CD63-eGFP+ B16F10 bearing mice (treated with PBS-Lip or Clo-Lip s.c.) and imaged at the indicated depth below the LN capsule (blue). Tumor-derived extracellular vesicles (tEVs) was shown as green fluorescence”. B. Quote from [34] “CD169 on subcapsular sinus macrophages prevents tumor derived exosomes decorated with sialic acid from accessing B cells that make pro-tumorigenic antibodies and possibly from accessing naive CD8+ T cells as well”. 
to autoantigens. In contrast, combining non-sensitized ApoVs with antigen-sensitized DCs significantly inhibited DC-mediated in vivo cytotoxicity, which occurred independently of CD169 expression. These results indicate that CD169 participates in antigen capture and immunosuppression in ApoV-mediated CTL immune responses. However, ApoVs inhibit DC-mediated immune responses independently of CD169 [22].

Muhsin-Sharafaldine et al. explored several aspects of the potential interactions between melanoma and CD169: (i) primary tumor growth, (ii) antitumor immune response induced by melanoma extracellular vesicles (apoptotic vesicles), and (iii) effects of local lymph node metastasis. No significant differences were found between these variables in comparing CD169-deficient mice and wild-type mice, indicating that CD169 molecules played insignificant roles in melanoma progression and the antitumor immune response caused by melanoma extracellular vesicles. However, macrophages remain in the marginal zone of the spleen and the subcapsular sinus of the lymph nodes, and their activities should be considered in current studies. Finally, studies indicated that CD169+ macrophages may have important effects on the immune response to melanoma, but this effect is unrelated to CD169 expression [23].

High numbers of infiltrating lymphocytes (especially CD8+ lymphocytes) in human tumor tissues are associated with good clinical prognoses in several malignant tumors. Recent studies in breast cancer models showed correlations between high numbers of infiltrating CD8+ lymphocytes in the tumor microenvironment and good outcomes from neoadjuvant chemotherapy [24-26]. These results suggest that CTLs are important mediators of antitumor immunity and human tumor cells. An in vitro study on the correlation between CD169+ macrophages and tumor cells in tumor-bearing mouse models showed that CD169+ macrophages phagocytosed tumor antigens and activated antigen-specific CD8+ cytotoxic T-cell proliferation [15].

A study in an early CD169-deficient mouse model showed that the lack of CD169 caused mild immune-related phenotypic changes such as a slight increase in CD8+ T-cells, a decrease in B220+ cells and remarkably decreased IgM in the spleen and lymph nodes [27]. Mice deficient in CD169 ligands showed fewer CD8+ T-cells, mainly due to apoptosis of the involved cells in the spleen [28].

Phagocytosis of the vesicles containing tumor antigens by CD169+ macrophages in the lymph nodes prevents the vesicles from contacting the DCs, thereby preventing the antigens from being presented to CTLs, thus inhibiting antitumor immunity [22]. Analysis via flow cytometry indicated that CD169+ macrophages in some lymph nodes could phagocytose dead tumor cells, whereas DCs could not [8]. In this murine model, CD169+ macrophages in the lymph nodes phagocytosed dead tumor cells and ultimately presented tumor antigens to CD8+ T-cells in the draining lymph nodes, thereby mediating antitumor immunity [8]. Studies have also found that CTLs mediated by DCs only respond to epitopes that evoke strong effects, whereas macrophage-induced CTLs respond to a wider range of epitopes [29].

In the tumor microenvironment, CD169 promotes CTL response. Zhang et al. found significantly fewer CD169+ macrophages in tissues with tumor infiltration than in tissues without tumor infiltration. Moreover, the number of infiltrating CD169+ macrophages was positively correlated with clinical prognosis. CD169+ macrophage infiltration in tumors enhanced the proliferation, cytotoxicity and cytokine production of CD8+ T-cells. This process is presumed to be related to CD43 on the T lymphocyte surface. CD43 can also act as a ligand for CD169, and both molecules may be coactivated to produce the above effects [18].

The CousEnS group found that B lymphocytes and humoral immunity participate in tumor development by activating M2 or tumor-promoting myeloid-derived cells. They also found that anti-CD20 monoclonal antibody, which can deplete B-cells, improved the efficacy of platinum- and paclitaxel-based chemotherapy in squamous cell carcinoma [30]. Complex mechanisms of cell-cell interactions exist among tumor cells, tumor-promoting B lymphocytes and macrophages.

An in vivo study in mice showed that CD169+ macrophages in the subcapsular sinus of the draining lymph nodes at the tumor site prevented extracellular vesicles derived from
malignant melanomas from reaching the lymph nodes, restricted the interactions between tumor extracellular vesicles and B-cells, reduced the production of tumor-promoting self-antibodies, and acted as a physical barrier or filter [31]. As a physical barrier, CD169+ macrophages in the subcapsular sinus of the lymph nodes prevent B-cells from assisting tumor progression and thus function in antitumor immunity (Figure 3).

Prospects and research directions

However, the published research results have not concluded how CD169+ macrophages participate in anti-tumor immunity or their specific mechanisms. So, the general directions that may related to clinical practice in this area mainly focus on three parts. Firstly, due to the broad expression of CD169 ligands on tumor-derived extracellular vesicles, studies have shown that CD169 may be involved in different kinds of tumor-derived vesicle uptake. This procedure may change pre-metastasis environment, depleting these exosomes from circulation system is a possible option for blocking cancer metastasis. Secondly, CD169+ macrophages are involved in activating CTLs and inhibiting the progression of tumor-promoting B-cells, thereby participating in antitumor immunity. CD169+ macrophages may cross present antigen to effector cells by taking up extracellular vesicles from different sources. This procedure of CD169+ macrophages may be used in predicting active immune therapy outcome. Thirdly, in recent years, increasing studies on the relationship between CD169+ macrophages and malignant tumors outcomes have revealed that CD169+ macrophages mainly inhibit tumors. More study needs to be carried out to prove that whether CD169+ macrophages can serve as a favorable prognostic marker and participate in more types of malignant tumors anti-tumor activities.

Discussion

Many existing studies have shown that different macrophage subtypes play different roles in malignant tumor evolution, tumor immunity, and prognosis. In recent years, increasing studies on the relationship between CD169+ macrophages and malignant tumors have revealed that CD169+ macrophages mainly inhibit tumors. At present, few biomarkers can be directly used to evaluate antitumor immunity in the body. Studies on the mechanism of CD169+ macrophages in the lymph nodes in tumor immunity should be conducive to exploring and evaluating the process and state of the body’s antitumor immune response to further investigate and predict the methods and effects of antitumor immunotherapy, especially in active immune therapy. CD169+ macrophages density, especially in the lymph nodes, may serve as a favorable prognostic marker.

Acknowledgements

This study was supported by grants from the National Natural Science Foundation of China (No. 31271565), the CAMS Innovation Fund for Medical Sciences (CIFMS, 2016-12M-3-005), and the Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (No. 2018PT32014).

Disclosure of conflict of interest

None.

Address correspondence to: Ge Chen and Yupei Zhao, Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China. E-mail: chenge@pumch.cn (GC); zhao8028@263.net (YPZ)

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