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Role of Innate Immunity in Colorectal Cancer

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Chemotherapy and surgical resection are the mainstay of cancer treatment. Particularly for chemotherapy, although it is effective method to care, sometimes cure various cancers, there are many different status of cancer not being controlled by chemotherapy such as recurrence and resistance to chemotherapy. In order to overcome those difficulties during cancer therapy, immunotherapy targeting immune cells and immune associated factors to enhance cancer immunity has been highlighted. Innate immunity plays important roles on initial stage of cancer immunity that are detecting, killing cancer cells and initiating adaptive immunity for cancer. So many basic and clinical studies to manage innate immunity for cancer therapy have been going on, and most of them were to stimulate innate immune cells including dendritic cell, macrophage, monocyte, and natural killer cell in various ways. They showed promising results but still there are many things to be resolved before clinical application. Herein, I review the role of innate immune cells and therapeutic trials for colorectal cancer.

Key Words: Innate immunity, Colon cancer, Immunotherapy

INTRODUCTION

Recently Cancer immunity is highlighted particularly in the area of cancer therapeutic approaches. There have been many reports saying that inflammation and immunity are deeply involved in cancer development, response to chemotherapy and prognosis. Clinical and epidemiologic studies have suggested a strong association between chronic inflammation and cancer such as alcoholic liver disease, chronic pancreatitis, inflammatory bowel disease, and COPD. Such observation suggests that chronic inflammation is involved in tumor initiation, promotion, and progression which means normal cells are genetically altered by chronic inflammation so that they become malignant and, because of the chronic inflammation, small clusters of malignant cells are stimulated to grow and finally chronic inflammation makes growing tumor more aggressive.

On the other hand, immune surveillance is anti-tumor activity in our body. At the Initial stage of immune surveillance, innate immune cells detect tumor antigens, and attack cancer cells and process the tumor antigens and present it to the other immune cells especially for T-cells. And through the processes, immune cells directly or indirectly kill cancer cells. Therefore, we need to reduce the immunologic effects of chronic inflammation and increase or normalize immune surveillance to get better therapeutic results after various types of cancer treatment including immunotherapy. And one more reason why cancer immunity is highlighted is clinical needs for new therapy for non-responsive cancer patients with conventional chemoradiotherapy. In this review, we are going to look into the role of innate immunity in colorectal cancer, and particularly focus on innate immune cells in tumor microenvironments in colorectal cancer (CRC).

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Innate Immune Cell Response to Cancer Cell

When we look at the normal immune responses to tumors, all the processes are similar to pathogen related immune responses. If there is a tumor cells, first step is to recognize cancer cell and cancer antigens by NK cell killing. And macrophages phagocytose tumor cell debris. NK cell react to tumor cell instantly and kill them and stimulate the other immune cells to be activated. Macrophages release related cytokines

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and present tumor antigen to T-cells in the tissue where they present. Dendritic cells also phagocytose tumor antigens, and move to lymph node and present tumor antigen to T-cells, which are CD4+, CD8+ T-cells. CD4+ T cells are going to induce Th1 immune reaction which is anti-tumor activity, and CD8+ T-cells are cytotoxic so they can kill tumor cell directly. These are the basic normal immune responses to tumor, and we want to keep these healthy immune reaction constant.[1] But things does not always go easy to what they are supposed to be.

An important step in tumor progression is the evasion and suppression of host immune response. In normal microenvironment, effector cells such as cytotoxic T Lymphocyte (CTL) and NK cells are able to drive potent anti-tumor suppressive activities. There are many kinds of immune cells and they can be classified by their characteristics. Epithelial cells, monocytes, macrophages, dendritic cells, neutrophils, eosinophils, basophils and mast cells belong to innate immune cell group. Most of T, B cells belong to adaptive immunity. Interestingly, there are some cells like NK cells, NKT cells, γδ T cells and innate lymphoid cells that show ambiguous characteristics. Among these innate immune cells, myeloid lineage cells which are macrophages and dendritic cells are major cell components that orchestrate immune responses to tumor.[2]

1) Dendritic Cells

Dendtiric cells (DC), the major antigen-presenting cells (APC), which play a pivotal role in the induction of anti-tumor cytotoxic immune responses and further link the adaptive and innate immune systems. Similar to other malignancies, CRC antigens evoke DC recruitment, maturation, and cytokine release in order to generate antitumor Th1 immune response and CD8+ CTL responses to attack cancer cells. DC present antigen using MHC class II to T cell through T cell receptor (TCR), and co-stimulatory molecules like CD28, CD 80, 86 are required to activate T cell properly.

Activated T cells secret interferon (IFN)-γ and upregulate antigen specific TCR and kill tumor cells. During these process, IFN-γ induce tumor cells to express PD-L1 which is the target for immune check point inhibition, in this review, I am not going to go deep into immune checkpoint inhibition, but now PD-1 monoclonal Ab is approved in FDA for MSI-H CRC patients to block the interaction between T cells and tumor cells. Tumor cell debris destroyed by immune cells are used by dendritic cells for the expansion of this immune reaction and these cyclic response is called antigen cascade.

However, CRC cells use diverse strategies to inhibit the tumor specific immunity in order to escape attack and prolong survival in the tumor microenvironment. These escape mechanism is multifactorial and involves also defects of dendritic cells. Some soluble factors derived from both tumors and associated cells within the tumor microenvironment that interfere DC differentiation from precursors, thereby contributing to a loss of stimulatory APC activity in colon cancer patient, which includes decreased uptake, processing and presentation of antigens, lowered expression of costimulatory signals, inefficient migration toward specific chemokines, and decreased production of IL-12.

Among cytokines, IL-10 plays important role in DC defects. It blocks the monocyte-to-DC differentiation, driving the differentiation process toward the macrophage lineage and impair the potent APC function. Elevated levels of VEGF associate with increased number of immature APC with a suppressive phenotype in the circulation of cancer patients. Transforming growth factor (TGF)-b, produced by tumors, has been demonstrated to immobilize dendritic cells within tumors and inhibit DC migration from tumors to their draining lymph nodes.

The inhibition of differentiation of DC leads to develop regulatory DC. They show a weak capability to activate effector T cells, whereas they induce regulatory T cell (Treg) proliferation in order to promote immune tolerance.

These Loss of APC function associated with suppressing DC differentiation may significantly limit the induction of anti-tumor immune responses and contribute to tumor immune escape.[3]

There was a report evaluating the infiltration rate of mature and immature DC in colon cancer.[4]

They showed that patients with locally advanced tumors had significantly lower infiltration with matured DCs in tumor stroma, and in the invasive margin. The frequency of distant metastases was significantly associated with lower infiltration with mature DCs in invasive margin. On the survival data for tumor invasive margin infiltration with CD83-positive DCs which is maturation marker for DC, Mean survival of patients with high infiltration with mature DCs was significantly higher than that of the patients with low infiltration with CD83-positive DCs. This study claim that mature, well-functioned DC may be a prognostic marker for CRC and that keeping normal differentiation and maturation of DC, and function of DC seemed important in CRC patients.

Not only in the colon cancer tissue but also in the blood of CRC patients, circulating DCs showed significant changes in stage III-IV advanced CRC patients. There was a report showing a significant reduction of the DC number in total

and advanced CRC patients compared to healthy control. This reduction was totally recovered after complete resection of cancer, suggesting that a systemic immunosuppressive effect done by the cancer toward circulating DCs.[5]

2) DC Vaccine

Because of the relevance between DCs and CRC, as you saw in previous data, DC are considered as promising candidates in cancer vaccines for CRC patients. The goal of cancer immunotherapy is to induce efficient antigen specific CD4+ and cytotoxic CD8+T cells mediated anti-tumor immune response.

CRC is a good candidate for immunotherapy like DC vaccines because CRC express numerous tumor associated antigens. Such as CEA, WT1, mucin1 MAGE or P53. The concept of DC vaccines is as follows, monocyte in circulation are isolated with leukapheresis from CRC patient, differentiate them with growth factor such as granulocyte monocyte colony stimulating factor (GM-CSF) into mature DC. The DCs are challenged with Tumor antigen from the patients to get tumor specificity and they become specific tumor antigen loaded DCs. Tumor antigen loaded DC is a potent, and has ability to induce Th1 polarization and decrease the induction of T reg cell and myeloid derived suppressor cell (MDSC), which are immunosuppressive. DC vaccine that is ready is infused into the patient and induce anti-tumor activity to specific cancer.

There were some clinical trials about DC vaccine for cancer therapy.[6] They made a autologous DC vaccine modified with CEA and MUC1 antigens and compared the effects with GM-CSF as a control.

After DC vaccine injection, there was little bit more CEA specific immune response in DC vaccine patients, 26% versus 13%, but it was not significant. And recurrent free probability in CRC patients based on the presence of immune response, it looks little bit better in the patients with immune response during initial period, but disappointingly, the study ended up with no significant differences between two groups.

So far, In spite of available data, unfortunately DC vaccine results were not that good enough.

There are some thinkable reasons for that, even though we inject the activated DC which is antigen specific, tumor microenvironment that is not favorable to normal immune activation is still present around tumor tissue and injected DC might lose their activity in the tumor microenvironment. So to get further improved strategies we may need to use combination immunotherapeutic to modify tumor microenvironment, and other adjuvant to promote full activation of DC before injection while modifying DC with antigen. Now DC vaccine for CRC is on the investigation to find more efficient protocols and possible candidates.

3) Macrophages

Monocyte-macrophage lineage are one of the major components of the leukocyte infiltration in tumors. Macrophage which is recruited to tumor microenvironment are generally divided into 2 subtypes, M1 and M2 macrophages. M1 has antitumor activities secreting TNF-a, IL-12, reactive nitrogen, and oxygen intermediates. And it activates Th1 immune response which in turn stimulate tumor specific CTL. However, during tumor progression, macrophages shift towards M2 phenotype induced by the exposure to IL-4, 13, and IL-10 which are supplied from the tumor microenvironment.[2] And also tumor cells and other cells of tumor microenvironment recruit monocytes from circulation then differentiate into M2 macrophage. They secret large amount of growth factors, inflammatory factors, angiogenic factors and metalloprotease protein which in turn all together promote progression of tumors. These macrophage supporting tumor progression are called tumor associated macrophage (TAM). And TAMs are considered markers of poor prognosis in most cancers. And there have been also some reports saying TAM are associated with poor survival in CRC patients.

There are many signals from tumor microenvironment that make TAM immunosuppressive. Hypoxia and some chemokines and cytokine from tumor cells, and other soluble factors from Th2 cell and B cell affect TAM. Then TAM can show these abilities enhancing angiogenesis, proliferation, protecting cancer stem cell, invasion and metastasis, genetic instability and taming adaptive immunity.

TAM secrets and interacts with T cells and produces IL-10, TGF-b, arginase, IDO, prostaglandins that inhibit DC maturation and suppress T cells. Even more, TAM can express PD-L1 that inhibit T cell activation. So there are many immunologic agents being developed to normalize the interaction between TAM and tumor cell. And other agents to block the soluble factors from tumor microenvironment that make TAM protumaral and chemokines for TAM recruitment into the tumor tissue.[7]

Based on the most studies on association between TAM and cancer, TAM is immunosuppressive, and has protumoral activity, so some study claim that TAM is poor prognostic factor in CRC. But there are controversies for this topic, because there are also another studies mentioning anti-tumor activity of TAM in CRC as opposed to the others studies. Bogel, et al.[8] took the blood from normal patients, and isolate circulating monocytes and culture them either in breast cancer

cell supernatant or CRC cell supernatant. When the monocytes were cultured with colon cancer supernatant, they secreted much higher pro-inflammatory cytokines which have anti-tumor effects, and much less IL-10, anti-inflammatory cytokines, which have immunosuppressive effects. This means TAM in CRC microenvironment might have anti-tumor activity.

Another study was showing that the frequency of conventional (pro-inflammatory) macrophages was significantly increased in tumor tissue compared to healthy bowel tissue. And "Homeostatic" gut resident macrophages were decreased, "inflammatory" gut resident macrophages were increased. So they suggested that macrophages provide a pro-inflammatory environment within the colon tumor tissue and may explain why these cells are often associated with improved patient outcome in colorectal cancer.[9]

And in other papar evaluating CRC with liver metastasis patients, if there were high TAM infilaration that was CD68+ cells, disease free survival was improved compared to the patients who had lower infilaration of CD68+TAM.[10]

Long story short, TAM in CRC has still controversies that there is a gap between clinical data and mechanistic studies and furthermore, some results were opposite. So there are things to be elucidated where this discrepancy is coming from. It is probable that diverse roles of TAMs might be influenced by the different stages of CRC progression. Anyway, further studies are required to understand how the macrophage phenotype changes at different stages of CRC tumorigenesis and development.

4) MDSC (Myeloid Derived Suppressor Cell)

Myeloid suppressor cells are heterogeneous and immature subset of circulating myeloid cells. These cells can differentiate into macrophage, granulocyte, dendritic cells under physiologic condition, but under pathologic conditions, the differentiation of these cells is inhibited and resulting accumulation of MDSC in tumor microenvironment.

The regulatory function of MDSC in inhibiting anti-tumor immunity has been extensively shown in both in vitro and in vivo studies. MDSC inhibit both antigen specific and non-specific T cell activation in murine model. MDSC inhibit T cells by depletion of L-arginine within the tumor microenvironment, thus arresting T cells in G0-G1. Similarly, MDSC inhibit T cell activation by sequestering cystine. This makes T cells being unable to obtain cysteine, which is essential for antigen activation, proliferation, and differentiation.[11]

MDSC can also promote regulatory T cell which is immune suppressive. MDSC secret cytokines that polarize macrophage into M2, TAM that enhance tumor progression. And reduced CD4+ and CD8+ T cell homing to lymph nodes is affected by MDSC.

There was a study that showed accumulation of MDSC promote CRC progression. SMAD4 binds directly to the promotor region of CCL15, which is the chemokine that recruit CCR1+ MDSC, and negatively regulate the expression of CCL15. So if SMAD4 is deficient, CCL15 expression increase. And this would recruit CCR1+ MDSC in CRC tissue. When they inoculated CRC cell line into rectal mucosa of nude mice, in SMAD4 deficient cell line injected mice, where MDSCs were accumulated in CRC, CRC showed progression which means accumulation of MDSC promote CRC progression and CCL15 may become a prognostic marker and therapeutic target for MDSC in CRC as well.[12]

Another study was to see the effects of MDSC targeted peptibody therapy in multiple tumor models. When they apply antibody targeting MDSC, there were significant effects that number of MDSD in blood was decreased and tumor progression was also decreased.[13] MDSC is also considered as a marker and therapeutic target.

5) Natural Killer Cell

NK cell is an innate immune cell, heterogenous lymphocyte population that can kill cancer cells directly and promptly. Normal cell express MHC class I, and that matches to the inhibitory receptor on the surface of NK cells. Then NK cell does not do anything. But if there is no MHC I on the cells such as tumor cells, inhibitory pathway is not activated, then NK cell release granule to attack the cell that does not have MHC I.[14]

There was a study evaluating NK cell activity in CRC risk patients. They perform colonoscopy to identify CRC and they check level of IFN-g which is the cytokine secreted from NK cells. When they analyze the results, NK cell activity was significantly decreased in CRC patients compared to the patients without CRC. And The NK cell activity test identified subjects with CRC with 87.0% sensitivity, 60.8% specificity, a positive predictive value of 5.7%, and a negative predictive value of 99.4%. The odds ratio for detection of CRC in subjects with low NK cell activity versus subjects with higher NK cell activity was 10.3.[15] This study have shown that the association between NK cell activity and CRC quite clearly, but there are some important question firstly they did not mention whether the NK cell activity was a cause for CRC or effects from CRC. So we don't know low NK cell activity would be the cause of development of CRC. And secondly, patient's characteristics were hetero-

geneous. So there might be another reason for the patients to get lower level of NK cell activity. Anyway, they have shown the possibility of new screening marker by using innate immune cell activity and of new therapeutic target enhancing NK cell activity for CRC patients as well.

Concluding Remarks

CRC is a heterogeneous disease with genetic and epigenetic characterization such as mutation of oncogenes, microsatellite instability phenotype, chromosomal instability pathway, CpG island methylator phenotype and DNA hypomethylation. These genetic and epigenetic characterization lead to multiple mutations of oncogenes, resulting in immunogenic CRC. So some CRC patients have higher immunogenicity but others don't have it. For example MSI -H CRC patients are the one that can have the good results from anti-PD1 immunotherapy. MSI-L, MSS CRC patients don't have advantage from that.

Innate immune cells can have both pro- and anti-tumor effects depending on tumor microenvironment. Blocking pro-tumoric mechanism and enhancing anti-tumoric mechanism would be the target for colon cancer immunotherapy. Clinical data from innate immune-centered colon cancer therapy is not that satisfied so far. However, immunotherapy is a promising tool in colon cancer treatment but further studies are needed. Developing more efficient protocols and defining possible candidates for immunotherapy would be important to get better results of immunotherapy.

In the immune reaction, particularly in chronic immune response, there are some compensation effects through microenvironmental changes such as cytokine milieu, cell to cell interactions, and various feedback mechanisms, heterogeneous cell population and diverse response based on the change of microenvironment, so many variables are in the colon cancer and immune war fields. So multi-way combination including conventional cancer therapy would be better strategy for patients. We could put agent targeting the tumor cell directly and also we could add some agent to make microenvironment favorable to cancer therapy.

REFERENCES

1. Kerkar SP, Restifo NP. Cellular constituents of immune escape within the tumor microenvironment. Cancer Res 2012; 72:3125-3130.
2. Striz I, Brabcova E, Kolesar L, et al. Cytokine networking of innate immunity cells: a potential target of therapy. Clin Sci (Lond) 2014;126:593-612.
3. Legitimo A, Consolini R, Failli A, et al. Dendritic cell defects in the colorectal cancer. Hum Vaccin Immunother 2014;10: 3224-3235.
4. Gulubova MV, Ananiev JR, Vlaykova TI, et al. Role of dendritic cells in progression and clinical outcome of colon cancer. Int J Colorectal Dis 2012;27:159-169.
5. Orsini G, Legitimo A, Failli A, et al. Quantification of blood dendritic cells in colorectal cancer patients during the course of disease. Pathol Oncol Res 2014;20:267-276.
6. Morse MA, Niedzwiecki D, Marshall JL, et al. A randomized phase II study of immunization with dendritic cells modified with poxvectors encoding CEA and MUC1 compared with the same poxvectors plus GM-CSF for resected metastatic colorectal cancer. Ann Surg 2013;258:879-886.
7. Mantovani A, Marchesi F, Malesci A, et al. Tumour-associated macrophages as treatment targets in oncology. Nat Rev Clin Oncol 2017;14:399-416.
8. Bogels M, Braster R, Nijland PG, et al. Carcinoma origin dictates differential skewing of monocyte function. Oncoimmunology 2012;1:798-809.
9. Roslyn Kemp SN, Edward Taylor, Elliott Dunn, Fran Munro, Michael Black and John McCall. Colorectal tumoru associated macrophages are more pro-inflammatory than adjacent control bowel tissue macrophage populations (TUM6P.1003). J Immunol 2015;194(1 Supplement):1.
10. Cavnar MJ, Turcotte S, Katz SC, et al. Tumor-Associated Macrophage Infiltration in Colorectal Cancer Liver Metastases is Associated With Better Outcome. Ann Surg Oncol 2017; 24:1835-1842.
11. Najjar YG, Finke JH. Clinical perspectives on targeting of myeloid derived suppressor cells in the treatment of cancer. Front Oncol 2013;3:49.
12. Inamoto S, Itatani Y, Yamamoto T, et al. Loss of SMAD4 Promotes Colorectal Cancer Progression by Accumulation of Myeloid-Derived Suppressor Cells through the CCL15-CCR1 Chemokine Axis. Clin Cancer Res 2016;22:492-501.
13. Qin H, Lerman B, Sakamaki I, et al. Generation of a new therapeutic peptide that depletes myeloid-derived suppressor cells in tumor-bearing mice. Nat Med 2014;20:676-681.
14. Morvan MG, Lanier LL. NK cells and cancer: you can teach innate cells new tricks. Nat Rev Cancer 2016;16:7-19.
15. Jobin G, Rodriguez-Suarez R, Betito K. Association Between Natural Killer Cell Activity and Colorectal Cancer in High-Risk Subjects Undergoing Colonoscopy. Gastroenterology 2017;153:980-987.