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Dendritic cells and their associated pro-inflammatory cytokines augment to the inflammatory milieu in vitiligo skin

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ABSTRACT

Background and Aim: Vitiligo is a progressive, autoimmune, hypomelanotic acquired disorder of skin which is characterized by depigmentation. The initial immunological events of this diseases are still at enigma that includes breach of immune tolerance, and defect in antigen presentation. Hence, we aimed to explore role of Dendritic cells (DCs) and its associated cytokines in the pathogenesis of generalized vitiligo (GV) patients.
Methodology: For this case-control study, 20 active patients and controls were enrolled. Phenotypic characterization of myeloid and plasmacytoid DCs (mDCs, pDCs) were done by flow-cytometry. Primary culture of DCs was done by monocyte differentiation supplemented with rIL-4 and rGM-CSF. Functional analysis DCs related cytokines and co-stimulatory molecules (CD80, CD40) was done by ELISA and qPCR respectively. Tissue localization of DCs was evaluated by immunohistochemistry.
Result: The frequency of mDCs (0.3715% *v/s* 0.188%) and pDCs (0.2331% *v/s* 0.1156%) were elevated in patients as compared to controls. Circulatory level of IL-12, TNF-α were significantly higher whereas IFN-α was decreased in patients than controls. Similar results were obtained in the culture supernatants of patients. Relative mRNA expression profiling of co-stimulatory molecules (CD80, CD40) were found to be up regulated in patient's skin. Tissue localization of Langerhans cells (Langerin, $CD1a^{+}$) were found to be significantly higher in patients.
Conclusion: Elevated frequency of mDCs and pDCs along with elevated levels of IL-12, TNF-α and CD80, CD40 may contribute in defective antigen presentation of DCs. Altered pro-inflammatory and anti-inflammatory cytokines along with tissue localization of Langerhans cells might be involved in the pathogenesis of GV. These DCs associated cytokines can be explored as a therapeutic target in future.

1. Introduction

Vitiligo is a progressive, idiopathic and hypomelanotic acquired disorder of skin. In this disease, defect or lack of melanosomes in melanocytes is evident at derma-epidermal basement membrane of skin, resulting in the clinical appearance of white patches [1]. The prevalence of vitiligo is 0.5–1% globally, whereas in India, 8.8% of population suffered from this disease which recorded to be highest in the world [2,3]. Vitiligo is a complex and multifactorial disease. Its pathogenesis involves several mechanisms such as oxidative stress, neural peptides and immune response to self-antigens [4-8]. One of the established mechanisms suggests that vitiligo is an autoimmune disease of skin where auto antibodies are generated against the melanocytes [9]. Aberrant immune response and defect in the initial step of the immune response i.e. antigen presentation might be complementing to the pathogenesis of vitiligo.

Dendritic cells (DCs) are well-defined antigen presenting cells of the immune system that also maintains the tolerance. It also recognizes, processes the antigen and modulates the activation of the T-cells. These activated T cells differentiate into mature Th1 and Th2 cells under the influence of cytokines such as IFN-α, TNF-α, and IL-12 [10]. Human DCs are mainly classified on the basis of surface markers, transcription factors, and tissue distribution. In Human DC cell subsets are mainly recognised as the Langerhans cells, Plasmacytoid DCs (pDCs), myeloid DCs (mDCs) [11]. mDCs are characterized by higher expression of the HLA-DR and CD11c. These cells activated by LPS and they secrete interleukin-

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Distorted frequency of dendritic cells and their associated stimulatory and inhibitory markers augment the pathogenesis of pemphigus vulgaris

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Abstract

The objective of this study was to investigate the frequency and functionality of DCs and its associated stimulatory and inhibitory markers in the pathogenesis of PV Active PV patients (n = 30) having both skin and oral lesions, and 30 healthy controls were recruited in the study. The frequency of DCs was determined by flow cytometry followed by the primary culture by using recombinant IL-4 (250 IU/ml) and GM-CSF (600 IU/ml). The culture supernatant was used for ELISA. RNA was isolated from sorted DCs and used for the mRNA expression of DC-associated stimulatory (CD40 and CD80) and inhibitory (PSGL1 and ILT3) markers. Tissue localization of Langerhans cells was done by immunohistochemistry. In this study, altered frequency of myeloid DC (mDC) and plasmacytoid DC (pDC) was seen in the circulation of PV patients. The primary culture of patient-derived DCs showed anomalous cytokine profiling. In the culture supernatant of DCs, elevated levels of TNF-α and IL-12 were detected in PV patients. Meanwhile, reverse trend was found in the case of IFN-α and IL-10 cytokine levels. Similarly, a discrepancy in the expression of DC-associated stimulatory (CD40 and CD80) and inhibitory (PSGL1 and ILT3) markers suggested their possible involvement in the immunopathogenesis of PV. An elevated number of tissue localizing Langerhans cells was also observed in the perilesional skin. This study indicates the distorted frequency and functionality of DCs in the immunopathogenesis of PV. Targeting these functional markers in the future may generate novel therapeutic options for better management of PV.

Keywords: Co-stimulatory markers (CD40, CD80); Dendritic cells; Inhibitory markers (ILT3, PSGL1); Pemphigus vulgaris.

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RESEARCH

KCMF1 regulates autophagy and ion channels' function in renal cell carcinoma: a future therapeutic target

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Abstract

Introduction In RCC, systematic procedures such as surgery, chemo-radiation therapy, and application of target-based inhibitors increase the risk of several comorbidities such as chronic kidney disease, hemorrhage, and cardiac arrest that may increase the mortality rate. Even though immune-based checkpoint inhibitor therapies have an overall good response rate, it is restricted to only 30–40% of patients. Hence, an in-depth study of tumor pathophysiology in RCC is needed to identify the new therapeutic target. In RCC, persisted hypoxia is an essential phenomenon for tumor growth and progression. KCMF1 is a newly identified ubiquitin ligase whose domain interacts with destabilized proteins and reprogrammed the ubiquitin coding for lysosome-mediated degradation and autophagy under hypoxic conditions/oxidative stress and maintaining cellular homeostasis. But in RCC, the functional role of KCMF1 remains undefined to date.

Method We determined KCMF1 and its associated proteins RAD6 and UBR4 expression and their co-localization using confocal microscopy in tumor and non-tumor tissues samples. Further, immunofluorescence staining was performed to determine autophagy (LC3B, p62), hypoxia-inducible factor (HIF-1A) and ion channel markers (Kv1.3, KCNN4) in RCC patients (n−10). Inductively coupled plasma mass spectrophotometry (ICPMS) was performed to estimate the concentration of potassium (K^+), sodium (Na^+) and Zinc (zn^{2+}) in tumor and non-tumor cells of RCC patients (n−20). Lastly, images were analyzed using ZEN3.1, and ImageJ software.

Result and conclusion We observed a discrepancy in the formation of ubiquitin ligase, autophagosome via KCMF1, and ionic concentration in tumor cells, which might be one of the possible factors for cancer evolution. KCMF1-associated ubiquitin ligase system could be considered as a novel therapeutic target for RCC in the future.

Keywords KCMF1 · Ubiquitin ligase · Autophagy · Ions · Renal cell carcinoma

Introduction

According to the (Sung et al. 2021), there is an annual increment of 2% of new cases of renal cell carcinoma (RCC) worldwide (Sung et al. 2021). Till date, cytoreductive nephrectomy (CN) is still considered a gold-standard procedure to treat advanced RCC (Esagian et al. 2021). The CARMENA (Méjean et al. (2018)) and SURTIME (Swami etal. 2019)) phase III and phase II, respective clinical trials are a proof of it and also support nephrectomy for advanced RCC patients (Méjean et al. 2018; Swami et al. 2019). Nonetheless, surgery increases the comorbidities such as chronic kidney disease, hypertension, and morbid cardiac events which elevate the mortality rate in patients (Capitanio and Montorsi 2016; Abraham et al. 2016; Qu et al. 2016). Although plethora of therapies are available to treat RCC, none can cross the first-line, second-line, third-line, and fourth-line of treatment settings in RCC including checkpoint inhibitor therapy, restricted to a few patients (Yip et al. 2018; Ross and Jones 2017). Hence, identification of new targets is needed which can manage the tumor growth and severity in

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Disruption in networking of KCMF1 linked ubiquitin ligase impairs autophagy in CD8$^+$ memory T cells of patients with renal cell carcinoma

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ABSTRACT

Metastatic Renal Cell Carcinoma (mRCC) remains incurable, despite the current checkpoint-blockade-driven, limited overall response rate. The CD8$^+$ memory T cells can mount a rapid and an effective response. The ubiquitin ligase RAD6-KCMF1-UBR4-mediated regulation of autophagy in CD8$^+$ memory T cells in patients with renal cell carcinoma (RCC) remains unexplored. Consequently, flow cytometry was used to study memory T cells, and their subsets, including activation and regulatory phenotypes in peripheral blood mononuclear cells (PBMCs). Expression of the ubiquitin ligase and autophagy was measured both at the cellular and molecular levels in memory T cells of patients with RCC. JC.1 staining and Annexin/PI assays were used to evaluate the memory T cells depolarization and apoptosis rates. The results indicated that the disruption of Ub-E2-E3 complex and impaired autophagy in memory T cells diminished their ability to survive and combat against tumor cells. Inhibition of memory T cells apoptosis by targeting E3 ubiquitin ligase or autophagy pathways can be explored as a potential therapeutic strategy to improve the long-term survival of memory T cells in RCC.

1. Introduction

RCC is the most common kidney cancer, and treatments like radical and partial nephrectomy, as well as target-specific inhibitors for VEGF/PDGFR/mTOR pathway, have been utilized to increase disease-free survival in metastatic renal cell carcinoma [1]. However, these therapies are often inadequate and can lead to recurrence or progression of the cancer [2–5]. Recent studies conducted by the mRCC database consortium showed that the overall response rates (ORR) of checkpoint inhibitor therapies such as PD-L1 inhibitor (nivolumab) alone or in combination with VEGF inhibitor were between 31% andto 40% in patients. Similarly, ORR for second-line, third-line, and fourth-line treatments were reportedly 22%, 24%, and 19%, respectively [6–9], and despite the promising efficacy of these treatments, further research is needed to improve existing outcomes. ORR of checkpoint inhibitors therapies have not been met from the first line to fourth line of treatment in mRCC, indicating the current treatment regimen for RCC is inadequate in providing long-term durable response. Therefore, developing a therapeutic strategy with an effective, efficient and long-term immune response within the tumor microenvironment, is warranted.

CD8$^+$ memory T cells play a critical role in the body's immune response to pathogens and tumors, possessing properties of long-term maintenance and survival with involvement in antigenic stimulation, thereby maintaining immune memory [10]. However, CD8$^+$ memory T cells may become dysfunctional and lose their anti-tumor potential [11–13]. Understanding the mechanisms behind the maintenance and deterioration of CD8$^+$ memory T cells is critical for developing strategies to harness their full potential in fighting renal cancer. Potassium channel modulatory protein (KCMF1), a zinc finger protein, aids in regulating the turnover of misfolding protein aggregates and cellular organelles through the ubiquitination pathway under stressful conditions. KCMF1 protein interacts with Ubiquitin-conjugating enzyme E2 A (UBE2A/RAD6) and Ubiquitin Protein Ligase E3 Component N-Recognin 4 (UBR4) at the C- and N-terminal domains respectively, forming a ubiquitin ligase E2-E3 complex. The unique E2-E3 complex with a RING domain can reprogram the ubiquitin code of the destabilized proteins, redirecting them towards lysosomes for efficient degradation via autophagy instead of relying on UPS (Ubiquitin Proteasome system) [14,15]. The potential contribution of this complex in regulation of autophagy in CD8$^+$ memory T cells in RCC currently remains unexplored.

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Lymphoid tissue inducer cells in cancer: a potential therapeutic target

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Abstract

Tumor cells are dynamic in nature; these cells first acquire immune surveillance and then escape from the immune system. Hence, progressed cancer cells distribute and metastasize to other organs via blood vessels as well as from the lymphatic system. Prognosis and treatment of metastatic cancer patients remain a major challenge nowadays. Till now, lots of target -based and immune checkpoint blocker therapies are used to treat disease patients. But these therapies fail to control the dissemination and metastasis of cancer. Before designing a treatment regimen for metastatic patients, understanding the mechanism of tumor cells spreading within lymph vessels remain undetermined. Construction of lymphoid structures since embryonic to adult stage are depend upon LTi. Foundation of lymph node, payer patches and TLO is initiated and regulated through these cells in any part of the body. During tumor growth, newly developed lymph node contained MDSCs and Treg cells which inhibit the immune response and promote tumor invasion and metastasis. LTi reconstituted lymph node can be used for both early and high risk detection of cancers. High and low risk of tumor growth and invasion depend upon the location and composition of immune cells within lymph nodes. However, LTi are not reported as predictive marker in cancer till date. Recent reports in cancer indicate that LTi cells are engaged in the spreading of tumor cells into a lymphatic vessel. Through this review we are trying to brief the development and role of the LTi in immune system during homeostasis and cancer.

Keywords: Cancer; Lymphoid tissue inducer cells (LTi); Secondary lymphoid organ (SLO); Tertiary lymphoid organ (TLO).

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