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DENDRITIC CELLS IN REGENERATIVE MEDICINE: FROM THE VIEW OF METABOLOMICS

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ABSTRACT
Dendritic cells (DCs) are professional antigen-presenting cells that are central regulators of the adaptive immune system. DCs process and present the captured antigen via major histocompatibility complexes (MHC), initiating the antigen-specific immune responses, which is crucial to the performance of biomaterials in regenerative medicine. The connection between metabolism and DC functions has been recently established. Though with decades of research, much detail surrounding DCs remains unclear. Metabolomics, focusing on small molecular metabolites and their functions in the organism, has been applied in research of DCs, providing new evidence of metabolites affecting the maturation of DCs. This new knowledge could provide some new insights into designing and modifying biomaterials, promoting the development of regenerative medicine.

KEYWORDS: Dendritic cells; Regenerative medicine; Metabolomic studies.

INTRODUCTION
Dendritic cells (DCs) are known as the professional antigen-presenting cells (APCs) derived from bone marrow and play a central role in the immune system.[1] The discovery of DCs in 1973 was awarded the Nobel Prize for Medicine in 2011.

Immunity is the result of a complex interaction between the innate immune system and the adaptive immune system (antigen-specific).[2] DCs are capable of sensing their environment to capture invading antigens and to present the relative information to lymphocytes, especially T cells. Therefore, DCs provide an essential bridge between innate and adaptive immune responses.

Regenerative medicine suggests a novel treatment for diseases by restoring normal functions of cells, tissues, or organs.[3] Biomaterials implanted in the body consist of a large part of regenerative medicine, and their performances highly depend on the host response which DCs play an important part in by maturation and antigen-presentation.

Although after decades of research, much detail of DCs remains unclear. The connection between metabolism and the functions of DCs was recently established.[4] Metabolomics is a rising omics focusing on small molecular metabolites in cells, tissues, or the whole organism, which has been widely applied, especially in the studies of metabolic diseases. Since it is particularly powerful when investigating metabolism, metabolomics shows great potential in providing new knowledge for DCs.

Dendritic Cells Subsets
DCs, which originated from common dendritic cell progenitor (CDP), can be divided into conventional DCs (cDCs) and plasmacytoid DCs (pDCs).[5] Conventional DCs can be further divided into conventional type 1 DCs (cDC1) and conventional type 2 DCs (cDC2). Every subset of DCs expresses distinct markers and has different functions in the immune system controlled by different transcriptional factors (Table 1).

Table 1: Three major subsets of dendritic cells.

Subsets	Differential markers (human)	Transcriptional factors
cDC1	CD141/BDCA3	ID2
	CLEC9A	IRF8
	CADM1/NECL2	BATF3
	BTLA	
	CD26	
cDC2	CD1c/BDCA1	ZEB2
	CD2	IRF4
	FcεR1	Notch2/KLF4
	SIRPA	
pDCs	CD123	E2-2
	CD303/BDCA2	ZEB2
	CD304/BDCA4	IRF8
		IRF4

With a high expression of CD141 (BDCA3), cDC1 is capable of cross-presenting exogenous antigen via major histocompatibility complex class Ⅰ (MHC Ⅰ), activating $CD8^+$ T cells and promoting T helper type 1 (Th1) and natural killer responses.[6] It is reported that cDC1 is indispensable for regulating cancer immunity and immune cell composition in the tumor microenvironment, and therefore, fundamental for cancer immunotherapy.[7] Similar to cDC1, cDC2 also regulates immune response and maintains tissue homeostasis.[8] Recognized by CD1c (BDCA1), cDC2 displays enhanced major histocompatibility complex class Ⅱ (MHC Ⅱ) antigen presentation and tends to activate $CD4^+$ T cells.[9]

Expressing CD123, pDCs are characterized by their capabilities of rapid and vast production of type Ⅰ interferon (IFN), responding to single-stranded viral RNA and DNA.[10] Besides antiviral immunity, pDCs also show an important effect in immune tolerance, inflammation, and tumor microenvironments.[11]

There are other subsets of DCs besides those mentioned above. For example, Langerhans cells (LCs) originate from macrophage lineage with migratory capabilities.[9,12] Resided in basal layers of stratified epithelial tissues, LCs can sense environmental signals promptly.[13] LCs play an essential role in maintaining epidermal health and also capable of responding to certain intracellular pathogens under inflammatory circumstances.[9,14]

Dendritic Cells Process And Present Antigens Via Mhc Complexes

DCs can initiate an immune response by presenting the captured antigen in the form of peptide-MHC (p-MHC) molecule complexes to T cells. Classically, the two classes of MHC, MHC Ⅰ, and MHC Ⅱ are involved in the endogenous and exogenous antigen-presenting process respectively (Figure 1).[15] DCs can also capture antigens from extracellular space converting into peptides and loading to MHC Ⅰ, which is known as "cross-presentation".[16, 17]

Endogenous antigens, mostly proteins, are first digested by proteasomes into peptides with lower molecular weight. Through transporter associated with antigen processing (TAP), peptides are then translocated into the endoplasmic reticulum (ER) where they combine with MHC Ⅰ forming p-MHC Ⅰ. The p-MHC Ⅰ complexes are transported towards the plasma membrane by the secretory pathway, presenting antigens to $CD8^+$ T cells.[18] On the other hand, exogenous antigens enter DCs by endocytosis forming endosomes, which are degraded into class Ⅱ-associate invariant chain peptide (CLIP). MHC Ⅱ, synthesized in ER, is translocated to the endosomes and combine with CLIP. The resulting p-MHC Ⅱ complexes in the endosome are transported to the plasma membrane by exocytosis, displaying to $CD4^+$ T cells.[19]

The adapt immune responses initiated by DCs are closely associated with the maturation of DCs. In their resting state, immature DCs are characterized by low expression of surface MHC Ⅱ and co-stimulatory molecules, such as CD80, CD86, and CD40. Immature DCs are still capable of capturing antigens but with limited capacity for secreting cytokine, inducing immune tolerance.[20, 21] In response to activation by infection, injury, or vaccination, DCs go through a complex process, giving them the ability to induce the clonal expansion of naïve T cells and the following differentiation into effector T cells. Though with the downregulation of antigen-capture activity, mature DCs express higher levels of surface MHC Ⅱ and cytokines, for example, CD 40.[2, 22]

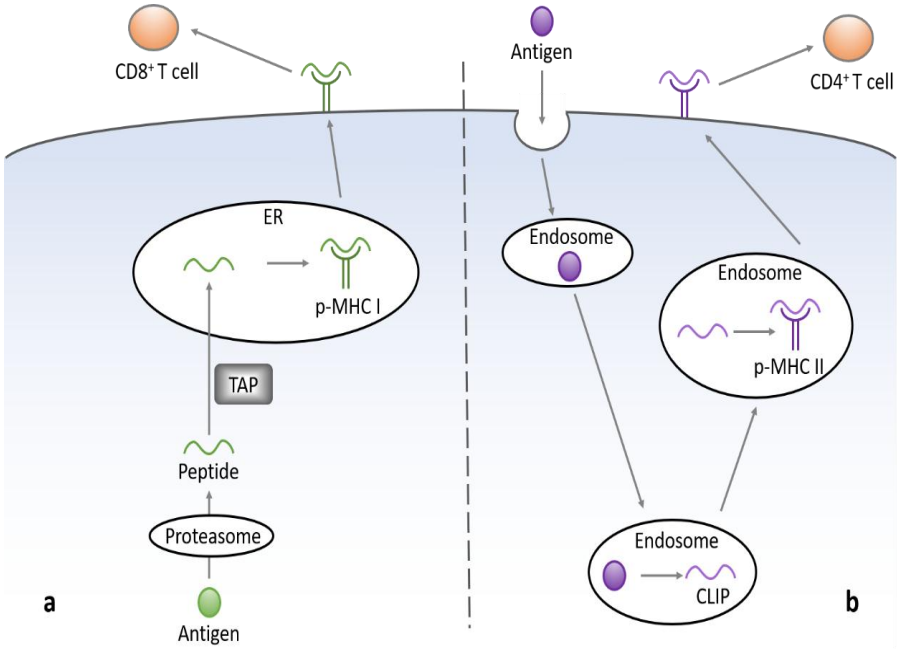

Figure 1: Antigen-presenting process of DCs involving MHC Ⅰ (a) and MHC Ⅱ (b).

Dendritic Cells In Regenerative Medicine

Regenerative medicine represents a novel direction for treating challenging diseases by focusing on replacing or regenerating cells, tissues, or organs to restore or establish their normal functions.[23] Products used for regenerative medicine generally fit in two strategies according to their target processes. One is to stimulate regenerative responses exogenously, where the implanted products (includes cells and tissues) are supposed to replace the damaged or dysfunctional tissue. The other is to stimulate endogenous regenerative responses where the delivered products (includes cells, biological factors, and genetic or epigenetic modifications) are intended to enhance the efficiency of endogenous reparative processes.[24, 25]

Dendritic cells are closely linked to regenerative medicine, especially in the host response to foreign biomaterials. Biomaterials implanted in the body are involved in the processes of regenerative medicine,[26] and their performances are largely dependent on their interactions with the host immune system. As the most potent antigen-presenting cell, DCs could interact directly with the biomaterials and generate a series of immune responses which would be crucial for the functions of biomaterials.[27, 28]

Increasing evidence suggested that the interaction between DCs and biomaterials can be affected by the physical and chemical properties of the biomaterials,[29] Properties that affects the maturation of DCs include biomaterial composition,[30] surface modification,[31, 32] surface hydrophobicity,[33] surface roughness,[34] spatial structure,[35, 36] molecular weight,[37] and so on. To evaluate how the surface modification affects DCs maturation, self-assembled monolayers (SAMs) modified with CH_3, OH, COOH, or NH_2 were used to treat DCs *in vitro*.[31] The results suggested that CH_3 SAMs may be more suited for tissue regenerative medicine as a scaffold to advance host acceptance with less mature DCs, while others could enhance protective immune responses.

Metabolism Affecting Dendritic Cells

The function of DCs is significantly influenced by the microenvironment they reside in.[38] The idea of a connection between immune cell function and metabolism has attracted the attention of researchers, resulting in the rising field of immunometabolism. Recent findings suggest that metabolic processes, such as glycolysis, fatty acid metabolism, and the Krebs cycle, show specific effects on DCs.[4, 39] The manipulation of these pathways can significantly alter the functions of DCs, which would be a highly useful tool in regenerative medicine.

Citrate, an intermediate of the Krebs cycle (also known as the tricarboxylic acid cycle), is considered as one of the key modulators of DCs maturation.[40-42] The maturation of DCs could result in a significant increase of glucose consumption and lactic acid production, suggesting the up-regulation of glycolysis.[43, 44] DCs maturation also causes the Krebs cycle to rewire, which eventually leads to the accumulation of citrate.[45]

Citrate provides a bridge between carbohydrate and fatty acid metabolism (Figure 2). Citrate in mitochondria is exported to the cytoplasm by citrate carrier (CIC), also known as solute carrier family 25 member 1 (SLC25A1).[46] Cytosol citrate is then processed by ATP-citrate lyase (ACLY) into acetyl-coenzyme A (acetyl-CoA) which is further processed into malonyl-CoA by acetyl-CoA carboxylase (ACC). Malonyl-CoA can be combined into fatty acids which can further combine into phospholipids forming membranes.[47] Increased

membranes are crucial for ER and Golgi to satisfy the increasing need of protein production and secretion in mature DCs.

Besides, citrate-derived itaconate is also an immunomodulator.[45, 48] In the mitochondrial matrix, citrate can be transformed into *cis*-citrate which is then processed into itaconate by immune-responsive gene 1 protein (IRG1) (Figure 2).[49] Itaconate is reported to be anti-inflammatory, which can neutralize the pro-inflammatory effect of succinate by inhibiting succinate dehydrogenase (SDH).[50, 51] However, another study also shows that itaconate is only a weak competitive inhibitor of SDH,[52] suggesting that further investigation on this matter is needed.

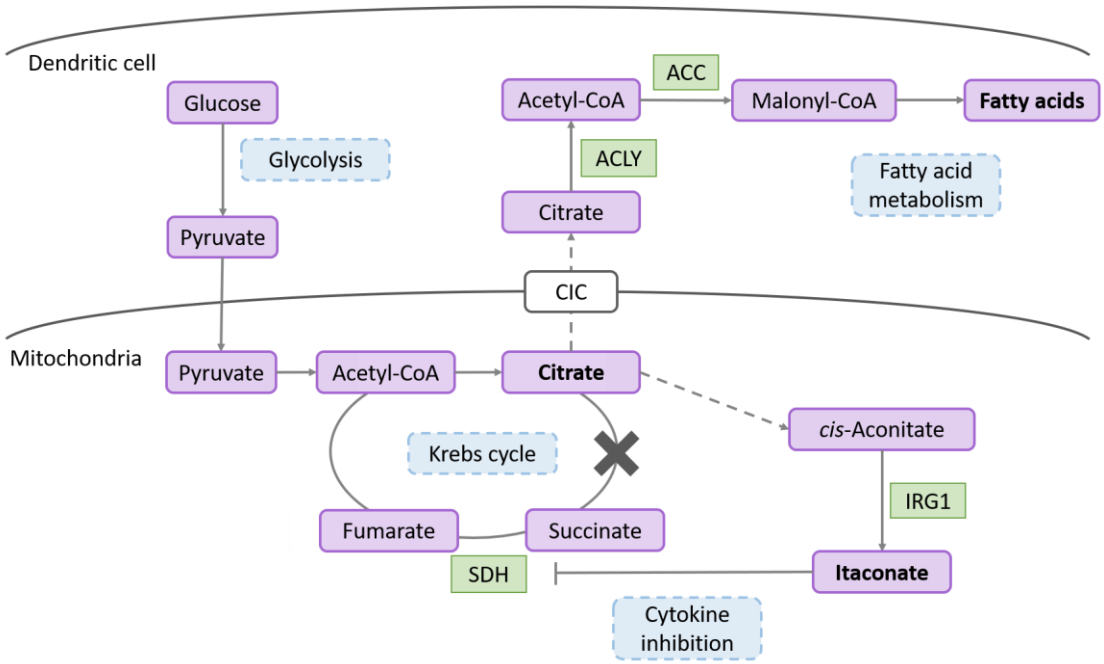

Figure 2: Citrate as key modulator of DCs maturation.

Metabolomics Studies On Dendritic Cells

Metabolomics is defined as the comprehensive profiling of small molecule metabolites in cells, tissues, or whole organisms. With rapid technological evolution, metabolomics has provided new insights in the research of complex metabolic diseases.[53, 54] The detection of metabolites is generally achieved by nuclear magnetic resonance (NMR), or mass spectrometry (MS) coupled with liquid chromatography (LC) or gas chromatography (GC).[55] The General workflow of the metabolomics study consists of sample collection, detection, and data processing.

The metabolomics approach has been applied in the research of immune cells, especially DCs, providing new knowledge of immune responses. It was reported that liver kinase B1 (LKB1) could regulate the metabolism of Treg cells, contributing to immune tolerance and homeostasis. Metabolomic profiling in LKB1-deficient Treg cells reveals a significant reduction of metabolic intermediates associated with tricarboxylic acid (TCA) cycle and purine and pyrimidine metabolism, and non-significant changes in glycolysis.[56] Metabolome analysis on pollen extracts identified adenosine as a potent immunoregulatory metabolite which posed differential effects on DCs.[57] Comprehensive metabolomics analysis was used in identifying alarmin uric acid as the key signal of peanut allergy. Further study also elaborated that alarmin uric acid could activate DCs in the development of allergic sensitization to peanuts.[58] It was suggested that active vitamin D3 could regulate DCs in inducing functional Treg cells. When identified the enzyme 6-phosphofructo-2-kinase/fructose-2,6-biophosphatase 4 (PFKFB4) as a transcriptional target of active vitamin D3, tracer metabolomics reveals that PFKFB4 activity was essential for glucose metabolism, especially for glucose oxidation, which highlights the alteration in glucose metabolism as the center of the immunomodulatory effects induced by active vitamin D3.[59]

Perspectives

As antigen-presenting cells, dendritic cells have significant importance in our immune system by connecting the innate and adaptive immunity. For regenerative medicine, biomaterials, including biological factors, cells, tissues, even organs, are intended to stimulate regeneration to restore the functions of dysfunctional organs. And their performance highly depends on the host responses which can be triggered by DCs. The maturation and antigen presentation of DCs can be affected by the properties of biomaterials. Though with numerous studies, the mechanisms of DCs maturation is not yet fully understand. Metabolomics, a

study based on the metabolites, has already provided many insights on other metabolic diseases and may shed some light on the mechanism of DCs maturation. These insights could help design and modify biomaterials in regenerative medicine to better fit its purpose.

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