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ANALYSIS OF THE FUNCTIONAL ROLE OF MICRORNAS IN REGULATING MACROPHAGE MEDIATED INFLAMMATORY RESPONSES

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1. INTRODUCTION

Macrophages are tissue differentiated cells that perform a variety of homeostatic and immune regulatory functions. They differentiate from peripheral blood monocytes that migrate across various tissues to carry out diverse physiological functions. Macrophages primarily balance cellular homeostasis by clearing extraneous materials like cellular debris and 'effete' or apoptotic cells. They are also chiefly involved in remodelling of damaged tissue by forming ECM (extracellular matrix) and supply of adequate amount of blood and oxygen to the damaged site. In addition to these functions macrophages also perform a crucial process of recycling the functional material that becomes a remarkable metabolic contribution without which the host would not survive.

Apart from these 'janitorial' functions they also execute 'effector' immunity functions to provide defence against malicious particulates thus forming a barrier across the cell surface. Any dys-regulation in their performances would lead to fatal inflammatory diseases. In the coming sections we shall comprehensively discuss the diverse roles of macrophages, their pathophysiological and regulatory aspects.

2. Macrophages and their regulatory role in inflammation and diseases

Macrophages are tissue differentiated monocytes that perform a variety of homeostatic and immune-regulatory functions. They respond to surrounding milieu and can alter their phenotype and cellular repertoire to suit their functional requirements. Such a plastic behavior of macrophages is termed as macrophage polarization. Tight balance between different activated/polarized states of macrophages is essential to maintain immune homeostasis and dys-regulation of their functionality may lead to fatal inflammatory related

diseases. The macrophage differentiation, proliferation, activation and pathophysiological roles will help usin understanding the disease mechanism.

3. Macrophage development and differentiation

Macrophages belong to mononuclear phagocytic system (MPS) which is the product of a regulated process of differentiation. Initially all the immune cells originate from a committed lineage of hematopoietic stem cells (HSC) in the bone marrow which subsequently undergo a series of differentiation steps to give rise to myeloid progenitor cells. These cells act as common myeloid progenitors (CMP) for a variety of cell types including neutrophils, eosinophils, basophils, monocytes, dendritic cells and mast cells depending on the growth factor they receive (Figure A).

For instance, in response to macrophage colony stimulating factor (M-CSF), myeloid progenitor cells commit to form a monocytic lineage for which they primarily differentiate into monoblasts followed by pro-monocytes where they exit the bone marrow and enter into the blood stream to finally differentiate into monocytes. Ly6C ^{hi} monocyte (lymphocyte antigen 6 complex, locus C1) populations are shown to actively extravasate into various tissues to differentiate into macrophages either in steady state or in response to an injury or infection to perform prodigious functions such as tissue remodelling, clearance of pathogens, and maintenance of cellular homeostasis.

Two theories were proposed to explain macrophage development and differentiation; firstly macrophages were believed to originate from reticuloendothelial system (RES). This theory was widely accepted for about half a century, until van Furth et al., in 1972 proposed macrophage generation from mononuclear phagocytic system (MPS). According to them all the macrophages are derived from monocytes and not from endothelial cells, reticulum cells or fibroblasts of RES and they believed that there are two major subpopulations of macrophages; tissue resident macrophages which were identified to extravasate in normal steady state or unstimulated cells and were grouped into major family of histiocytes or tissues macrophages and ii) exudate macrophages which were differentiated from increased populations of monocytes during inflammatory stimuli.

However, many recent evidence indicated that monocytes do not significantly contribute to tissue macrophages rather majority of them are already formed during embryonic development and are maintained in adults by their self-renewal property. According to this, erythro-myeloid

progenitor cells (EMP) in the yolk sac migrate and colonize in the foetal liver during very early stages of embryonic development. Additionally, it was reported that macrophages can be directly formed in the yolk sac in Myb (transcription factor) independent manner while HSCs in the foetal liver produce macrophages in Myb dependent fashion. These cells subsequently enter into the developing tissues to form tissue resident macrophages (Figure B).

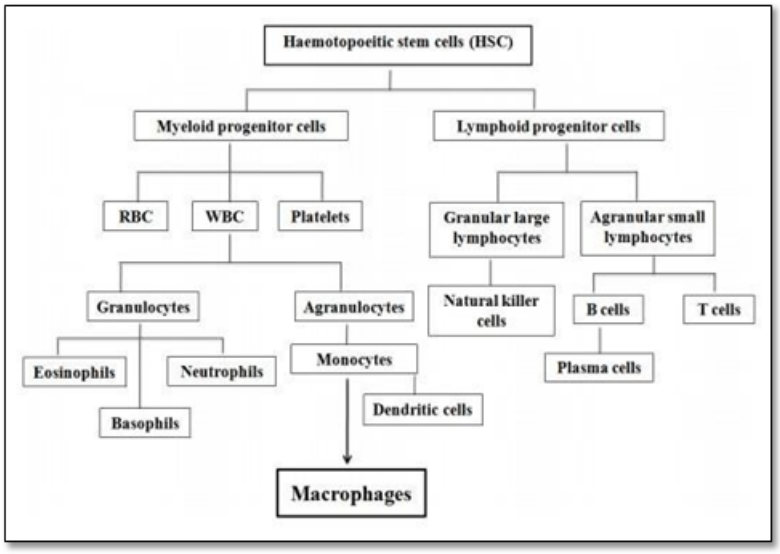


Figure A. Macrophage development from hematopoetic stem cells. Macrophages are differentiated from monocytes via myeloid progenitor cells. They constitute a large group of agranulocytes which are evolved to perform diverse functions in the tissues.

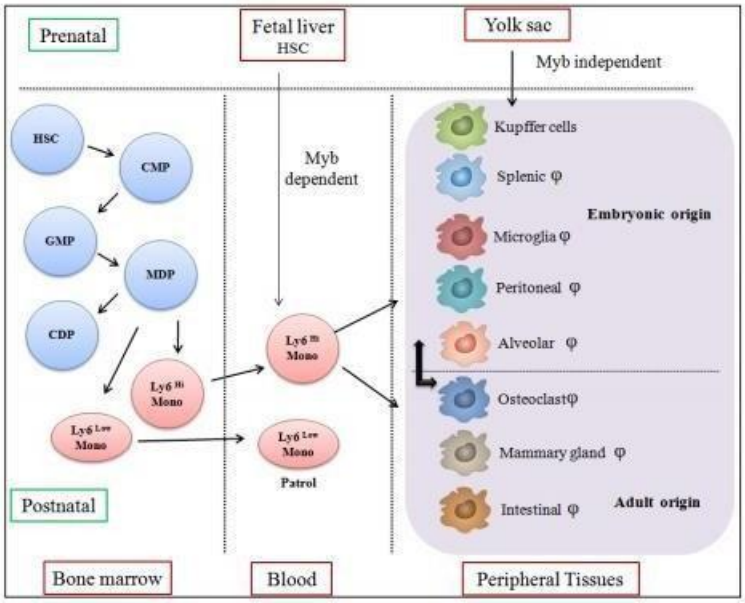
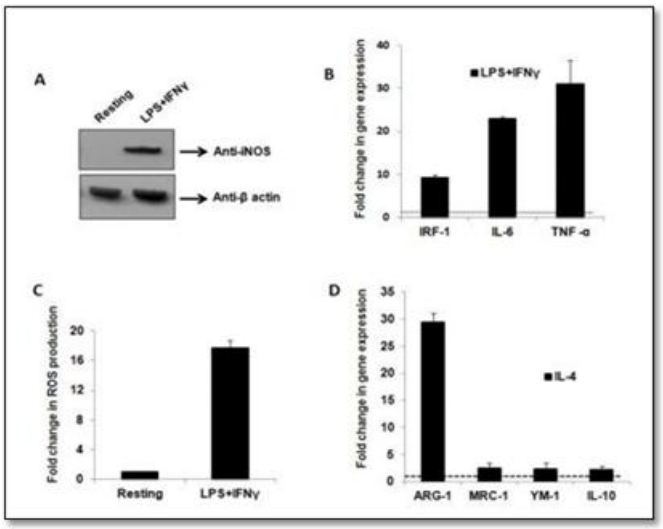


Figure B. Macrophage differentiation and tissue distribution in embryonic and adult cells.

In embryonic cells, macrophages differentiate directly from yolk sac in Myb independent fashion while HSCs in the foetal liver produce monocytes which later differentiate into macrophages in Myb dependent manner. Further HSCs in the bone marrow gradually differentiate into macrophages in both pre and postnatal stages. The macrophages which are formed in early stages get distributed across various peripheral tissues and are characterized by self-renewal and longevity. On the contrary macrophages developed during adult stages are primarily involved in regulating inflammation and usually have short life span. φ is the representation for macrophages, CMP-common myeloid progenitors, GMP-Granulocyte myeloid progenitor, MDP-macrophage or dendritic cell progenitor, CDP-common dendritic cell progenitor.

4. LPS+IFNγ and IL-4 are potent inflammatory insults

Stimulation of macrophage with 1 µg/ml of LPS and 100 ng/ml of IFNγ resulted in enhanced expression of several M (LPS+IFNγ) markers: iNOS (an enzyme involved in catalyzing nitric oxide (NO) production from arginase) (Figure 2.1A), cytokines (IRF-1, IL-6 and TNF-α) (Figure 2.1B) and ROS generation (Figure 2.1C) in LPS+IFNγ exposed macrophages suggesting that the conditions were optimal for the polarization of macrophages by LPS and IFNγ towards M (LPS+IFNγ) phenotype. Moreover, IL-4 treated macrophages displayed enhanced expression of arginase (ARG-1), mannose receptor C type lectin (MRC-1), YM-1 and IL-10 expression that are the characteristic of repair capable anti-inflammatory macrophages (Figure C).



CONCLUSION

Unrestrained inflammation frequently observed in chronic inflammatory diseases such as

insulin resistance, Type 2 Diabetes (T2D) and several types of cancers is prominently due to imbalances in different activation states of macrophages. Delineation of the regulatory mechanisms underlying macrophage polarization may help us to better understand the pathophysiological basis of inflammation linked diseases. MicroRNAs are posttranscriptional regulatory molecules that drive distinct biological processes such as proliferation, cell survival, differentiation and inflammation. However, the functional role of microRNAs in inflammation induced insulin resistance is poorly studied. Thus, there is a necessity to study the involvement of microRNAs in inflammation induced insulin resistance (IR), as IR is the leading cause of diabetes. With this objective we sought to understand the regulatory role of microRNAs in macrophage polarization and insulin resistance.

Our microRNA microarray data revealed several microRNAs that were differentially regulated in polarized macrophages. Among them, we investigated the functional roles of two microRNAs: miR-712 and miR-16 whose expression levels were down-regulated in macrophages exposed to pro-inflammatory stimuli such as LPS+IFNγ and palmitate. Additionally, we have observed that miR-16 expression is also down-regulated in palmitate exposed myoblasts, ER stress inducer (tunicamycin and thapsigargin) treated myoblasts and insulin responsive tissues of high sucrose diet (HSD) induced insulin resistant rats. On the other hand unlike in macrophages, miR-712 expression levels were observed to be increased in myoblasts upon exposure to stress inducers such as palmitate, iv tunicamycin and thapsigargin pointing to potential cell specific effects. Investigating the direct role of miR-712 in skeletal muscle may help us in better understanding of insulin mediated effects in physiology and disease.

We next noted that ectopic expression of miR-712 and miR-16 in macrophages reduced the production of pro-inflammatory cytokines such as TNF-α, IL-6 and IFN-β which in turn led to improved insulin sensitivity in insulin resistant (IR) skeletal myoblasts suggesting reduced paracrine inhibitory effects of LPS+IFNγ polarized macrophages on skeletal myoblasts insulin sensitivity. In addition we observed that forced expression of miR-16 directly in myoblasts augmented insulin stimulated glucose uptake via upregulation of two key players: GLUT4 and MEF2A that are involved in insulin stimulated glucose uptake.

Mechanistic analysis revealed LRRK2 (a serine / threonine protein kinase associated with inflammatory diseases such as Crohn's and Parkinson's disease) as the target of miR-712. Further over-expression of miR-712 resulted in reduced phosphorylation of p38 and ERK1/2,

key players involved in inflammatory gene expression suggesting that miR-712 is positioned to control macrophage mediated pro-inflammatory responses. Collectively, our data demonstrates the pivotal roles of microRNAs miR-712 and miR-16 in alleviating inflammation induced insulin resistance for the first time. Exploring the pathophysiological roles of these microRNAs may further help us in understanding the progression and treatment of T2D.

MicroRNA-712 and miR-16 dampen macrophage mediated pro-inflammatory responses and improve insulin mediated glucose uptake in myoblasts. (A) miR712 expression is down-regulated in LPS +IFNγ polarized macrophages. Ectopic expression of miR-712 attenuated macrophage pro-inflammatory responses and their paracrine inhibitory effects on myoblast insulin sensitivity. Mechanistic analysis showed that miR-712 directly targets LRRK2 resulting in decreased phosphorylation of p38 and ERK1/2 in macrophages. (B) miR-16 expression is down-regulated in LPS+IFNγ and palmitate stimulated macrophages and in palmitate, tunicamycin and thapsigargin treated myoblasts. Importantly, forced expression of miR-16 into macrophages improved myoblast insulin sensitivity by enhancing GLUT4 and MEF2A expression levels.

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