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Crosstalk between Notch signaling and nuclear receptors, Nur77 and PPARγ, in macrophage polarization and its impact upon macrophages-adipocytes interactions

Obesity causes many serious health-related problems among world population in recent decades. In Thailand, approximately six million Thais are facing obesity problem. Obesity is one of the conditions in aberrant metabolic syndrome characterized by abnormal in energy utilization and storage. Recent evidence reveals that obesity involves a chronic low grade inflammation in adipose tissues, but the precise mechanism how obesity triggers inflammation remains unclear. However, many evidences indicate that the chronic imbalance of overnutrition state can activate Toll-like receptor 4 (TLR4)-pathogen sensing molecules in adipose tissues, liver and macrophages to produce proinflammatory cytokines.

Macrophages can be classified in a simple way into two types in human that is M1 or classical activated macrophages and M2 or alternatively activated macrophages. To correctly identify the types of human macrophages, more than one marker are often used and in some instances the classification is still controversial. Nevertheless, in M1 macrophages, upregulation of CD86, major histocompatibility complex class II (MHC class II), and downregulation of mannose receptor, the increase production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNFα), IL-6, IL-1β, inducible nitric oxide synthase (iNOS) in response to GM-CSF, IFNγ and inflammatory stimuli such as LPS are often used as markers. For M2 macrophages, peroxisome proliferator-activated receptor gamma (PPARγ), transglutaminase2 (TGM2), and the up-regulation of IL-10 production in the presenting of M-CSF, IL-4 and IL-13 are often used as markers.

Adipose tissues compose of adipocytes, the main cellular components, which have the important role in energy storage and endocrine activity. Other cell types that localize in adipose tissues are adipocyte precursor cells, fibroblasts, vascular cells and immune cells (e.g. macrophages and T cells). Secreted proteins from these cells are required to maintain homeostasis in adipose tissues. In the lean state, predominant immune cells are $CD4^+$ T helper 2 (Th2) cells, regulatory T (Treg) cells, eosinophils and alternatively activated macrophages. Interleukin-4 (IL-4) and IL-13 from Th2 and eosinophils play a critical role to drive macrophages to become alternatively activated macrophages which mainly produce IL-10 similar to Treg. Secretion from these cells sustains adipose tissue homeostasis and insulin-sensitive phenotype. In obesity, immune cells are newly recruited to adipose tissues. Major components change to $CD4^+$ Th1, $CD8^+$ effector T cells, increased B lymphocytes and classical activated macrophages, and decrease Treg and alternatively activated macrophage. The event leads to cytokine profile switches from anti-inflammatory to pro-inflammatory cytokines. These changes interfere with insulin signaling pathway leading to unresponsiveness of adipocytes to insulin. Interestingly, many evidence indicated that macrophage development and function are strongly associated with cholesterol-induced atherogenesis, which one of the important clinical complication.

Because lipid metabolism is important in regulating inflammation in adipose tissues, particularly in macrophages, this research will focus on two nuclear receptors, Nur77 and PPARγ, that are expressed and involved in lipid recognition/regulation in macrophages. Nur77 is an orphan nuclear receptor which no known endogenous ligands. Its transcriptional activity depends on the expression of the receptor, post-translational modification and subcellular localization. Nur77 expression can be induced by several stimuli such as growth factors, inflammatory stimuli, cytokines, peptide hormones and cellular stress. Recent studies reveal that macrophages in atherosclerosis plaque expressed Nur77. The inflammatory stimuli such as LPS and oxLDL can induce Nur77 expression *in vitro* in macrophages through nuclear factor-κb (NF-κb) dependent pathway. Furthermore, in Nur77 knockout mice fed with high fat diet, the peritoneal macrophages polarized to M1 in LPS response. Therefore, Nur77 is involved in M1/M2 polarization but the detailed mechanisms are largely unknown.

PPARγ plays an important role in regulating lipid metabolism in adipose tissues, macrophages and dendritic cells. PPARγ can be activated by many ligand such as ox-LDL, rosiglitazone, prostacyclin, leukotriene B4 and, 4-hydroxy-2-nonenal. This activation results in increasing in expression of PPARγ target genes such as haptoglobin and lipoprotein lipase. Moreover, activation by PPARγ ligand leads to elevation of CD14, CD36, CD11b and CD11c

expression that are monocytic/macrophage markers in macrophages. More importantly, M2 macrophages which were stimulated with IL-4 upregulated PPARγ. While IFNγ suppressed PPARγ expression in macrophage. Therefore, it is possible that anti-inflammatory activity of M2 macrophages might be mediated by PPARγ.

Another pathway that has influence to regulate inflammatory response is Notch signaling pathway. Notch signaling plays critical roles in regulating development, differentiation, cell proliferation and inflammation in various cell types. Previous reports indicated that Notch signaling fine tune the appropriate response of TLR4 downstream signaling. For example, Notch signaling inhibitor had potential upregulation of IL-10 and downregulation of IL-6, IL-12, iNOS and TNFα in macrophage responded to LPS.

Although Notch signaling pathway, Nur77 and PPARγ have been suggested to regulate macrophage functions, especially macrophage polarization. However, it is not known what the impact of this crosstalk may have upon adipocytes function during metabolic disorders. To explore the crosstalk between these signaling pathways in macrophages and adipocytes in the context of macrophages polarization, ImmunoTools reagents will be used to investigate the human macrophage marker and their function by flow cytometry and/or ELISA. This research will provide a better understanding of the interaction between these signaling pathways in the regulation of macrophages polarization that effect to insulin responsiveness in adipocytes and may help develop more effective control of obesity-induced inflammation.