Review

The Roles of Hypoxia Signaling in the Pathogenesis of Cardiovascular Diseases

Hajime Abe¹, Hiroaki Semba¹,² and Norihiko Takeda¹

¹Department of Cardiovascular Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
²Department of Cardiovascular Medicine, The Cardiovascular Institute, Tokyo, Japan

The circulatory system distributes blood flow to each tissue and transports oxygen and nutrients. Peripheral circulation is required to maintain the physiological function in each tissue. Disturbance of circulation, therefore, decreases oxygen delivery, leading to tissue hypoxia which takes place in several cardiovascular disorders including atherosclerosis, pulmonary arterial hypertension and heart failure. While tissue hypoxia can be induced because of cardiovascular disorders, hypoxia signaling itself has a potential to modulate tissue remodeling processes or the severity of the cardiovascular disorders. Hypoxia inducible factor-1α (HIF-1α) and HIF-2α belongs to a group of transcription factors which mediate most of the cellular responses to hypoxia at a transcriptional level. We, and others, have reported that HIF-α signaling plays a critical role in the initiation or the regulation of inflammation. HIF-α signaling contributes to the tissue remodeling processes; thus it has a potential to become a therapeutic target. Elucidation of the molecular link, therefore, between hypoxia signaling and tissue remodeling will greatly help us to understand the pathophysiology of the cardiovascular disorders. The purpose of this review is to give a brief overview of the current understanding about the function HIF-α in inflammation processes especially by focusing on its roles in macrophages. In addition, the pathophysiological roles of hypoxia signaling for the development of cardiovascular disease will be discussed.

Key words: Cardiovascular diseases, Inflammation, Hypoxia, HIF-1α

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Introduction

While molecular oxygen is required to maintain the homeostasis in each cell, the oxygen consumption rate greatly varies depending on each tissue. For instance, the brain consumes approximately 3 ml O₂/min/100g tissue. The oxygen consumption rate in the heart is even larger at a rate of 8–15 ml O₂/min/100g tissue. It should be noted that it could rise up to 70 ml O₂/min/100g during vigorous exercise period.⁰ In general, the oxygen concentration below the tissue-specific physiological level is called hypoxia.

In the hypoxic environment, each cell exhibits several types of responses at transcriptional, translational or post-translational levels. Most of the gene expressions are, at the transcriptional level, significantly suppressed in the hypoxic environment. In contrast, the expression of a group of genes is significantly enhanced in hypoxia. These genes are termed as hypoxia-inducible genes. Representatives of hypoxia-inducible genes are genes related to angiogenesis (vascular endothelial growth factor-α, Vegf-α)⁶, erythropoiesis (erythropoietin, Epo)⁴, cellular metabolism (pyruvate dehydrogenase kinase, isofrom 1, Pdk 1, or lactate dehydrogenase-a, Ldh-a)⁵, ⁶ and inflammation (inducible nitric oxide synthase, iNOS)⁷. Hypoxia-inducible factor-1α (HIF-1α) and HIF-2α act as transcriptional mediators in their hypoxic induction.⁸ ⁹ The activity of HIF-α is regulated at a post-translational level. In normoxic conditions, HIF-α protein is hydroxylated through an oxygen dependent process, and is degraded through a ubiquitin-proteasome system.¹⁰ In contrast, the HIF-α protein is stabilized in...
hypoxic condition and translocated into the nucleus. After forming a heterodimer complex with HIF-1β, HIF-α binds to the hypoxia responsive elements (HRE) with a core consensus sequence (RCGTG), and activates the transcription of hypoxia-inducible genes11, 12(Fig. 1).

Sterile Inflammation in Cardiovascular Diseases

Most basic or clinical researches support the notion that an inflammatory process underlies the development of atherosclerotic processes. Monocytes are recruited in the subendothelial space of the arterial wall and differentiate into macrophage at the initial phase of atherosclerosis. Repeated innate immune responses, in turn, promote the deposition of lipid-loaded macrophages or foam cells, which secrete pro-inflammatory mediators and result in plaque destabilization13, 14. Consistent with these findings, the serum levels of inflammatory markers, including Creative protein (CRP) and interleukin-6 (IL-6), are elevated in atherosclerotic patients15-17.

The inflammatory process also contributes to the development of vascular remodeling in systemic hypertension or pulmonary artery hypertension (PAH)18, 19. An elevated serum level of CRP precedes the new onset of systemic hypertension in an elderly healthy population20, 21. Similarly, serum level of pro-inflammatory cytokines including interleukin 6 (IL-6) is elevated in PAH patients, and the cytokine abundance has a significant impact on the subsequent prognosis of PAH patients22-24.

Heart failure is a condition in which the heart can’t pump enough amount of blood to meet the body’s needs. Heart failure with reduced ejection fraction (HFrEF; also known as systolic heart failure) develops after myocardial infarction or as one of the manifestation of cardiomyopathy. In contrast, heart failure with preserved ejection fraction (HFpEF) occurs in patients with systemic hypertension. It should be noted that the serum levels of inflammatory markers are increased in both HFrEF and HFpEF patients25-27. Importantly, the serum levels of CRP, IL-6 and tumor necrosis factor alpha (TNF-α) are associated with their prognosis28-33.

These observations clearly indicate that an inflammatory signal plays a critical role during the processes of cardiovascular remodeling. It still remains unclear, however, why sterile inflammation develops in the vessel wall or in the cardiac tissue. Moreover, it should also be elucidated whether these inflammatory processes play an adaptive or mal-adaptive function.

HIF-α Switching in M1 / M2 Macrophage

Previously, it has been formerly considered that macrophages consist of a single cell population, and simply activate inflammatory processes in response to the tissue injury or infection34. A number of recent studies have revealed clearly that macrophages are composed of heterogeneous cell populations. Currently, several types of classification exist in defining each macrophage population based on its origin, loca-
tion or the function. Tissue macrophages may exist as a resident macrophage or may differentiate from monocyte populations\textsuperscript{(39)}. One macrophage population activates inflammation (pro-inflammatory), but the other suppresses or resolves the inflammatory processes (anti-inflammatory)\textsuperscript{(35-37)}. A number of definitions still exist regarding the pro- or anti-inflammatory macrophage population. Among them, it has been widely used for easy understanding of macrophage heterogeneity including M1 (pro-inflammatory) and M2 polarization (anti-inflammatory).

In \textit{in vitro} experiments, M1 is usually induced by a combination of Th1 cytokine, interferon-\textgamma (IFN-\textgamma) and a ligand for toll-like receptor 4, lipopolysaccharide (LPS). IFN-\textgamma and LPS robustly induce the expression of pro-inflammatory genes including inducible nitric oxide (NO) synthase (iNOS), and eliciting the production of NO. NO is one of the critical mediators in inflammation. Thus, the iNOS gene is considered as a classical M1 marker gene. On the other hand, M2 macrophase is induced by Th2 cytokines such as interleukin-4 (IL-4) or IL-13. Arginase 1 (Arg1) or the mannose receptor expression is highly expressed in M2 macrophages, thus these genes are known as M2 marker genes\textsuperscript{(38)}.

It should be noted that both iNOS and ARG1 enzymes catalyze and compete for the same metabolic substrate, \textit{l}-arginine. While iNOS in M1 macrophage produces NO from \textit{l}-arginine and strikingly promotes the inflammatory processes, ARG1 in M2 macrophage suppresses NO production\textsuperscript{(39)}. The antagonistic activity, therefore, between iNOS and Arg1 crucially regulates the production of NO.

The roles of HIF-1\textalpha in M1 macrophage activation have been extensively investigated. LPS or IFN-\textgamma mediated HIF-1\textalpha induction is required for iNOS gene expression in M1 macrophages\textsuperscript{(40, 41)}. In agreement with this, the severity of septic shock was significantly attenuated in myeloid-specific HIF-1\textalpha deficient (LysM-cre;HIF-1\textalpha \textsuperscript{fl/fl}) mice\textsuperscript{(42, 43)}. Moreover, chemically induced cutaneous inflammation or experimental arthritis was also attenuated in HIF-1\textalpha deficient mice\textsuperscript{(44)}. These results indicate that HIF-1\textalpha plays a critical role in M1 macrophage activation.

In contrast to the pro-inflammatory processes those are activated in M1 macrophages, little is known about its resolution process. We examined the expression of HIF-1\textalpha and HIF-2\textalpha in murine macrophages, and found that HIF-1\textalpha and HIF-2\textalpha are specifically expressed in M1 and M2 macrophages, respectively\textsuperscript{(45)}. While LPS or IFN-\textgamma significantly upregulated HIF-1\textalpha protein abundance, LPS and IFN-\textgamma strikingly suppressed HIF-2\textalpha gene expression. In contrast, IL-4 or IL-13 significantly increased HIF-2\textalpha protein abundance in hypoxia. Importantly, we revealed that HIF-2\textalpha induces Arg1 gene expression in M2 macrophages. While both iNOS and Arg1 gene expression increase in hypoxia, iNOS and Arg1 utilize distinct isoform of HIF-\textalpha in its hypoxic induction. Through a loss-of-function approach, we identified that HIF-1\textalpha potentiates, but HIF-2\textalpha suppresses NO production \textit{in vivo}. These results revealed that the balance between HIF-1\textalpha and HIF-2\textalpha, named as HIF-\textalpha switching, critically determine the on/off regulation of NO production (Fig. 2).

### The Role of Active Glycolysis in Macrophage Migration

In addition to its activation, migration is another important function of macrophages initiating inflammation\textsuperscript{(42, 45)}. The processes of cell migration consist of a series of dynamic remodeling in actin cytoskeleton. While lamellipodia is an actin projection on the leading edge of the migrating cell, filopodia is a slender projection that extends beyond the lamellipodia\textsuperscript{(46)}. During cell migration, dynamic polymerization and depolymerization takes place through an ATP dependent manner. It should be noted, therefore, that the cell migration process consumes abundant ATP in the cytosol\textsuperscript{(47, 48)}.

ATP can be synthesized through at least two metabolic pathways, including glycolysis in the cytoplasm and tricarboxylic cycle (TCA cycle) in the mitochondria. In glycolysis, two molecules of ATP are synthesized from one glucose molecule, accompanying the generation of lactate. Glucose can also be metabolized through a TCA cycle in the mitochondria via oxidative phosphorylation. Mitochondrial electron transport chain produces 36 molecules of ATP from glucose through an oxygen dependent manner.

While the oxygen concentration inside the blood vessel remains high, its concentration is strikingly decreased in the inflammatory area\textsuperscript{(49)}. During the migration processes of monocyte derived macrophages, the oxygen concentration gradually decreases as macrophages migrate from the blood stream into the inflammatory area. It has been well documented that mitochondrial activity is significantly suppressed in hypoxic condition, which is termed as the Pasteur effect or classic glycolysis\textsuperscript{(50)}. ATP production in hypoxic condition should also be decreased. Given that macrophage migration consumes abundant ATP, how is it that macrophage can migrate under hypoxic environment?

We initially investigated which energy substrate is required for macrophage migration in hypoxia. Using Boyden chamber assay, we identified that glu-
cose, but not glutamine, is critically required for macrophage mobilization in hypoxia 51). Intriguingly, dichloroacetate (DCA), a chemical inhibitor of pyruvate dehydrogenase kinase (PDK) significantly suppressed macrophage migration capacity. These results indicated that glycolysis, but not mitochondrial respiration plays a critical role in maintaining macrophage migration capacity under hypoxic environment.

To further investigate the link between glycolytic metabolism and macrophage migration, we examined the intracellular localization of glycolytic enzymes in macrophages. Pyruvate kinase muscle isozyme (PKM2) belongs to glycolytic enzymes, and is responsible for glycolytic ATP synthesis in the cytosol. Intriguingly, PKM2 co-localizes with F-actin in filopodia and lamellipodia in primary macrophages. These results implied that glycolysis, but not mitochondrial respiration plays a critical role in maintaining macrophage migration capacity under hypoxic environment.

We also examined the molecular processes by which hypoxia signaling suppresses glucose oxidation in primary macrophages. We established a novel experimental system in which we could measure the oxygen consumption rate in hypoxic environment. Based on these approaches, we identified a novel mode of glycolytic reprogramming which takes place in primary macrophages, termed as active glycolysis (Fig. 4). In active glycolysis, HIF-1α mediated Pdk1 induction actively elicits glycolytic reprogramming even in the presence of mitochondrial electron transport chain activity.

Collectively, these results indicated that cytosolic distribution of intracellular ATP accelerates macrophage motility. Intriguingly, active glycolysis occurs not only in primary macrophages, but also in primary hepatocytes, indicating that this metabolic alteration may be one of the common features in non-malignant cells.

**Hypoxia Signaling in Atherosclerosis**

Chronic inflammation in the vessel walls underlies the development of atherosclerosis. Various types of the cells including endothelial cells, smooth muscle cells, fibroblasts, monocytes/macrophages and T lymphocytes are involved in atheroma plaque formation. Among them, monocyte/macrophages predominantly promote the progression of atheroma. Foam cells, one of the macrophages in atheroma which is surrounded by the lipid, elicits a fatty streak formation in the vessel walls 54).

The cells of the blood vessel wall depend on the...
oxygen supply from the luminal blood or the adventitial vasa vasorum. In developed atherosclerosis, oxygen consumption rate increases in vessel wall, leading to the occurrence of tissue hypoxia at the plaque lesion. Pimonidazole immunohistochemistry, a hypoxia marker, in human atheroma patients revealed that a hypoxic area exists in the macrophage-rich center of the plaque lesion\textsuperscript{55}. Intriguingly, HIF-1α is expressed in the macrophages of human atheroma plaques\textsuperscript{55-57}.

The low-density lipoprotein receptor in deficient mice (Ldlr \textsuperscript{-/-}) is commonly used as a model of atheroma progression. Using this animal model, the roles of macrophage HIF-1α in plaque progression were examined. Bone marrow transplantation of myeloid-specific HIF-1α deficient mice, but not of wild type mice, significantly decreased plaque burden in the
aorta of Ldlr −/− mice. The expression of the genes related to the inflammation or M1 macrophage accumulation was strikingly suppressed in HIF-1α deficient mice58.

Lectin like oxidized low density lipoprotein (LDL) receptor-1 (Lox-1) is the major receptor for oxidatively modified low density lipoproteins (OxLDLs)59, and strikingly promotes the progression of atherosclerosis60. While Lox-1 is expressed in macrophages, endothelial cells or smooth muscle cells61, 62, its expression is increased in the hypoxic environment59. In agreement with this, macrophage lipid content is significantly increased in hypoxia through a Lox-1 dependent manner. Importantly, HIF-1α content is significantly increased in hypoxia through a but that in turn, HIF-1α is not just a consequence of increased plaque burden, demonstration the tissue hypoxia in the plaque lesion is not just a consequence of increased plaque burden, but that in turn, HIF-1α signaling promotes M1 macrophage activation at the center of atheroma plaque.

The roles of HIF-1α signaling, however, in plaque formation seem to be more complicated than expected. CD11c is known as one of the surface markers in antigen-presenting cells (APCs). Conditional ablation of HIF-1α in APCs significantly augments the severity of atheroma formation in Ldlr −/− mice63. Accumulation of Th1 at the plaque area was significantly accelerated in APC specific HIF-1α deficient mice.

Collectively, the roles of HIF-1α signaling in each cell during atherogenensis has to be examined in more detail. It still remains unclear as to the molecular processes by which inflammation takes place in the vessel wall. Is it induced through an antigen-dependent or independent process? If there is a specific antigen, what are they? Additional experiments by focusing in the hypoxic signaling will help to elucidate the molecular link between sterile inflammation in atheroma formation.

The Roles of Hypoxia Signaling in Vascular Remodeling

We have shown that the balance between HIF-1α and HIF-2α, namely HIF-α switching, critically regulates the production of NO in primary macrophages. NO also acts as a powerful vasodilator in the circulatory system. NO mediated increase of cyclic guanosine monophosphate (cGMP) in smooth muscle cells regulates vascular tone64,66. NO could also elicit vasodilation through a cGMP-independent fashion including S-nitrosylation of target proteins, activation of sarco/endoplasmic reticulum calcium ATPase or production of cyclic inosine monophosphate67.

Skin is one of the largest organs, and critically regulates systemic vascular resistance through the production of NO. Vasodilation in the skin also works as a radiator and helps to maintain core body temperature. While its vascular tone has to be tightly regulated depending on the external environment, its molecular process still remains unclear. We therefore hypothesized that HIF-α switching regulates vascular tone in the skin, and tested the roles of keratinotype HIF-α signaling in vascular function. We generated keratinocyte-specific HIF-1α and HIF-2α deficient mice (K14-cre; HIF-1α fl/fl or HIF-2α fl/fl) and measured systemic blood pressure (BP). While systemic BP is elevated in HIF-1α deficient mice, it is decreased in HIF-2α deficient mice68. Cardiac fibrosis elicited by AngiotensinII infusion was also attenuated in HIF-2α deficient mice. These results indicate that HIF-α switching in the skin critically regulates vascular tone in the skin. Consistent with our hypothesis, the core body temperature in HIF-1α deficient mice was elevated when the mice were exposed to the warm environment. Intriguingly, decrease of HIF-1α, but elevation of HIF-2α expression was detected in hypertensive patients, indicating that HIF-α in the skin could contribute in the regulation of systemic BP.

Pulmonary arterial hypertension (PAH) is manifested by an increased BP in pulmonary artery, resulting in the right ventricular heart failure69,71. It is also known that a hypoxic environment elicits pulmonary vasoconstriction and arterial remodeling. While hypoxia signaling seems to play pivotal roles in PAH72,73, the precise roles of HIF-α in pulmonary arterial remodeling have been unclear. Hypoxic exposure is commonly used as a murine model of PAH. Recently, the roles of HIF-α switching were examined using hypoxia induced PAH model64. Pulmonary endothelial specific HIF-2α deficient mice (L1-cre;HIF-2α fl/fl) exhibited tolerance to hypoxia induced PAH compared to control mice or HIF-1α deficient mice. Notably, PA remodeling was significantly attenuated in HIF-2α deficient mice. As a molecular mechanism, HIF-2α mediated induction of arginase-1 critically regulates NO production in pulmonary vasculature.

These results clearly indicated that HIF-2α-Ariginase1 axis could become a therapeutic target to improve the NO availability of the pulmonary arteries or systemic circulation. Recently, HIF-2α antagonist was synthesized by using a structure-based design approach, and is currently used to treat patients with renal cell carcinoma75,76. It seems tempting, therefore, to test the therapeutic efficacy of HIF-2α antagonists in PAH. Elucidating the molecular process by which HIF-2α signal is activated in systemic hypertension or PAH will also help to understand the pathophysiology
of vascular remodeling in more detail.

**Hypoxia Signaling and Cardiac Remodeling**

Hypoxia signaling, as described in the previous section, plays a critical role in the inflammatory process or intracellular metabolism. Importantly, both of them strikingly affect the cardiac function. While heart failure is predominantly a hemodynamic condition, inflammatory signal is strikingly activated in heart failure patients. Serum level of inflammatory cytokines including tumor necrosis factor (TNF)-α or interleukin-6 (IL-6) is significantly elevated in heart failure patients. Importantly, the level of these inflammatory cytokines correlated with the severity of the heart failure. At a histological level, it has also been shown that inflammatory cells including macrophages accumulate to the cardiac tissues of human heart failure subjects. The roles of inflammatory processes in myocardial infarction have been studied using in vivo murine model of myocardial infarction. Two types of monocytes/macrophages, including Ly-6C<sup>hi</sup> and Ly-6C<sup>lo</sup>, sequentially accumulated in response to the myocardial death. While Ly-6C<sup>hi</sup> monocytes/macrophages engulf the injured tissues, Ly-6C<sup>lo</sup> monocytes/macrophages promoted angiogenesis, and scar formation. These results demonstrate that each population of inflammatory cells exerts a distinct function in cardiac remodeling. These results raised the hypothesis that sterile inflammation underlies the development of heart failure. Two multicenter clinical trials, however, using anti-TNF-α antibody demonstrated that inhibition of TNF-α did not improve the clinical courses of heart failure patients. These results indicate that further study is required to fully elucidate and identify the role of each inflammatory cell in cardiac remodeling.

In physiological condition, the myocardium predominantly utilizes free fatty acids as its energy substrate, and acquires ATP through the oxidative phosphorylation. Molecular oxygen is required to maintain the activity of mitochondrial electron transport chain. Notably, cardiomyocytes change their energy substrates from fatty acids to glucose in response to the mechanical or ischemic stresses. We previously reported that this metabolic alteration could become a helpful diagnostic tool in the evaluation of the cardiac function.

It still remains unclear whether the metabolic reprogramming in cardiomyocytes is an adaptive or maladaptive process in maintaining cardiac function. It has been shown that tissue hypoxia develops during cardiac remodeling processes. Moreover, HIF-1α in murine cardiomyocytes plays an important role in modulating its intracellular metabolism by activating PPARgamma. Therefore, elucidation of the hypoxia signaling in cardiomyocytes will help us to understand roles of metabolic alteration in cardiac function.

**Conclusion**

Tissue hypoxia seems to be one of the common features in cardiovascular disorders including atherosclerosis, vascular remodeling and heart failure. Notably, HIF-α signal has a potential to become a therapeutic target in the managing cardiovascular remodeling. While a number of questions remain unsolved as to the roles of inflammation or metabolic alteration in cardiovascular disorders, further study on the hypoxia signaling will help us to understand its pathological processes in more detail.

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**References**

1) West JB. Cardiac energetics and myocardial oxygen consumption. Physiologic basis of medical practice. Williams and Wilkins. Baltimore, Maryland, USA. 1991: 250-260
2) Braunwald E. Coronary blood flow and myocardial ischemia. Heart disease: A textbook of cardiovascular medicine. W.B. Saunders Company. Philadelphia, Pennsylvania, USA. 2001: 1161-1183
3) Tuder RM, Flook BE, Voelkel NF. Increased gene expression for vegf and the vegf receptors kdr/flk and flt in lungs exposed to acute or to chronic hypoxia. Modulation of gene expression by nitric oxide. J Clin Invest. 1995; 95: 1798-1807
4) Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol. 1992; 12: 5447-5454
5) Kim JW, Tchernyshyov I, Semenza GL, Dang CV. Hif-1α-mediated expression of pyruvate dehydrogenase kinase: A metabolic switch required for cellular adaptation to
hypoxia. Cell metabolism. 2006; 3: 177-185
6) McClelland GB, Brooks GA. Changes in mct 1, mct 4, and ldh expression are tissue specific in rats after long-term hypoxic barorach. Journal of applied physiology (Bethesda, Md. : 1985). 2002; 92: 1573-1584
7) Melillo G, Musso T, Sica A, Taylor LS, Cox GW, Varesio L. A hypoxia-responsive element mediates a novel pathway of activation of the inducible nitric oxide synthase promoter. J Exp Med. 1995; 182: 1683-1693
8) Tian H, McKnight SL, Russell DW. Endothelial pas domain protein 1 (epas1), a transcription factor selectively expressed in endothelial cells. Genes Dev. 1997; 11: 72-82
9) Wang GL, Semenza GL. General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. Proc Natl Acad Sci U S A. 1993; 90: 4304-4308
10) Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wyckoff CC, Pugh CW, Maher ER, Ratcliffe PJ. The tumour suppressor protein vhl targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature. 1999; 399: 271-275
11) Hu CJ, Wang LY, Chodosh LA, Keith B, Simon MC. Differential roles of hypoxia-inducible factor 1alpha (hif-1alpha) and hif-2alpha in hypoxic gene regulation. Mol Cell Biol. 2003; 23: 9361-9374
12) Schodel J, Oikonomopoulos S, Ragoussis J, Pugh CW, Ratcliffe PJ, Mole DR. High-resolution genome-wide mapping of hif-binding sites by chip-seq. Blood. 2011; 117: e207-217
13) Libby P. Inflammation in atherosclerosis. Nature. 2002; 420: 868-874
14) Gautier EL, Huby T, Witztum JL, Ouzilleau B, Miller ER, Saint-Charles F, Aucouturier P, Chapman MJ, Lesnik Emilie D. Increased interleukin-1 and interleukin-6 serum concentrations in severe primary pulmonary hypertension. European journal of heart failure. 1999; 119: 1795-1804
15) Ridker PM. Clinical application of c-reactive protein for cardiovascular disease detection and prevention. Circulation. 2003; 107: 363-369
16) Biausi LM, Liuoz G, Fantuzzi G, Caligiuri G, Rebuzzi AG, Ginneti F, Dinarello CA, Maseri A. Increasing levels of interleukin (il)-1ra and il-6 during the first 2 days of hospitalization in unstable angina are associated with increased risk of in-hospital coronary events. Circulation. 1999; 99: 2079-2084
17) Valgimigli M, Cecchi C, Malagutti P, Merli E, Soukho-movskaia O, Francolini G, Ciccitelli G, Oliareas A, Par-nillo G, Percoco G, Guardigli G, Mele D, Pirani R, Fer-rari R. Tumor necrosis factor-alpha receptor 1 is a major predictor of mortality and new-onset heart failure in patients with acute myocardial infarction: The cytokine-activation and long-term prognosis in myocardial infarction (c-alpha) study. Circulation. 2005; 111: 863-870
18) Pullamsetti SS, Savai R. Macrophage regulation during vascular remodeling: Implications for pulmonary hypertension therapy. American journal of respiratory cell and molecular biology. 2017; 56: 556-558
19) Fujiu K, Wang J, Nagai R. Cardioprotective function of cardiac macrophages. Cardiovasc Res. 2014; 102: 232-239
20) Dauphinot V, Roche F, Kossovsky MP, Schott AM, Pichot V, Gaspoz JM, Gosse P, Barthelemy JC. C-reactive protein implications in new-onset hypertension in a healthy population initially aged 65 years: The proof study. Journal of hypertension. 2009; 27: 736-743
21) Mattace-Raso FU, Verwoert GC, Hofman A, Witteman JC. Inflammation and incident-isolated systolic hypertension in older adults: The rotterdam study. Journal of hypertension. 2010; 28: 892-895
22) Dorfmuller P, Perros F, Balabanian K, Humbert M. Inflammation in pulmonary arterial hypertension. The European respiratory journal. 2003; 22: 358-363
23) Humbert M, Monti G, Brenot F, Sitbon O, Portier A, Grangeot-Keros L, Duroux P, Galanau P, Simonneau G, Emilie D. Increased interleukin-1 and interleukin-6 serum concentrations in severe primary pulmonary hypertension. American journal of respiratory and critical care medicine. 1995; 151: 1628-1631
24) Soon E, Holmes AM, Treacy CM, Doughty NJ, Southgate L, Machado RD, Trembath RC, Jennings S, Barker L, Nicklin P, Walker C, Budd DC, Pepke-Zaba J, Morrell NW. Elevated levels of inflammatory cytokines predict survival in idiopathic and familial pulmonary arterial hypertension. Circulation. 2010; 122: 920-927
25) Raymond RJ, Dehmer GJ, Theoharides TC, Deligiannis EN. Elevated interleukin-6 levels in patients with asymptomatic left ventricular systolic dysfunction. Am Heart J. 2001; 141: 435-438
26) Kosmala W, Derzhko R, Przewlocka-Kosmala M, Orda A, Mazurek W. Plasma levels of tnf-alpha, il-6, and il-10 and their relationship with left ventricular diastolic function in patients with stable angina pectoris and preserved left ventricular systolic performance. Coronary artery disease. 2008; 19: 375-382
27) Williams ES, Shah SJ, Ali S, Na BY, Schiller NB, Whooley MA. C-reactive protein, diastolic dysfunction, and risk of heart failure in patients with coronary disease: Heart and soul study. European journal of heart failure. 2008; 10: 63-69
28) Torre-Amione G, Kapadia S, Benedict C, Oral H, Young JB, Mann DL. Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: A report from the studies of left ventricular dysfunction (solvd). Journal of the American College of Cardiology. 1996; 27: 1201-1206
29) Tsutamoto T, Hisanaga T, Wada A, Maeda K, Ohrnishi M, Fuku D, Mabuchi N, Sawaki M, Kinoshita M. Interleukin-6 spillover in the peripheral circulation increases with the severity of heart failure, and the high plasma level of interleukin-6 is an important prognostic predictor in patients with congestive heart failure. Journal of the American College of Cardiology. 1998; 31: 391-398
30) Roig E, Orus J, Pare C, Azqueta M, Filella X, Perez-Villa F, Heras M, Sanz G. Serum interleukin-6 in congestive heart failure secondary to idiopathic dilated cardiomyopa-thy. Am J Cardiol. 1998; 82: 688-690, a688
31) Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. N Engl J Med. 1990; 323: 236-241
32) Miettinen KH, Lassus J, Harjola VP, Siirila-Waris K, Melin J, Punnonen KR, Nieminen MS, Laakso M, Peuh-kurinen KJ. Prognostic role of pro- and anti-inflamma-tory cytokines and their polymorphisms in acute decompensated heart failure. European journal of heart failure.
MacMicking JD, Nathan C, Hom G, Chartrain N, Cramer T, Yamanishi Y, Clausen BE, Forster I, Pawlinski T, Takeda N, O’Dea EL, Doedens A, Kim JW, Weidemann Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel Bonello S, Zahringer C, BelAiba RS, Djordjevic T, Hess J, El Kasmi KC, Qualls JE, Pesce JT, Smith AM, Thompson Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Mosser DM, Edwards JP. Exploring the full spectrum of Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003; 3: 23-35 Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2008; 8: 958-969 Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel Bonello S, Zahringer C, BelAiba RS, Djordjevic T, Hess J, El Kasmi KC, Qualls JE, Pesce JT, Smith AM, Thompson Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Mosser DM, Edwards JP. Exploring the full spectrum of Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003; 3: 23-35 Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2008; 8: 958-969 Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel Bonello S, Zahringer C, BelAiba RS, Djordjevic T, Hess J, El Kasmi KC, Qualls JE, Pesce JT, Smith AM, Thompson Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Mosser DM, Edwards JP. Exploring the full spectrum of Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003; 3: 23-35 Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2008; 8: 958-969 Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel Bonello S, Zahringer C, BelAiba RS, Djordjevic T, Hess J, El Kasmi KC, Qualls JE, Pesce JT, Smith AM, Thompson Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Mosser DM, Edwards JP. Exploring the full spectrum of Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003; 3: 23-35 Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2008; 8: 958-969 Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel Bonello S, Zahringer C, BelAiba RS, Djordjevic T, Hess J, El Kasmi KC, Qualls JE, Pesce JT, Smith AM, Thompson Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Mosser DM, Edwards JP. Exploring the full spectrum of Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003; 3: 23-35 Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2008; 8: 958-969 Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel Bonello S, Zahringer C, BelAiba RS, Djordjevic T, Hess J, El Kasmi KC, Qualls JE, Pesce JT, Smith AM, Thompson Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Mosser DM, Edwards JP. Exploring the full spectrum of Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003; 3: 23-35 Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2008; 8: 958-969 Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel Bonello S, Zahringer C, BelAiba RS, Djordjevic T, Hess J, El Kasmi KC, Qualls JE, Pesce JT, Smith AM, Thompson Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Mosser DM, Edwards JP. Exploring the full spectrum of Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003; 3: 23-35 Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2008; 8: 958-969 Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel Bonello S, Zahringer C, BelAiba RS, Djordjevic T, Hess J, El Kasmi KC, Qualls JE, Pesce JT, Smith AM, Thompson Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Mosser DM, Edwards JP. Exploring the full spectrum of Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003; 3: 23-35 Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2008; 8: 958-969 Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel Bonello S, Zahringer C, BelAiba RS, Djordjevic T, Hess J, El Kasmi KC, Qualls JE, Pesce JT, Smith AM, Thompson Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Mosser DM, Edwards JP. Exploring the full spectrum of Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003; 3: 23-35 Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2008; 8: 958-969 Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel Bonello S, Zahringer C, BelAiba RS, Djordjevic T, Hess J, El Kasmi KC, Qualls JE, Pesce JT, Smith AM, Thompson Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Mosser DM, Edwards JP. Exploring the full spectrum of Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003; 3: 23-35 Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2008; 8: 958-969 Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel Bonello S, Zahringer C, BelAiba RS, Djordjevic T, Hess J, El Kasmi KC, Qualls JE, Pesce JT, Smith AM, Thompson Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya Mosser DM, Edwards JP. Exploring the full spectrum of Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003; 3: 23-35 Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2008; 8: 958-969.
1989; 320: 915-924
60) Chen M, Masaki T, Sawamura T. Lox-1, the receptor for oxidized low-density lipoprotein identified from endothelial cells: Implications in endothelial dysfunction and atherosclerosis. Pharmacology & therapeutics. 2002; 95: 89-100

61) Draude G, Hrboticky N, Lorenz RL. The expression of the lectin-like oxidized low-density lipoprotein receptor (lox-1) on human vascular smooth muscle cells and monocytes and its down-regulation by lovastatin. Biochemical pharmacology. 1999; 57: 383-386

62) Yoshida H, Kondratenko N, Green S, Steinberg D, Quehenberger O. Identification of the lectin-like receptor for oxidized low-density lipoprotein in human macrophages and its potential role as a scavenger receptor. The Biochemical journal. 1998; 334 (Pt 1): 9-13

63) Chaudhari SM, Sluimer JC, Koch M, Theelen TL, Mantey HD, Busch M, Caballero-Franco C, Vogel F, Cochain C, Pelisek J, Daemen MJ, Lutz MB, Gorlach A, Kissler S, Hermanns HM, Zernecke A. Deficiency of hif1alpha in antigen-presenting cells aggravates atherosclerosis and type 1 t-helper cell responses in mice. Arteriosclerosis, thrombosis, and vascular biology. 2015; 35: 2316-2325

64) Arnold WP, Mittal CK, Katsuki S, Murad F. Nitric oxide activates guanylate cyclase and increases guanosine 3': 5'-cyclic monophosphate levels in various tissue preparations. Proc Natl Acad Sci U S A. 1977; 74: 3203-3207

65) Denninger JW, Marletta MA. Guanylate cyclase and the No/cgmp signaling pathway. Biochimica et biophysica acta. 1999; 1411: 334-350

66) Hussain MB, Hobbs AJ, MacAllister RJ. Autoregulation of nitric oxide-soluble guanylate cyclase-cyclic gmp signaling in mouse thoracic aorta. British journal of pharmacology. 1999; 128: 1082-1088

67) Zhao Y, Vanhoucke PM, Leung SW. Vascular nitric oxide: Beyond enos. Journal of pharmacological sciences. 2015; 129: 83-94

68) Cowburn AS, Takeda N, Boutin AT, Kim JW, Sterling JC, Zhao Y, Vanhoutte PM, Leung SW. Vascular nitric oxide: Beyond enos. Journal of pharmacological sciences. 2015; 9: 331-342

69) Hussain MB, Hobbs AJ, MacAllister RJ. Autoregulation of nitric oxide-soluble guanylate cyclase-cyclic gmp signaling in mouse thoracic aorta. British journal of pharmacology. 1999; 128: 1082-1088

70) Carbone R, Bossone E, Bottino G, Monselise A, Rubenfire M. Secondary pulmonary hypertension--diagnosis and management. European review for medical and pharmacological sciences. 2005; 9: 331-342

71) Naeije R. Pulmonary hypertension and right heart failure in chronic obstructive pulmonary disease. Proceedings of the American Thoracic Society. 2005; 2: 20-22

72) Gilroy J, Cahalan JL, Berman R, Newman M. Cardiac and pulmonary complications in duchenne's progressive muscular dystrophy. Circulation. 1963; 27: 484-493

73) Arias-Stella J, Saldana M. The terminal portion of the pulmonary arterial tree in people native to high altitudes. Circulation. 1963; 28: 915-925

74) Cowburn AS, Crosby A, Macias D, Branco C, Colaco RD, Southwood M, Toshner M, Crotty Alexander LE, Morrell NW, Chilvers ER, Johnson RS. Hif2alpha-arginase axis is essential for the development of pulmonary hypertension. Proc Natl Acad Sci U S A. 2016; 113: 8801-8806

75) Cho H, Du X, Rizzi JP, Libezon E, Chakraborty AA, Gao W, Carvo I, Signoretti S, Bruck RK, Josey JA, Wallace EM, Kaelin WG. On-target efficacy of a hif-2alpha antagonist in preclinical kidney cancer models. Nature. 2016; 539: 107-111

76) Chen W, Hill H, Christie A, Kim MS, Holloman E, Pavia-Jimenez A, Homayoun F, Ma Y, Patel N, Yell P, Hao G, Yousuf Q, Joyce A, Pedrosa I, Geiger H, Zhang H, Chang J, Gardner KH, Bruck RK, Reeves C, Hwang TH, Courtneay K, Frenkel E, Sun X, Zojwalla N, Wong T, Rizzi JP, Wallace EM, Josey JA, Xie Y, Xie XJ, Kapur P, McKay RM, Brugarolas J. Targeting renal cell carcinoma with a hif-2 antagonist. Nature. 2016; 539: 112-117

77) Yndestad A, Damas JK, Oie E, Ueland T, Gullestad L, Aukrust P. Role of inflammation in the progression of heart failure. Current cardiology reports. 2007; 9: 236-241

78) Anker SD, von Haehling S. Inflammatory mediators in chronic heart failure: An overview. Heart (British Cardiac Society). 2004; 90: 464-470

79) Rauchhaus M, Doehner W, Francis DP, Davos C, Kemp M, Liebenthal C, Niebauer J, Hooper J, Volk HD, Coats AJ, Anker SD. Plasma cytokine parameters and mortality in patients with chronic heart failure. Circulation. 2000; 102: 3060-3067

80) Seta Y, Shan K, Bozkurt B, Oral H, Mann DL. Basic mechanisms in heart failure: The cytokine hypothesis. Journal of cardiac failure. 1996; 2: 243-249

81) Azzawi M, Kan SW, Hillier V, Vonan N, Hutchinson IV, Hasleton PS. The distribution of cardiac macrophages in myocardial ischaemia and cardiomyopathy. Histopathology. 2005; 46: 314-319

82) Frangogiannis NG. The immune system and cardiac repair. Pharmacological research. 2008; 58: 88-111

83) Frangogiannis NG. The inflammatory response in myocardial injury, repair, and remodelling. Nature reviews. Cardiology. 2014; 11: 255-265

84) Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling--concepts and clinical implications: A consensus paper from an international forum on cardiac remodeling. Behalf of the American College of Cardiology. 2000; 35: 569-582

85) Hilgendorf I, Gerhardt LM, Tan TC, Winter C, Holderried TA, Chousterman BG, Iwamoto Y, Liao R, Zirlik A, Scherer-Crosbie M, Hedrick CC, Libby P, Nahrendorf M, Weissleder R, Swirski FK. The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. J Exp Med. 2007; 204: 3037-3047

86) Nahrendorf M, Swirski FK, Aikawa E, Stangenberg L, Wurdinger T, Figueiredo JL, Libby P, Weissleder R, Pittet MJ. The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. J Exp Med. 2007; 204: 3037-3047

87) Nahrendorf M, Pittet MJ, Swirski FK. Monocytes: Protagonists of infarct inflammation and repair after myocardial infarction. Circulation. 2010; 121: 2437-2445

88) Mann DL, McMurray JJ, Packer M, Swedberg K, Borer
89) Chung ES, Packer M, Lo KH, Fasanmade AA, Willerson JT, Investigators A-TTACHF. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-alpha, in patients with moderate-to-severe heart failure: Results of the anti-tnf therapy against congestive heart failure (attach) trial. Circulation. 2003; 107: 3133-3140

90) Knapp FJ, Goodman M, Callahan A, Kirsch G. Radioiodinated 15-(p-iodophenyl)-3,3-dimethylpentadecanoic acid: A useful new agent to evaluate myocardial fatty acid uptake. J Nucl Med. 1986; 27: 521-531

91) Stanley W, Lopaschuk G, Hall J, McCormack J. Regulation of myocardial carbohydrate metabolism under normal and ischaemic conditions. Potential for pharmacological interventions. Cardiovasc Res. 1997; 33: 243-257

92) Dwivedi G, Al-Shehri H, deKemp RA, Ali I, Alghamdi AA, Klein R, Scullion A, Ruddy TD, Beanlands RS, Chow BJ. Scar imaging using multislice computed tomography versus metabolic imaging by f-18 fdg positron emission tomography: A pilot study. Int J Cardiol. 2013; 168: 739-745

93) Abe H, Iguchi N, Utanohara Y, Inoue K, Takamisawa I, Seki A, Tanizaki K, Takeda N, Tohbaru T, Asano R, Nagayama M, Takayama M, Umemura J, Sumiyoshi T, Tomoike H. Non-invasive diagnosis of coronary artery disease by 123i-bmipp/201tlcl dual myocardial spect in patients with heart failure. Int J Cardiol. 2014; 176: 969-974

94) Sano M, Minamino T, Toko H, Miyauchi H, Orimo M, Qin Y, Akazawa H, Tateno K, Kayama Y, Harada M, Shimizu I, Asahara T, Hamada H, Tomita S, Molkentin JD, Zou Y, Komuro I. P53-induced inhibition of hif-1 causes cardiac dysfunction during pressure overload. Nature. 2007; 446: 444-448

95) Shyu KG, Liou JY, Wang BW, Fang WJ, Chang H. Carvedilol prevents cardiac hypertrophy and overexpression of hypoxia-inducible factor-1alpha and vascular endothelial growth factor in pressure-overloaded rat heart. J Biomed Sci. 2005; 12: 409-420

96) Wei H, Bedja D, Koitabashi N, Xing D, Chen J, Fox-Talbot K, Rouf R, Chen S, Steenbergen C, Harmon JW, Dietz HC, Gabrielson KL, Kass DA, Semenza GL. Endothelial expression of hypoxia-inducible factor 1 protects the murine heart and aorta from pressure overload by suppression of tgf-beta signaling. Proc Natl Acad Sci U S A. 2012; 109: E841-850

97) Krishnan J, Suter M, Windak R, Krebs T, Felley A, Montessuit C, Tokarska-Schlattner M, Aasum E, Bogdanova A, Periardi E, Periardi JC, Larsen T, Pedrazzini T, Krek W. Activation of a hifalpha-ppargamma axis underlies the integration of glycolytic and lipid anabolic pathways in pathologic cardiac hypertrophy. Cell metabolism. 2009; 9: 512-524