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Transflammation in Tissue Regeneration and Response to Injury:

How cell-autonomous inflammatory signaling mediates cell plasticity

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Abstract

Inflammation is a first responder against injury and infection and is also critical for the regeneration and repair of tissue after injury. The role of professional immune cells in tissue healing is well characterized. Professional immune cells respond to pathogens with humoral and cytotoxic responses; remove cellular debris through efferocytosis; secrete angiogenic cytokines and growth factors to repair the microvasculature and parenchyma. However, non-immune cells are also capable of responding to damage or pathogens. Non-immune somatic cells express pattern recognition receptors (PRRs) to detect pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). The PRRs activation leads to the release of inflammatory cytokines required for tissue defense and repair. Notably, the activation of PRRs also triggers epigenetic changes that promote DNA accessibility and cellular plasticity. Thus, non-immune cells directly respond to the local inflammatory cues and can undergo phenotypic modifications or even cell lineage transitions to facilitate tissue regeneration. This review will focus on the novel role of cell-autonomous inflammatory signaling in mediating cell plasticity, a process which is termed transflammation. We will discuss the regulation of this process by changes in the functions and expression levels of epigenetic modifiers, as well as metabolic and ROS/RNS-mediated epigenetic modulation of DNA accessibility during cell fate transition. We will highlight the recent technological developments in detecting cell plasticity and potential therapeutic applications of transflammation in tissue regeneration.

Keywords

Transflammation; innate immunity; DNA accessibility; epigenetics; metabolism; ROS; RNS; single-cell omics; regeneration

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The Discovery of Transflammation

This story of transflammation has its origin in 2006 when Shinyi Yamanaka electrified the field of stem cell biology with his contribution to discovering the induced pluripotent stem cells (iPSCs)[1]. Dr. Yamanaka used a retroviral vector to forcibly express the transcriptional factors Oct4, Sox2, KLF4, and c-MYC in mouse fibroblasts to induce pluripotency. To avoid the use of an integrating vector in human cells (which could engender safety concerns for regenerative medicine applications), we generated cell-permeable peptides of the Yamanaka factors to enable nuclear reprogramming. Despite evidence that the cell-permeable peptides could enter the nuclei of human fibroblasts, no efficient induction of pluripotency genes was achieved, nor induced pluripotent stem cells were derived. Based on the earlier findings that empty virions could alter the phenotype of human cells[2–4], we hypothesized that the retroviral vector used by Yamanaka played a role in reprogramming. To test this hypothesis, in addition to exposing human fibroblasts to our cell-permeant proteins, we added a retroviral vector encoding green fluorescent protein (GFP) and we were stunned to observe an efficient induction of pluripotency genes by this combination. We went on to demonstrate that the retroviral vector used to deliver the Yamanaka factors induced inflammatory signaling that was necessary for pluripotency induction. Specifically, stimulation of toll-like receptor 3 (TLR3) by the retroviral vector activated NFkB and IRF3, which then triggered global changes in the expression of epigenetic modifiers responsible for DNA accessibility[5,6]. This increase in DNA accessibility presumably gave the Yamanaka factors access to the promoter regions of the pluripotency genes required for iPSC formation. We could increase the generation of iPSCs with inflammatory activation and could abrogate iPSC production by blocking inflammatory activation. Thus, inflammatory signaling is necessary for efficient nuclear reprogramming of fibroblasts to iPSCs, as described in our Cell paper that was published the same month that Yamanaka garnered the Nobel Prize for his work[6]. The process whereby cell-autonomous inflammatory signaling enhances DNA accessibility to promote phenotypic plasticity has been termed “transflammation”[7].

Transflammation has proven to be critical in other cell fate transitions besides nuclear reprogramming of somatic cells to iPSC. We have observed that this process is also involved in transdifferentiation. Transdifferentiation is defined as the process by which one somatic cell directly transforms into another somatic cell lineage without going through the stem-cell stage[8].

Our group was the first to show that transdifferentiation of fibroblasts to endothelial cells (Mesenchymal-endothelial transition, MEndoT) can be induced in cell culture or mice in response to limb ischemia, which requires activation of inflammatory signaling[6,9–11]. In these studies, we focused on the transdifferentiation of fibroblasts to endothelial cells, but it seems likely that the transdifferentiation of any somatic cell to another lineage requires inflammatory signaling. In this conceptual framework, inflammatory signaling increases DNA accessibility to a broader genetic repertoire. This genetic repertoire may encode cell cycle proteins, growth factors, and cytoskeletal proteins, in the case of quiescent cells that are expected to become proliferative and migratory to heal a wound (e.g., keratinocytes at the edge of a cutaneous laceration). The genetic repertoire may include lineage factors to support transdifferentiation to another cell type (as when fibroblasts transdifferentiate into

endothelial cells to support angiogenesis). During physiological conditions, the operational efficiency of a cell mandates the repression of genes that are not involved in cell identity or homeostatic processes of that cell lineage. However, when exposed to injury or invading pathogens, the ability of a somatic cell to rapidly change its genetic program and phenotype is adaptive. Thus, activation of PRRs induces cellular plasticity as well as cytokines for defense and regeneration, a parsimonious utilization of inflammatory signaling.

Since our first description of transflammation a decade ago, much has been learned about the process. Firstly, activation of cell surface PRRs such as TLRs or retinoic acid-inducible gene protein (RIG1)-like receptors[5] has been confirmed to lead to NFKb and IRF-3 mediated alterations in expression and activities of epigenetic modifiers, thereby promoting DNA accessibility. The activation of transflammation is then associated with an upregulation of histone acetyltransferases (HATs), downregulation of histone deacetylases (HDACs)[6], an increase in histone acetylation, and other epigenetic modifications associated with chromatin accessibility (e.g. H3K4me3). In addition, inducible nitric oxide synthase (iNOS) has been found to translocate to the nucleus during transdifferentiation. As described below, iNOS binds to repressive epigenetic modifiers, reducing their binding to and suppressive effect on the chromatin[12,13]. Metabolic regulation has also been found during transdifferentiation. The work of Lai et al. revealed a glycolytic shift during cell fate transitions that generate more of the substrate for histone acetylation, indicating that metabolism is coupled to epigenetic alterations[14]. These changes in epigenetic modifiers have the effect of globally altering histone markings to increase DNA accessibility and phenotypic fluidity. Thus, a cellular challenge, such as hypoxia, generates DAMPs which the cell senses, and responds to, with an increase in epigenetic plasticity that permits cellular plasticity and physiological adaptation. Furthermore, the most recent lineage tracing studies have shown evidence of this phenomenon in vivo, in the form of angiogenic transdifferentiation in a mouse hindlimb ischemic revascularization model[10]. The suppression of innate immune signaling by dexamethasone treatment or p65 conditional knockout in fibroblast cells impeded tissue regeneration which further supported the notion that transflammation is a key factor in cellular reprogramming.

The current understanding of the mechanisms underlying transflammation and cell plasticity is discussed in more detail below. (Figure1)

Inflammatory signaling in other types of transdifferentiation.

Whereas our work has focused on the role of transflammation in the nuclear reprogramming of fibroblasts to pluripotency, or to their transdifferentiation to endothelial cells or MEndoT, it is likely that the phenomenon of transflammation also applies to other cell fate transitions. Indeed, Dzau and colleagues have shown that activation of innate immunity is required for fibroblast to cardiomyocyte transdifferentiation[15]. In addition, the maturation of the induced cardiomyocytes requires TLR3-mediated NFKb activity[16]. These studies suggested that the transdifferentiation of any somatic cell to another lineage may require inflammatory signaling. This response to inflammatory signaling may be a primordial mechanism that provides cells with the ability to respond to a challenge with increased adaptability.

The association of inflammatory signaling with pathological transdifferentiation, i.e. epithelial-mesenchymal transition (EMT) or endothelial-mesenchymal transition (EndoMT), has been described in chronic diseases like cancer[17–20] and organ fibrosis[21–27]. In cancers, the chronic inflammation in the tumor microenvironment causes sustained activation of immune-responsive transcriptional factors (TF)s, including TGF-β and NF-KB, which collaboratively initiate the epithelial transformation and facilitate the dissemination of different cancers[18,28–30] by activating the expression of EMT-TFs, such as TWIST, SLUG[31], and SNAIL[17]. Similar mechanisms are reported in EndoMT[32–34] which contributes to the production of Cancer-Associated-Fibroblasts (CAFs)[35] to further supports tumor growth and metastasis[36]. Tissue fibrosis is also characterized as a common feature of a variety of chronic pathological processes, affecting major organs including the heart[37,38], kidney[39,40], liver[41–43], and lung[44,45], etc. In all cases, inflammation response is considered to play a role in the disease progression[46,47]. However, the cellular origins of the activated fibroblasts remain controversial[48,49]. Seminal works have connected pathological transdifferentiation with cardiovascular fibrotic events. Specifically, EMT[50,51] and EndoMT[52,53] are reported to be involved in the response to cardiac injury, mediated by inflammatory signaling and TGF-β[54–56]. These mechanisms may also underlie cardiac fibrosis in diabetic[57,58] and hypertrophic[59,60] conditions. Some forms of cardiac fibrosis may occur in the absence of EndoMT such as post-transverse aortic constriction (TAC)[61,62] or myocardial infarction (MI) surgery[63–65]. Moreover, other forms of transdifferentiation including macrophages to myofibroblasts transition (MMT) are also reported in fibrotic disorders[66–68]. So, more lineage tracing work *in vivo* in combine with fate mapping using single-cell sequencing analysis will be needed to confirm the role of pathological transdifferentiation under different disease conditions.

Immunometabolism in cell fate transition

Metabolic pathways provide the energy and building blocks through catabolism and anabolism to meet the diverse demands of cellular processes[69,70]. Cellular metabolism also provides intermediates as the substrate for epigenetic events that are required for cell fate transition[71–75]. Several of the tricarboxylic acid metabolites mediate the activities of the chromatin-modifying proteins. An example of a metabolite with an epigenetic role is acetyl-coA[76,77]. It is generated through the metabolism of several precursors, including fatty acids, acetate, pyruvate, and glutamine. Acetyl-CoA can then be used for lipid synthesis and protein acetylation. In the nucleus, acetyl-CoA is utilized in histone acetylation, a major regulatory process in chromatin configuration and gene expression. Histone acetylation neutralizes the positive charge of the lysine on the histone tail and decreases the interaction between histone and DNA, which makes DNA more accessible for the transcriptional machinary[78,79]. The enzymes that are responsible for acetyl-CoA synthesis, including ATP-citrate lyase (ACL)[80] and acetyl-CoA synthetase (ACSS)[81], have become therapeutic targets[82] in diseases including cancer[83–85] and atherosclerosis[86–89] to reduce histone acetylation and cellular activation in those conditions.

Immunometabolism describes the intricate interplay between metabolism and immune cell activation and differentiation, also termed intrinsic immunometabolism. For example, the M2 macrophage relies on β-oxidation. Inhibition of fatty acid oxidation is sufficient to

alter macrophage polarization, switching the immune-repressive M2 phenotype to the pro-inflammatory M1 phenotype[90–92]. Furthermore, the metabolic-epigenetic axis is known to be involved in T cell fate determination, including effector[93,94], Treg[95–97], memory[98,99], and exhausted[100,101] T cells. Additionally, studies performed to explore the role of immune cells in systemic metabolism, including obesity[102] and diabetes[103,104], termed the extrinsic immunometabolism, have further unveiled a crucial role of chronic inflammation in insulin resistance[105,106] and other metabolic diseases[107–109]. However, although many studies have characterized the molecular circuits traversing the reciprocal relationship between inflammation and metabolism, much less attention has been focused on metabolism and inflammatory signaling in non-immune cells. Here, the interaction between inflammatory activation and metabolism appears to be crucial for cell function, cell identity, and cell fate transition.

One example of inflammatory signaling and cell plasticity in non-professional immune cells is the endothelial-to-mesenchymal transition (EndoMT) that contributes to atherosclerosis, pulmonary hypertension, and cardiac fibrosis[110,111]. In those pathological conditions, the endothelial cells persistently express a high level of pro-inflammatory leukocyte adhesion molecules and growth factors that induce endothelial dysfunction, and which promote a mesenchymal phenotypic switch. Recent studies highlight a role for metabolism, in that fatty acid metabolism provides an essential pool of acetyl-CoA to maintain endothelial cell identity[112].

In the transdifferentiation of fibroblasts to endothelial cells[11], metabolic-epigenetic coupling plays a critical role[14]. Specifically, upon TLR3 activation, a rapid Warburg effect is triggered, in which glycolysis exceeds oxidative phosphorylation, coupled with a non-canonical tricarboxylic acid cycle (TCA cycle) in which glucose-derived citrate accumulates and is exported out of the mitochondria through citrate transporter, Slc25a1. Concurrently there is an increase in the expression of ATP-citrate lyase (ACL) in the nucleus, which converts citrate to acetyl-CoA. Acetyl-CoA is the substrate for histone acetylation which increases DNA accessibility to facilitate cellular reprogramming. These observations represented a novel metabolism-driven signaling cascade across mitochondria, cytoplasm, and nucleus, linking metabolism with cell-autonomous inflammatory signaling, epigenetic regulation, and cell plasticity which may serve as a general mechanism in many inflammation-induced cell fate transitions[113–116] (Figure2). A similar non-canonical TCA cycle phenomenon was later observed in embryonic stem cells (ESCs) with [U-13C] glucose tracing assay[117]. In this study, Arnold et al. observed ESCs prefer a non-canonical TCA cycle in which the mitochondrial citrate tends to be shunted into the cytoplasm where it is converted to oxaloacetate and malate. Malate is then transported back to mitochondria through Slc25a1, the citrate/malate antiporter, to replenish mitochondrial oxaloacetate. They documented that the utilization of the non-canonical TCA cycle in naïve ES cells was increased when the cells exited the naïve state. Similarly, during the differentiation from muscle myoblast to myotubes, the utilization of the canonical pathway increased, suggesting a dynamic regulation of metabolism during cell fate transition. Together with the findings from earlier paper[14], this non-canonical TCA cycle may play an important role in cell fate transitions.

The role of ROS/RNS in the cell plasticity

It is well-known that inflammation induces the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS)[118–120]. These free radicals are key signaling molecules that drive many homeostatic as well as pathophysiological processes[121]. They also have a prominent impact on the cell fate transition[122,123].

ROS plays essential roles in signaling to maintain cellular homeostasis[124–126]. A low and modest level of ROS is known to be beneficial for survival and proliferation[127] in many cells including T cells[128,129] and cancer cells[130] which normally rely on glycolysis for energy expenditure. Similarly, iPSCs are known to be better cultured under conditions that favor glycolysis[131]. A study from Gang et. al has observed that ROS production is transiently increased during nuclear reprogramming to generate iPSCs[132] using mice embryonic fibroblasts carrying the doxycycline-inducible Yamanaka cassette (Oct4, Sox2, Klf4, and c-Myc). Upon the initiation of nuclear reprogramming, there is a transient spike in ROS generation which subsides at a later phase of the process. Inhibition of ROS generation by knockdown of NADPH oxidase 2 (Nox2), or the use of ROS scavengers, at the onset of nuclear reprogramming, abrogated iPSC formation. Furthermore, this phenomenon is mediated by NF-KB signaling which is in line with our initial finding about the function of innate immune activation during nuclear reprogramming[6]. The inhibition of NFKB phosphorylation by BAY117085 decreases the early upregulation of Nox and iPSC formation. Later in the process, the generation of ROS subsides, and here the administration of antioxidants enhances reprogramming. Interestingly, the overproduction of ROS by Nox overexpression or a high dose of hydrogen peroxide, even at the initial stage of reprogramming, impairs iPSC formation. These observations suggest that there is an optimal range of ROS generation for effective nuclear reprogramming and are consistent with the Goldilocks' zone for inflammatory signaling and cell fate plasticity described below.

Reactive nitrogen species are another form of free radical. They are generated through 3 major nitric oxide synthases (NOS), including neuronal isoform (nNOS), endothelial isoform (eNOS), and the inducible isoform, iNOS[133]. The activation of iNOS is a notable feature of inflammation[134]. The nitric oxide (NO) generated by NOS enzymes is important for many cellular processes maintaining vascular homeostasis[135–137]. The reduced expression of eNOS and reduced bioavailability of NO are often associated with cardiovascular diseases including atherosclerosis, hypertension, and aging[138,139]. The NOS enzymes exert their influence in part by post-translational modification of proteins through S-Nitrosylation[140] which was found to occur on epigenetic modifiers during cell fate transitions. Specifically, during the induction of pluripotency through nuclear reprogramming, an increased iNOS expression was observed. Furthermore, iNOS translocated to the nucleus to bind and S-nitrosylates MTA3, which is a component of the nucleosome remodeling and deacetylase (NuRD) complex[141]. The MTA3 S-nitrosylation is associated with reduced association of the NuRD complex with chromatin; reduced HDAC activity; increased coverage of the chromatin with the active marker H3K27ac and decreased coverage with the repressive marker H3K27me3 on promoter regions of pluripotency genes[12]. Similarly, iNOS is induced and NO generation is increased, during transdifferentiation of fibroblasts to endothelial cells. Concurrently, S-nitrosylation of

polycomb repressive complex protein, RING1A[142], was observed, which causes dissociation of the repressive polycomb complex from chromatin to increase epigenetic plasticity[13].

Goldilocks zone of inflammatory signaling in regeneration

In view of the many facets of immune signaling in physiological and pathological conditions, it is easy to speculate that the timing and the intensity of inflammatory signaling modulate the downstream effects of immune activation. Indeed, the observations during the induction of pluripotency, or transdifferentiation, strongly suggest a Goldilocks zone for innate immune signaling to activate cellular plasticity and tissue regeneration[143]. In these studies, a range of doses of TLR3 agonist Poly I: C was used to activate inflammatory signaling during cell fate transition. Distinct outcomes are observed for different doses of the TLR3 agonist, and intermediate doses of Poly I: C (10 to 100 ng/ml) enhances cell fate transitions, whereas doses above this level impair reprogramming efficiency[12]. This discordance may be partially explained by an overproduction of ROS which was previously observed to impair reprogramming to iPSCs[132]. Furthermore, during nuclear reprogramming, the repressive epigenetic factor, MTA3, and NuRD complex activity are downregulated with 30 ng/ml Poly I: C, while upregulated with 1000ng/ml Poly I: C. Also, the MNase digestion assay which reflects the DNA accessibility showed the highest mononucleosome to dinucleosome and mononucleosome to trinucleosome ratios in the optimal range of innate immune signaling. These data indicate that there is an optimal dose of inflammatory signaling that can increase DNA accessibility for phenotypic fluidity.

It is also possible that sub-optimal inflammatory signaling fails to orchestrate the energy supply/expenditure as well as the metabolic-epigenetic coupled regulation during cellular reprogramming[144]. For example, under chronic inflammatory conditions, e.g., atherosclerosis, ACL is highly expressed, and its knockdown generates a more favorable plaque phenotype[145]. The Goldilocks zone of inflammatory signaling may be reflected in the apparently contradictory observations about the role of inflammation in tissue regeneration. For example, the poor wound healing in the patient who receives intensive steroid therapy[146,147] may be due to impaired inflammatory activation of DNA accessibility and regenerative cellular plasticity. In other words, these patients have inadequate stimulation of the epigenetic and metabolic mechanisms required for an open chromatin state and transcriptional activation of regenerative pathways. On the contrary, the patient with a diabetic foot ulcer[148,149] may have failed to heal due to excessive inflammatory activation, which may also impair DNA accessibility and cellular plasticity (Figure 3). Understanding how to manipulate inflammatory signaling, with attention to spatiotemporal control, may provide a new avenue in the management of non-healing wounds. In this regard, novel anti-inflammatory drugs have exciting applications for cardiovascular disease[150], but there should be a heightened level of concern in using such agents in patients in the setting of surgery or trauma[150,151].

New technologies for characterizing mechanisms of cell plasticity

Cell plasticity in response to a pathogenic challenge or injury is required for tissue defense and regeneration. Elucidation of the mechanisms undergirding cell plasticity should lead

to major advances in regenerative medicine. The technology of single-cell multi-omics has transformed the field of regenerative medicine. The traditional approaches to identifying cell subpopulations are largely dependent on the low throughput and low-resolution flow cytometry or immunostaining with known cell identity markers. Furthermore, bulk RNA sequencing may dilute rare transcriptional events and cellular subpopulations that are important in regeneration. With the rapid development of single-cell multi-omics and computational analysis tools, genetic and epigenetic information can be profiled for the same cell[152], and databases from different experiments can be further integrated[153]. Now spatiotemporal transcriptomics can profile genetic information on tissue sections with the resolution of a few cells. In combination with single-cell RNA sequencing datasets, single-cell resolution transcriptomics can be generated from tissue slides[154].

These advances in technology have led to fruitful discoveries. For example, a single cell RNA seq study in a murine model of experimental myocardial infarction identified an endothelial subpopulation that transiently expresses mesenchymal signatures early after the surgery[155]. This observation supports the concept of an endothelial-mesenchymal transition which contributes to tissue remodeling and fibrosis post-cardiac ischemia, a concept that has been debated for years[155]. Another single-cell RNA seq study of endothelial zonation in the mouse brain also finds evidence for the transdifferentiation of endothelial cells to other cell types and the metabolic underpinnings which can be potentially harnessed for therapeutic strategies[156]. As another example, eight divergent subpopulations of fibroblasts in a model of hindlimb ischemia mouse model are discovered when combining single-cell RNA seq with lineage tracing strategy. Furthermore, with experimental induction of limb ischemia, two of these fibroblast subpopulations (clusters 5 and 8) increased significantly and appeared to contribute to angiogenesis. These clusters were then isolated using specific surface markers, and cultured ex vivo. Cluster 8 generated angiogenic cytokines, whereas cluster 5 expressed some endothelial identity genes, and in Matrigel, formed tubes and expressed endothelial surface markers suggestive of transdifferentiation to an angiogenic phenotype. In the murine ischemic hindlimb model, inhibition of inflammatory signaling markedly reduced the number of these “angiogenic fibroblasts”; impaired wound healing in the ischemic limb; and reduced perfusion recovery[10]. This study may explain the different responses of fibroblasts to the same stimuli. The ability of a fibroblast to transdifferentiate may be pre-determined by the epigenetic and transcriptional heterogeneity of the fibroblasts. With the unprecedented development of single cell-resolution transcriptome and epitranscriptome profiling and analysis methods[157], it will be possible to predict and target the specific sub-clusters that drive the pathophysiological processes.

Single-cell proteomics and metabolomics are the newer members of the single-cell omics field. The single-cell proteomics that characterizes the amount, the post-translational modification, and the kinetics of the thousands of proteins at the same time are complementary to and synergistic with, transcriptomic studies[158]. This approach mainly includes single-cell barcoding, which is similar to single-cell RNA seq but with a mass spectrometry version (isobaric tags), in a nanoliter-scale reaction system called nanoPOTS for protein lysis, followed by ultra-high-resolution mass spectrometry[159]. Advances have been made by combining nanoPOTS with laser-capture microdissection, mass spectrometry, and a newly developed computational algorithm, HIT-MAP, which adds spatial information

in proteomics studies[160]. The single-cell metabolomics is the newest single-cell omics technology and provides additional single-cell phenotypic information[161]. Unlike the previously described omics, single-cell metabolomics doesn't involve a barcoding process. Instead, matrix-assisted laser desorption/ionization mass spectrometry (MALDI–MS) is integrated with an imaging system to identify single cells. Then the identified cell is irradiated with a UV laser beam to ionize analytes for assessment by mass spectrometry[162]. These advances in single-cell omics, together with new bioinformatic approaches (such as RNA velocity algorithms) will provide more comprehensive transcriptional, epigenetic, and metabolic profiles to characterize the determinants of cell identity and plasticity. These fundamental insights will no doubt contribute to the development of novel therapeutic avenues.

Conclusion and Discussion

Whereas the role of professional immune cells in tissue repair and regeneration has been well-characterized, non-immune cells are also capable of a response to injury, in part by sensing the molecular patterns presented by pathogens or damaged tissue. Essentially, the stimulation of pattern recognition receptors by damage-associated or pathogen-associated molecular patterns activates inflammatory signaling which triggers a cascade of cellular signaling (mediated by RNS, ROS, and metabolites) to cause changes in epigenetic modifiers that increase DNA accessibility. The cell is now in a state of epigenetic plasticity that permits phenotypic fluidity. However, the trajectory of the transition is dependent upon the milieu. In a setting where ischemia triggers the generation of angiogenic cytokines, a subset of fibroblasts may become endothelial cells. However, it is also possible that, if the ischemia is too severe, or the inflammatory signaling is too profound, the transdifferentiation of fibroblasts to endothelial cells may be abrogated. Indeed, we have shown that there is a Goldilocks zone for inflammatory signaling in cell fate transitions. With excessive inflammatory signaling, the generation of induced pluripotent stem cells from fibroblasts is attenuated. With excessive production of ROS, fibroblast reprogramming to pluripotency is reduced[12,132]. Furthermore, other factors in the milieu, such as intercellular or tissue-derived signals generated by cytokines, neurohormonal factors, exosomes, or alterations in the composition of the extracellular matrix will contribute to modifying the trajectory and direction of cell fate transitions. For example, in a murine model of myocardial ischemia, activation of the TLR3 pathway by mechanical stimulation releases exosomes containing angiogenic microRNA from endothelial cells to stimulate angiogenesis[163,164].

Based on our studies and others, we believe that inflammatory signaling has epigenetic effects to increase DNA accessibility, whereas cell fate is determined by the microenvironment. Differences in the microenvironment may play a critical role in the different responses observed in myocardial versus limb ischemia. Whereas transdifferentiation of cardiac fibroblasts to endothelial cells in the setting of myocardial ischemia is controversial[63,64,155,165,166], the contribution of fibroblast transdifferentiation to endothelial cells in the recovery from limb ischemia has been convincingly shown[10]. This difference may be due to the fact that there is a greater capacity for arteriogenesis and collateralization in the limb in comparison to the heart. In C57BL6J mice, femoral artery ligation reduces limb blood flow by 80%. However, this ischemic challenge triggers a robust

arteriogenic and angiogenic response that largely recovers perfusion within 2 to 3 weeks. Conversely, with ligation of the left anterior descending (LAD) coronary artery, there is extensive necrosis and scarring of the myocardium, with little perfusion recovery[167 168]. The profound ischemic insult of LAD ligation, combined with the ongoing metabolic demand of the working heart, may limit the degree of recovery of the microvasculature.

Ours and others' recent findings about the role of metabolism and ROS/RNS in the process of transflammation also suggested a new path toward developing therapeutic strategies targeting these pathways to enhance DNA accessibility, cellular plasticity, and tissue repair. For example, pharmacotherapeutics that increase ACL activity and histone acetylation may be useful for tissue regeneration. As another example, spatiotemporal control of iNOS activity and S-nitrosylation may have regenerative medicine applications. As we begin to identify subpopulations of regenerative cells by single-cell omics and the determinants of their fate and function, better markers of tissue health and regeneration will be developed to orchestrate a regenerative response. The single-cell characterization of these subpopulations by transcriptional, epigenetic, proteomic, and metabolomic approaches will lead to novel strategies to target and manipulate those populations to enhance tissue repair and regeneration.

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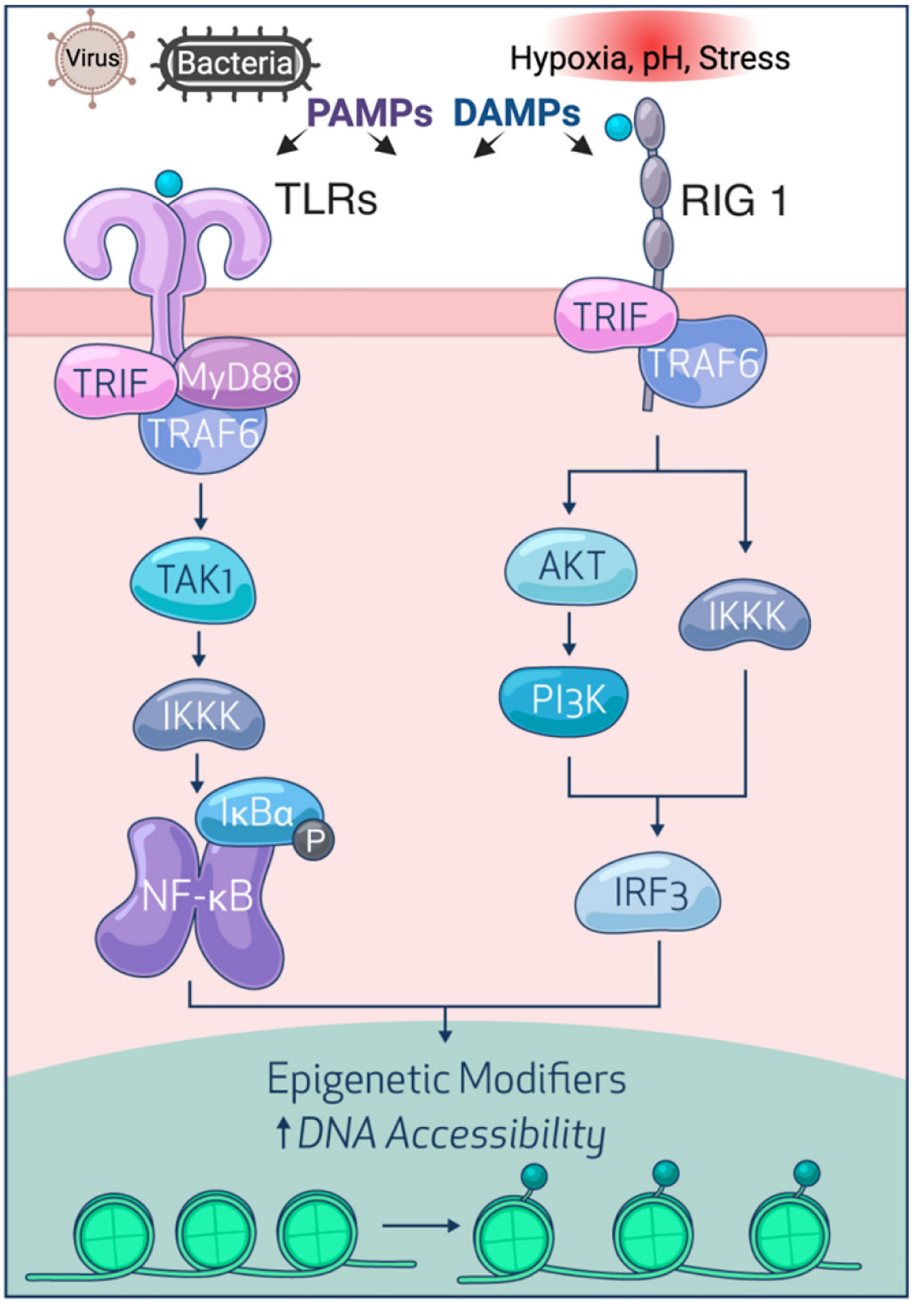

Figure1.

Transflammation. Viruses and bacteria produce PAMPs while hypoxia, pH, stress, and other injurious stimuli generate DAMPs, both of which trigger innate immune signaling by intracellular or transmembrane PRCs such as TLRs and RIG-I. Subsequently, NF-kB or IRF3 are activated and translocated to the nucleus to alter the expression of epigenetic modifiers, thereby increasing DNA accessibility to facilitate the cell fate transition.

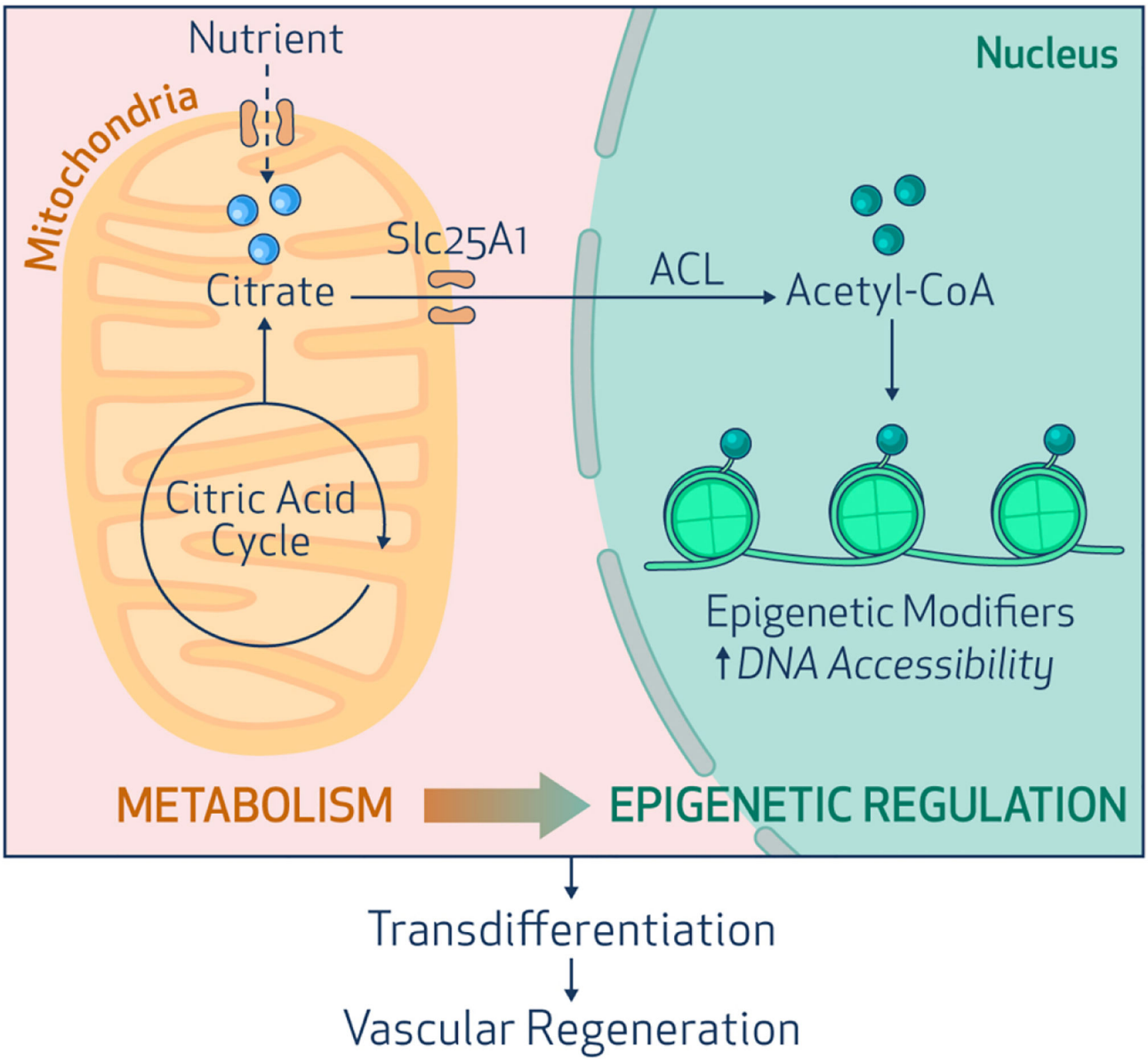

Figure 2.

A metabolic-epigenetic axis controls cell fate transition. Innate immune activation induces glycolysis and facilitates mitochondrial export of citrate to the nucleus. In the nucleus, ATP-Citrate Lyase (ACL) converts citrate to acetyl-CoA. There it serves as the substrate for histone acetylation to increase DNA accessibility, and facilitate cell fate transitions, as in vascular regeneration.

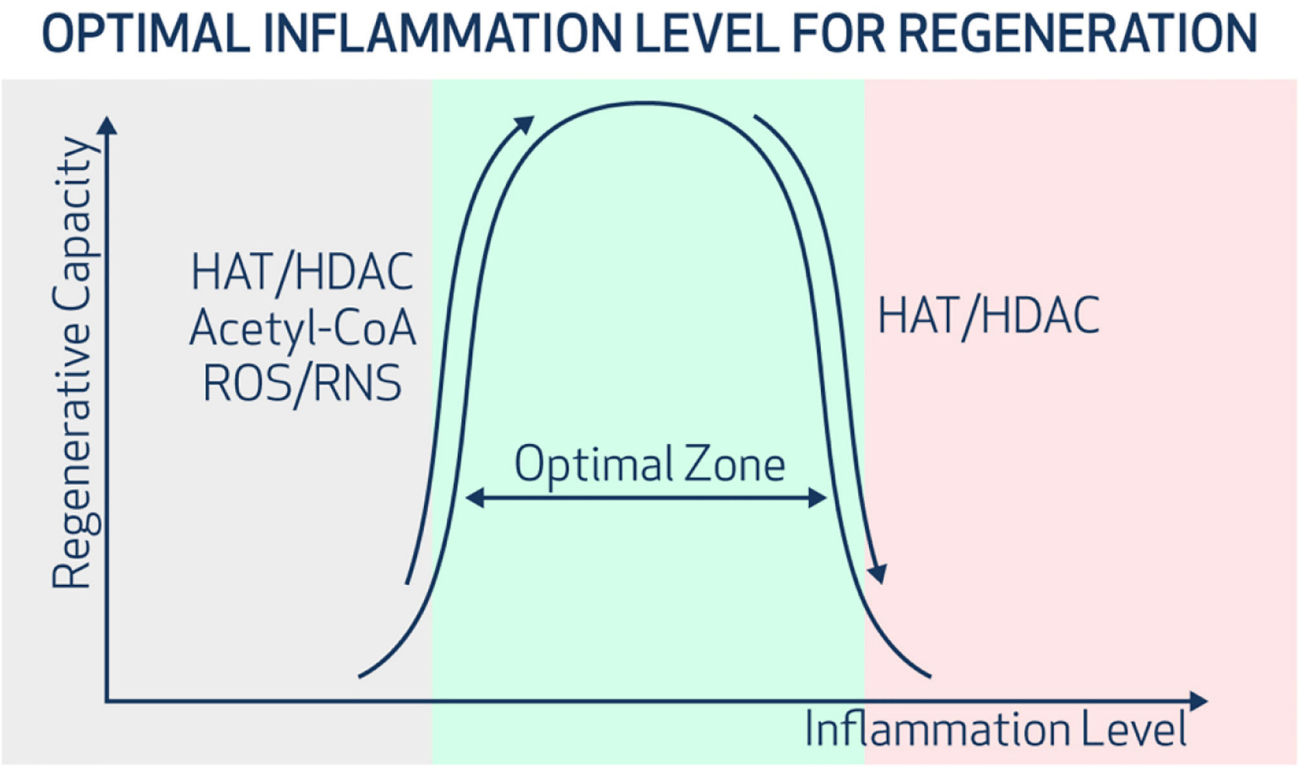

Figure 3.

The Goldilocks zone of inflammatory signaling in regeneration. An optimal intensity of inflammatory signaling increases DNA accessibility and cellular plasticity. Insufficient or excessive inflammation reduces DNA accessibility and limits cellular adaptability to injury or stress. HAT/HDAC represents the ratio of gene expression of histone acetyltransferases (HAT) to histone deacetylases (HDAC). A higher HAT/HDAC ratio suggested a more open chromatin and higher DNA accessibility. ROS=reactive oxygen species. RNS = reactive nitrogen species.