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Transflammation: Innate immune signaling in nuclear reprogramming☆

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ABSTRACT

Induction of pluripotency in somatic cells by retroviral overexpression of four transcription factors has revolutionized the field of stem cell biology and regenerative medicine. The efficient induction of pluripotency requires the activation of innate immune signaling in a process termed "transflammation" (Lee et al., 2012). Specifically, the stimulation of pattern recognition receptors (PRRs) causes global alterations in the expression and activity of epigenetic modifiers to favor an open chromatin configuration. Activation of toll-like receptors (TLR) or RIG-1-like receptors (RLR) (Sayed et al. 2017) trigger signaling cascades that result in NFκB or IRF-3 mediated changes in epigenetic plasticity that facilitate reprogramming. Another form of nuclear reprogramming is so-called direct reprogramming or transdifferentiation of one somatic cell to another lineage. We have shown that transdifferentiation of human fibroblasts to endothelial cells also involves transflammation (Sayed et al., 2015). Recently, we also identified reactive oxygen species (ROS) (Zhou et al. 2016) and reactive nitrogen species (RNS) (Meng et al., 2016) as mediators of innate immune signaling in nuclear reprogramming. Innate immune signaling plays a key role in nuclear reprogramming by regulating DNA accessibility (Fig. 1). Here, we review recent progress of innate immunity signaling in nuclear reprogramming and epigenetic plasticity.

Contents

Abbreviations: PRR, pattern recognition receptor; TLR, toll-like receptor; RIG-1, retinoid acid-inducible gene I; RLR, RIG-1-like receptor; ROS, reactive oxygen species; RNS, reactive nitrogen species; iPSC, induced pluripotent stem cell; ESC, embryonic stem cell; MHC, major histocompatibility complex; MEndoT, mesenchymal-to-endothelial transition; EndoMT, endothelial-to-mesenchymal transition; PAMP, pathogen-associated molecular pattern; NLR, nucleotide-binding oligomerization domain-like receptors; DAMP, damage-associated molecular pattern; HMGB1, high mobility group box 1; RAGE, receptor for advanced glycation end products; dsRNA, double-stranded RNA; ssRNA, single-stranded RNA; MyD88, myeloid differentiation primary response gene 88; TRIF, TIR-domain-containing adapter-inducing interferon-β; polyI:C, polyinosinic-polycytidylic acid; IPS-1, interferon-beta promoter stimulator 1; NO, nitric oxide; iNOS, inducible nitric oxide synthase; HDAC, histone deacetylase; HAT, histone acetyltransferase; PRC1, polycomb repressive complex 1.

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1. Introduction

Nuclear reprogramming describes a global change in the epigenetic landscape and transcriptional profile of one cell type so that it resembles that of another cell type [6,7]. Often, the term nuclear reprogramming is used in literature to describe the generation of induced pluripotent stem cells (iPSCs) from somatic cells by overexpression of four pluripotent transcription factors (Oct4, Sox2, Klf4 and cMyc) [8,9]. The iPSCs that are generated are similar to embryonic stem cells (ESCs). They can replicate indefinitely and are capable of differentiating into cells from each of the three germ layers (endoderm, ectoderm and mesoderm). This breakthrough discovery has many applications in biotechnology and regenerative medicine [10,11]. The iPSCs generated from patient-derived somatic cells are useful for understanding the pathobiology of a disease; for use in cell therapy or tissue regeneration; or for generating high throughput cellular assays for screening of molecules that therapeutically modulate the pathology.

Transdifferentiation is another form of nuclear reprogramming, which describes the metamorphosis of a somatic cell of another lineage. Transdifferentiation may happen in physiological conditions such as mesenchymal to endothelial transition in embryonic development [12] and in regeneration [13]. Transdifferentiation may also contribute to disease, such as the transformation of squamous esophageal epithelial cells to a cell type that resembles the columnar epithelium of the intestine. This so-called Barrett's esophagus occurs in the setting of chronic inflammation induced by gastroesophageal reflux, and is a pre-malignant change [7,14]. However, the process of transdifferentiation may also be wielded with therapeutic intent. Therapeutic modulation of this process may be useful in diseases characterized by loss of certain type of cells that can be replenished by transdifferentiation [3].

Nuclear reprogramming may also be used to describe the process by which a senescent cell is transformed into a juvenile cell of the same lineage, for example, the transformation of a senescent human endothelial cell to a juvenile endothelial cell [15]. Such a transformation requires the same global changes in the activity and expression of epigenetic modifiers as other forms of reprogramming.

We discovered that innate immune signaling facilitates nuclear reprogramming in reprogramming to pluripotency [1] and in transdifferentiation [3]. We identified that the innate immune activation was required for the changes in the expression and activity of epigenetic modifiers that make the chromatin more accessible to transcriptional factors [1–5]. We have coined the term “transflammation” to describe the process by which innate immune signaling promotes epigenetic plasticity. Here we review recent progress in our understanding of how innate immunity signaling facilitates nuclear reprogramming, both in the reprogramming to pluripotency or in transdifferentiation. We also discuss the potential role of innate immune signaling and transflammation in tissue regeneration.

1.1. Nuclear reprogramming to pluripotency

Nuclear reprogramming of a somatic cell to pluripotency was first achieved using nuclear transfer. In this procedure, the nucleus of a somatic cell is placed into an enucleated egg [6,16]. The somatic cell nucleus was acted upon by all of the transcriptional factors and other regulators in the egg cytoplasm, leading to the reprogramming of the somatic cell nucleus to a pluripotent state. This transformed chimeric cell is now capable of proliferating and differentiating into all three germ layers. John Gurdon used this approach to clone a frog from somatic cells of a *Xenopus* tadpole [6,16]. Many years later, Yamanaka and colleagues defined a limited set of transcriptional factors (Oct4, Sox2, Klf4 and cMyc) that could induce pluripotency when overexpressed in a somatic cell [9]. They termed these cells “induced pluripotent stem cells” or iPSCs. The ability to generate iPSCs from somatic cells highlighted the fact that somatic cell phenotype is fluid and cell fate can be manipulated by the overexpression of lineage specific or pluripotency transcription factors. For their seminal discoveries that revealed the mechanisms of pluripotency induction, Drs. Gurdon and Yamanaka received the Nobel Prize in Physiology or Medicine in 2012.

The iPSCs derived are highly similar to ESC in terms of the capacity for indefinite self-renewal and the potential to differentiate into any somatic cell type in all three germ layers [8,9]. Thus human iPSCs can be used as a surrogate for human ESCs in studies of pluripotency and differentiation, which avoids the ethical issues surrounding the latter cells. Notably, patient-specific iPSCs may be generated from somatic cells that are easily obtained (e.g. skin fibroblasts), and then differentiated into cells that are less easily obtained (e.g. neurons) so as to understand the mechanism of disease (e.g. a neurological disease that is not well characterized) or to test the efficacy of a potential medication. To the extent that the patient's disorder has a genetic basis, the generation of patient-specific cells is likely to replicate the genetic substrate for the pathobiology. Once the pathophysiology is understood, such iPSC-derived cells may be used to develop cellular assays for high throughput screening for therapeutic molecules [17–26]. Finally, iPSCs provide a renewable source of any cell type for regenerative medicine applications, thus it is possible to generate the large number of cells required to have a therapeutic effect.

Patient-specific iPSC-derived cells may have application in regenerative medicine. One could potentially use such cells to replace or regenerate tissues or organs to restore or reestablish normal cell function [27]. These derived cells are autologous and would not solicit immune reactions. However, for logistical reasons, it may be more feasible to utilize therapeutic cells derived from banks of allogeneic iPSCs that express the major histocompatibility (MHC) antigens most frequently represented in the population [28,29]. Patients requiring a cell therapy would undergo testing to identify their MHC profile, and receive an iPSC-derived cell therapy using matched cells. Such therapy might be administered as the cells alone; cells bioengineered into a matrix or device; or a cell-derived product.

1.2. Transdifferentiation

Transdifferentiation, or so called direct reprogramming, is manifested by a change in the epigenetic and transcriptional profile of a somatic cell as it undergoes a metamorphosis toward a different lineage [30]. This form of nuclear reprogramming was first accomplished by cell fusion. The experimental fusion of a liver and muscle cell caused the liver cell to express muscle proteins [31]. The overexpression of lineage specific transcription factor has similar effect. In this case, overexpression of MyoD, a muscle specific transcription factor, in the liver cells recapitulated many of the same effects as cell fusion with a skeletal muscle cell [32]. Transdifferentiation via the overexpression of specific lineage determination factors has now been broadly applied to induce many cell lineages from other somatic cells. For example, transdifferentiation of fibroblasts into cardiomyocytes [33], endothelial cells [34], and neurons [35] has been achieved in vitro.

Although transdifferentiation is a common feature in developmental stages in mammals, its role in adult mammalian system was not clearly defined until the development of lineage tracing technologies. Transdifferentiation is now known to be involved in multiple physiological and pathological processes. For example, in the myocardium, there is a physiological transdifferentiation of cardiac fibroblasts to endothelial cells (mesenchyme-to-endothelial transition or MEndoT), which contributes to the angiogenic response to hypoxia [13]. By contrast, a pathological endothelial-to-mesenchyme (EndoMT) participates in the reduction in capillary density and increase in cardiac fibrosis that occurs in diabetes mellitus [36]. In addition, therapeutic transdifferentiation in vivo has been accomplished using forced overexpression of lineage factors. Therapeutic transdifferentiation to the desired cell type showed

promise in ameliorating organ injury [37,38]. Small molecule based methods for therapeutic transdifferentiation could obviate some of the problems (e.g. concerns with integration of viral DNA; adverse immune responses) attending the use of viral vectors [39,40].

In some applications, by comparison to the use of iPSCs, transdifferentiation might be a simpler approach to generating therapeutic cells. The direct reprogramming avoids the steps required for induction of pluripotency, the differentiation of the iPSCs, and the selection of therapeutic cells from a heterogeneous mixture [7]. Also, iPSCs must be generated ex vivo and differentiated to the therapeutic cell type prior to administration (to avoid the adverse event of teratoma formation). Among the hurdles to iPSC-derived cell therapy is how to deliver the cells where they are needed, in the number that is necessary (too many cells could invite ischemia-induced apoptosis), and in a fashion that recreates the normal tissue architecture [7]. It seems ideal to infuse small molecules into the tissue or organ that may promote transdifferentiation of resident cells (e.g. fibroblasts) to a therapeutic cell type (e.g. endothelial cells) in the physiological milieu.

However, until recently transdifferentiation has largely been accomplished using viral vectors that encode lineage determination factors. A more tractable approach for clinical use would be a small molecule strategy that could avoid the concerns of integration of exogenous DNA, with potential disruption of normal gene function. Our recent discovery that innate immune signaling promotes nuclear reprogramming has provided a new approach for therapeutic transdifferentiation that can be achieved by small molecules [3]. Thus the understanding of innate immune signaling and its effects on the epigenetic landscape provides novel insights for regenerative medicine applications.

2. Innate immune signaling

Innate immune response is the host's first line of defense against invading pathogens. Pathogens are recognized by the host as they display pathogen-associated molecular patterns (PAMPs) [41,42]. These PAMPs are a group of highly conserved structures that are present in large groups of microorganisms [43], which are recognized by pattern-recognition receptors (PRRs) on mammalian cells. The binding of PAMPs to PRRs initiates a signaling cascade that triggers the synthesis and release of cytokines that promote a local inflammatory response [43,44].

The PRRs are comprised mainly of Toll-like receptors (TLRs) [45–47] as well as cytosolic PRRs which include the retinoid acid-inducible gene I (RIG-1)-like receptors (RLRs) [48] and the nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) [49]. TLRs recognize microbes in endosomes and on cell surface, whereas RLRs and NLRs detect cytosolic microbial components.

In addition, endogenous components derived from dying host cells may display damage-associated molecular patterns (DAMPs) that can also bind to and activate PRRs [50]. DAMPs released by cells during stress can also activate TLRs [51]. Well-known examples of DAMPs are high mobility group box 1 (HMGB1), S100 proteins and nucleic acids like mitochondrial DNA and cytosolic RNA [52–54]. Proteins belonging to the S100 family members such as S100A8 and S100A9 also bind to the receptor for advanced glycation end products (RAGE) [55].

The toll receptors were first identified as sensors of pathogens in Drosophila [56]. TLR homologues were subsequently identified in mammals. So far 13 TLR members have been discovered. These receptors can be classified into two large groups based on their locations: those on the cell surface (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10) and those in intracellular compartments such as the endosome (TLR3, TLR7, TLR8, TLR9, TLR11, TLR12, and TLR13) [57]. Cell surface TLRs generally recognize microbial membrane components such as lipids and lipoproteins. For example, TLR4 recognizes bacterial lipopolysaccharide (LPS). By contrast, the intracellular TLRs recognize pathogenic RNA or DNA. For example, TLR3 recognizes viral double-stranded RNA (dsRNA) [58]. TLR7 and TLR8 recognize single-stranded RNA (ssRNA) [59,60]. TLR9 recognizes unmethylated CpG-DNA [61].

TLRs are type I integral membrane glycoproteins, comprised of an exodomain containing leucine-rich-repeat motifs, a membrane-spanning segment and a signaling domain (TIR domain) homologous to that of the IL1 receptor. The activation of TLRs by their ligand (PAMPs) triggers monomers of the TLRs to form active homodimers or heterodimers. Adaptor proteins are recruited subsequently, triggering the downstream signaling cascades. Several adaptor proteins have been shown to bind to the TIR domain so as to mediate the downstream signaling, including myeloid differentiation primary response gene 88 (MyD88) and TIR-domain- containing adapter-inducing interferon-β (TRIF) [62,63]. MyD88 is the adaptor for all TLRs except for TLR3, which signals through TRIF. MyD88 and TRIF activate proinflammatory signaling pathways including NFκB, AP1, ERK and p38, and anti-viral signaling pathways through IRFs [64].

TLR3 is responsible for viral recognition [58] and signals through the TRIF adaptor following recognition of dsRNA. Polyinosinic-polycytidylic acid (polyI:C), a synthetic analog of dsRNA, can also binds to TLR3 to activate the TLR3–TRIF pathway [62,65]. In addition to activating TLR3, viral RNA can also stimulate the retinoic acid-inducible gene 1-like receptors (RLR) to mediate host antiviral responses [66,67]. In human cells, the family is represented by RIG-1, MDA5 and LGP2. Each of the three family members signal through the adaptor protein interferon-beta promoter stimulator 1 (IPS-1) [68]. Activation of RLRs induce type I interferon (IFNα/β) and interferon-inducible genes via activation of IRF3, IRF7 and NFκB [66]. NOD1 and NOD2 bind to cytosolic peptidoglycan fragments meso-DAP and muramyl dipeptide, respectively. A different set of NLRs induces caspase-1 activation through the action of inflammasomes [49].

These innate immune pathways ultimately induce the generation and release of inflammatory cytokines and chemokines that represent the first volley in the inflammatory response to a pathogen. Recently we have discovered another process that is sparked by innate immune signaling and which may be important for the response to a pathogen or damage. The process of transflammation results in a global change in the expression and activity of epigenetic modifiers that facilitates the phenotypic fluidity needed to respond to a cellular challenge [7].

3. The role of free radicals in innate immune signaling

Reactive oxygen species (ROS) are known downstream targets of innate immune signaling. Immune cells such as neutrophils and macrophages generate prodigious amounts of ROS when activated [69,70]. TLR engagement by ligands increases the expression and activity of NADPH oxidase to generate ROS [71,72]. ROS, in turn, mediate release of other DAMPs and promote sterile inflammation [73]. For example, ROS-induced oxidation of extracellular matrix proteins also generates DAMPs [74]. The transcription factor NFκB is considered as the master regulator of both innate and adaptive immune responses. Activation of NFκB by diverse proinflammatory stimuli including IL-1β requires ROS in part after activation of NADPH oxidases and mitochondrial ROS in certain contexts [75–77]. However, other evidences showed that the oxidative events triggered by those stimuli could be ROS space-temporal dependent or specific to components of the NFκB pathway process [78,79].

Evidences that reactive nitrogen species (RNS) might be involved in innate immunity came in the early 80s when it was shown that plasma nitrite and nitrate level increase in the setting of infection. These findings would be consistent with an increase in the endogenous production of nitric oxide (NO) [80]. Furthermore, it was shown that IL-2-mediated immune activation elevated serum and urinary nitrate levels in patients consistent with a cytokine-induced increase in NO synthesis [81]. In mouse, PAMPs interact with membrane bound or cytosolic pattern recognition receptors (PRRs) that trigger the activation of NFκB and the production of endogenous type I interferons like IFN-α/β. NFκB binds to the inducible nitric oxide synthase (iNOS) promoter to induce

the expression of iNOS resulting in high amounts of NO release from activated macrophages [82].

NO may S-nitrosylate cysteine residues of proteins to modify their function [83]. S-nitrosylation can regulate the NFκB activation pathway at several points, from the upstream molecules to the transcription factor itself. Most of the described S-nitrosylation targets like MyD88, CD40, IKKβ, p50, and p65 inhibit the pathway whereas the modification of surfactant protein-D and Src may activate it [84]. S-nitrosylation also inhibits the kinase activity of apoptosis signal regulation kinase 1 (ASK1) through inhibition of its binding to substrates [85]. ASK1 is an important regulator of the TRAF6-p38 MAPK pathway downstream of TLR4 and is also involved in modulation of both the NFκB and apoptotic pathways downstream of TLR2 [86]. Caspase-1 is involved in TLR2- and TLR4-mediated signal transduction of the MyD88-dependent pathway through the cleavage of the TIR domain-containing adaptor protein TIRAP [87]. Caspase-1 also undergoes S-nitrosylation at a cysteine residue within the enzymatic active site, suppressing its proteolytic activity [88]. It is evident that NO has multiple regulatory effects on TLR-mediated innate immune signaling through S-nitrosylation.

4. Innate immune signaling and nuclear reprogramming to pluripotency

The Nobel Prize for Physiology and Medicine in 2012 was awarded to Shinya Yamanaka for his demonstration of induced pluripotency. Dr. Yamanaka had generated mouse [9] and human [8] iPSCs by overexpressing four transcription factors in somatic murine and human fibroblasts using a retroviral vector. What he didn't know at the time was that the viral vector was half of the story. Our laboratory later discovered that the retroviral vectors used to transport Yamanaka factors play a critical role in the reprogramming to pluripotency [1]. Essentially, the Yamanaka factors alone (in the form of cell-permeant peptides) are not effective. However, when Yamanaka factors are delivered by retroviral vectors, the concurrent activation of innate immune signaling permits efficient reprogramming.

We showed that activation of the TLR3 receptor by the viral vector was critically involved in nuclear reprogramming [1]. When elements of the TLR3 signaling pathway were knocked down, reprogramming to pluripotency using the Yamanaka approach was dramatically suppressed. We also showed that innate immune activation was required for other reprogramming approaches as well [1]. Reprogramming using modified mRNA encoding the Yamanaka factors, or using the Yamanaka factors as cell permeant peptides, was also dependent upon activation of innate immunity. Furthermore, innate immune activation was also critical for reprogramming of murine embryonic fibroblasts carrying a doxycycline-inducible cassette encoding the Yamanaka factors. In this system, the addition of the TLR3 agonist polyI:C enhanced, whereas the NFκB decoy oligonucleotide suppressed, reprogramming to pluripotency [1].

We then identified that activation of innate immune signaling caused global changes in epigenetic modifiers that favored an open chromatin configuration. Activation of innate immune signaling caused a dramatic downregulation of epigenetic modifiers known to enforce suppressive epigenetic marks, such as Dot1L and members of the histone deacetylase family (HDAC 1, 5, 8, 9 and 10). Similarly, we also observed that with innate immune activation, epigenetic modifiers known to enhance gene activation were upregulated, including members of the histone acetyltransferase (HAT) family. These effects of TLR3 activation were associated with increased H3K4 trimethylation (signature of transcriptional activation) and decreased H3K27 trimethylation (signature of transcriptional repression) at the Oct4 and Sox2 promoters [1].

Activation of IRF3 through most PRRs also induces the transcription of type I IFNs such as IFN-α4 and IFN-β [89]. B18R, a viral-encoding protein that can block the activity of type I IFNs, has been shown to improve the efficiency of nuclear reprogramming to iPSCs in several studies [90,91], suggesting that type I IFNs signaling is inhibitory in reprogramming to iPSCs. Alternatively, these data may indicate the presence of a "Goldilock's zone" for innate immune activation and facilitation of reprogramming (see discussion below).

In addition to activating TLR3, viral RNA can also stimulate the RLR to mediate host antiviral responses [66,67]. In human cells, the RLR family comprises RIG-1, MDA5 and LGP2. The RLR family responds to dsRNA and polyI:C [92]. IPS-1 is an intermediary protein that is required for RLR-dependent IFN production in response to virus infection [93]. Knockdown of iPS-1, the common adaptor protein for the RLR family, substantially reduced nuclear reprogramming [2]. In addition, we found that LPS, an agonist of TLR4, also promotes the reprogramming mechanism (unpublished data).

Besides PAMPs, DAMP may also play a role in reprogramming. It has been shown that autophagy activation is necessary at the initial phase of cellular reprogramming [94]. HMGB1, one of the DAMP molecules, is known to enhance autophagy [95]. HMGB1 is also known to interact through RAGE and TLRs and activate NFκB [96] which could also lead to cellular reprogramming. Thus DAMPs may also promote reprogramming through innate immune activation.

Further, we identified that ROS signaling is activated early with nuclear reprogramming, and optimal levels of ROS signaling are essential to induce pluripotency [4]. It is well known that ROS signaling is triggered by activation of innate immunity [97]. Furthermore, NFκB is activated by ROS intermediates [76,98]. Interestingly, NFκB also induces the expression of Nox2, a key gene to generate ROS, in MEFs [99]. Thus, activation of innate immunity may induce a positive feedback cycle of ROS signaling. Furthermore, ROS signaling modulates various cellular processes including differentiation, senescence, apoptosis, and proliferation [100].

Although iPSCs have decreased ROS level compared with the parental somatic cells from which they are derived, our laboratory reports effective iPSCs generation begins with a burst of reactive oxygen species (ROS) in the initial stage of reprogramming from somatic cells [4]. NFκB, the downstream targets of innate immunity, is activated during this early phase of reprogramming, which is associated with the upregulation of Nox2 gene expression. Knock down or knockout of p22phox, a key component of Nox2 complex, drastically decreased reprogramming efficiency [4].

On the other hand, excessive ROS impairs nuclear reprogramming efficiency, as discussed below. Specifically, whereas Nox2 activity was important for reprogramming, and addition of lower concentrations of H_2O_2 enhanced reprogramming, overexpression of Nox2 or the addition of higher concentrations of H_2O_2 reduced the yield of iPSCs [4]. Evidence for the role of innate immune signaling in nuclear reprogramming has been enhanced by studies of cells from mice with knockdown of different proteins in the transflammation cascade (Table 1).

Table 1
Molecules involved in innate immune signaling and their role in reprogramming to pluripotency.

Molecules	Efficiency of nuclear reprogramming to iPSCs
TLR3	KD decreases reprogramming efficiency to iPSCs [1]
TRIF	KD decreases reprogramming efficiency to iPSCs [1]
IRF3	KD decreases reprogramming efficiency to iPSCs [1]
IKK	KD decreases reprogramming efficiency to iPSCs [1]
NFκB p65	NFκB p65 decoy decreases reprogramming efficiency to iPSCs [1]
iPS1	KD decreases reprogramming efficiency to iPSCs [2]
P22phox	KD/KO decreases reprogramming efficiency to iPSCs [4]
NOX2	Overexpression decreases reprogramming efficiency to iPSCs [4]
IFNα/β	Neutralizing IFNα/β by B18R promotes reprogramming efficiency [90,91]

5. The goldilocks zone for innate immunity in nuclear reprogramming

In concordance with the observation that an optimal level of intracellular ROS is required for efficient generation of iPSCs, we discovered a 'Goldilock's zone' for innate immune activation in nuclear reprogramming. When innate immune signaling is inhibited, so too is nuclear reprogramming. When innate immune signaling is excessive, this too abrogates nuclear reprogramming. Thus, there is an optimal level of innate immune activation required for the generation of iPSCs. We discovered the zone when we examined the dose-dependency of TLR3 stimulation on the yield of iPSCs from murine embryonic fibroblasts containing a doxycycline-inducible cassette encoding the Yamanaka factors. In this model, when doxycycline is added to the cells, iPSC colonies are observed within 2 weeks. In our experiments, the dox-inducible MEFs also carried a construct encoding a luciferase construct driven by an NFκB promoter. This construct permitted us to assess the level of innate immune activation. With doxycycline induction, the MEFs manifested a modest activation of NFκB, and colony formation after 3 weeks (unpublished data). The addition of polyI:C at relatively low dosage, increased NFκB activity as well as colony generation. At higher doses of polyI:C, we saw a further increase in NFκB activity, but a reduction in iPSC colony formation.

In parallel, we examined the effect of suppressing innate immunity on iPSC generation. We use an oligonucleotide decoy so as to scavenge NFκB. The oligonucleotide decoy caused a dose-dependent reduction of innate immune signaling (as assessed by the luciferase construct) and of iPSC colony formation. Indeed, colony generation could be nearly abolished when the dose of decoy was sufficient to abrogate NFκB signaling. Taken together, these studies revealed an optimal activation of innate immunity is necessary for maximal iPSC generation.

6. Innate immune signaling and transdifferentiation

A number of groups have forced transdifferentiation of one cell type into another cell lineage by using viral vectors to overexpress lineage determination factors [101,102]. Our work on innate immune signaling in nuclear reprogramming suggests that innate immune signaling is also opening up the chromatin to facilitate cell plasticity. This led us to ask if this process may also be involved in transdifferentiation. Indeed, we reported successful transdifferentiation of fibroblasts to endothelial cells through activation of innate immunity and endothelial development pathways [3]. Priming cells with polyI:C, together with endothelial growth factors, could directly convert a small percentage of the treated fibroblasts into endothelial cells. These fibroblast-derived induced endothelial cells (iECs) expressed all of the expected immunohistochemical markers; manifested endothelial functions not present in fibroblasts (e.g. generation of nitric oxide, formation of tubular networks in matrigel; uptake of acetylated LDL); and had a transcriptional profile (by RNA seq studies) that was highly similar to genuine endothelial cells [2]. Expected histone modifications were observed in the iECs (such as increased H3K4me3 and decreased H3K27me3 in endothelial specific gene promoters such as CD31).

In the absence of polyI:C priming, the endothelial growth factors alone cannot generate transdifferentiation, suggesting that transdifferentiation is absolutely dependent upon innate immune activation. Furthermore, innate immune activation is reduced by knocking down TLR3, as TLR3 KD fibroblasts manifested reduced transdifferentiation efficiency. More notably, iECs derived from these KD fibroblasts had markedly defective endothelial cell function. The RNA sequencing of these KD iECs revealed that they were incompletely reprogrammed [2].

A key player in innate immune signaling is iNOS. This enzyme is induced by NFκB to generate NO and RNS that play a major role in cell signaling and cellular defense. It is well known that innate immune activation of NFκB could bind to iNOS promoter to induce its gene

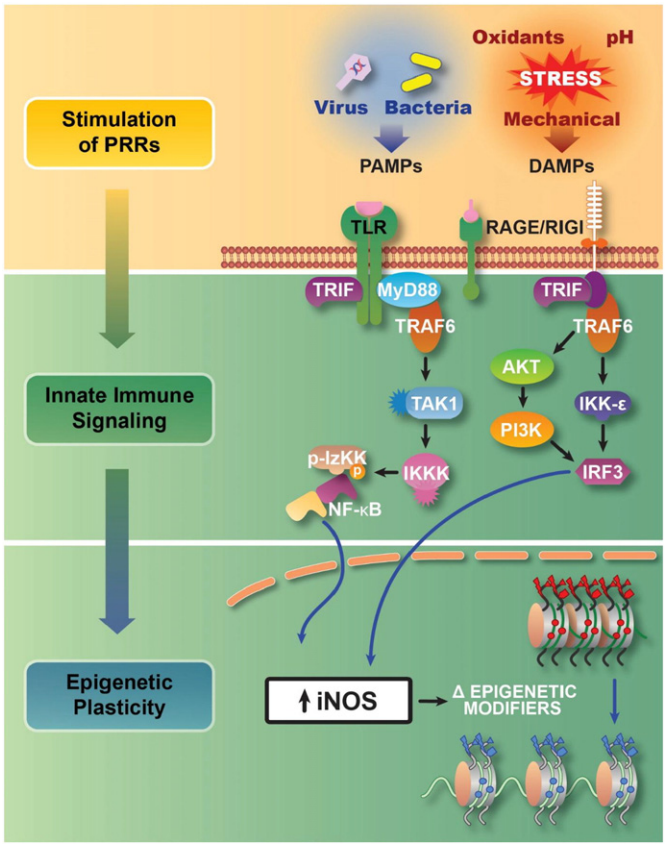

Fig. 1. Transflammation is an adaptive response to cellular challenges. Pathogen associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) activate pattern recognition receptors (PRRs) such as the toll-like receptors (TLRs) and the receptor for advanced glycation end products (RAGE) which trigger innate immune signaling that leads to the activation of NFκB and IRF-3. These transcriptional factors cause global changes in the expression of epigenetic modifiers (upregulation of histone methyltransferases, and downregulation of Dot1L and histone deacetylases). In addition, the expression of inducible nitric oxide synthase leads to the S-nitrosylation of epigenetic modifiers to alter their activity and association with the chromatin. These changes in the expression and activity of epigenetic modifiers increase the open probability state of the chromatin. The increase in DNA accessibility provides for phenotypic fluidity, and adaptive responses to the cellular challenge.

expression [103,104]. Subsequently, iNOS generates NO which can activate guanylate cyclase to produce the second messenger cGMP. In addition, NO may S-nitrosylate cysteine residues of proteins to modify their function [83].

We found that iNOS-derived NO is a major effector of transdifferentiation [5]. Stimulation of innate immune signaling using the TLR 3 agonist polyI:C induces iNOS at the earliest stage of transdifferentiation, weeks before iECs are generated. The induction of iNOS is associated with increases in intracellular NO, as assessed by fluorimetric assays. The critical importance of iNOS in transdifferentiation was revealed when we repeated our transdifferentiation protocol using pharmacological antagonists or genetic interventions to abrogate iNOS activity or expression. The iNOS antagonists significantly inhibited transdifferentiation, and the generation of iECs from iNOS −/− fibroblasts was nearly abolished [5]. Thus iNOS activity is required for transdifferentiation of fibroblasts to endothelial cells.

Furthermore, we observed that iNOS translocated to the nucleus during transdifferentiation. Although iNOS has been reported to translocate into the nucleus both in malignant [105] and normal cells [106,107] the biological implications of this translocation are obscure. We were curious to determine if this translocation played a role in S-nitrosylation of epigenetic modifiers. Several epigenetic modifiers have been shown to be capable of S-nitrosylation in screening assays [108–110], but whether the modifications modulate their function is

not clear. S-nitrosylation of histone deacetylase 2 (HDAC2) inhibits its activity and could favor chromatin configuration [111]. As expected, the induction of iNOS during transdifferentiation was associated with an increased level of protein S-nitrosylation. One of the proteins that we identified as being S-nitrosylated was RING1A, a key component of polycomb repressive complex 1 (PRC1). Our chromatin immunoprecipitation studies revealed that S-nitrosylation of RING1A caused it to dissociate from chromatin. This effect was associated with a reduction in H3K27 trimethylation, and an increase in H3K4 trimethylation [5]. These studies indicated that S-nitrosylation of RING1A impaired the repressive effects of PRC1, thereby facilitating transdifferentiation (Fig. 1).

7. Role of innate immune signaling in tissue regeneration

Lower vertebrates are well known for their ability to regenerate. Urodele amphibians can regenerate limbs, tails and other body parts after injury [112]. Zebrafish can regenerate the apex of the heart after its resection [113]. By contrast, mammals have limited ability to regenerate certain tissues. Adult humans can partially regenerate liver, skeletal muscle and the peripheral nervous system, but have very limited regenerative capacity in some organs, such as the heart [114]. Furthermore, any regenerative capacity of humans decreases substantially with age.

Tissue regeneration is a complex process that requires multiple cell types. Cell-mediated innate immune signaling is well known to play a diverse role in this process. The role of immune cells in regeneration has been nicely reviewed elsewhere [115,116]. To simplify a complex body of work, it may be said that an initial injury is associated with an influx of neutrophils. This cellular infiltrate is an initial response to injury. This early inflammatory response must be replaced by the infiltration of immunomodulatory M2 macrophages, which suppress inflammation by the release of IL-10 and other anti-inflammatory cytokines. In this regard, depleting neutrophils may enhance wound healing in certain contexts [117]. However, inhibition of the normal biphasic inflammatory process can substantially disrupt the response to tissue damage in such diverse murine models as ischemia/reperfusion injury [118], lung, kidney, muscle [119], intestine [120] and liver injury [121], as well as the regenerative response in the adult zebrafish brain [122].

The role of the cell-autonomous innate immune signaling is less well studied. However, our work suggests that the first response to injury may be the cell-autonomous activation of innate immune signaling, causing global changes in the activity and expression of epigenetic modifiers. We find that these changes promote an epigenetic plasticity that would favor phenotypic fluidity. Such phenotypic fluidity would certainly be an adaptive response to an injury or pathogenic challenge. To respond effectively to an injury, cells in the vicinity of the damage must change their basal phenotype from a quiescent to an activated state. Resident cells must proliferate, migrate and integrate into the newly forming tissue. Indeed, in the salamander, the blastema that forms after a limb amputation consists of skin, muscle, nerve and other tissue cells that have “de-differentiated”, and have substantially increased their rate of proliferation and migration [114]. As the limb bud grows, the cells that are behind the proliferating front begin to differentiate back into their original lineage, presumably guided by an epigenetic memory of their previous state. This process must necessarily involve an epigenetic plasticity that facilitates the dynamic changes in the transcriptional profile that facilitates the metamorphosis. Thus it seems likely that there is an important role for the cell-autonomous activation of innate immune signaling that provides epigenetic plasticity for non-immune cells at the site of the injury.

The role of innate immune signaling in the response to injury has been studied in murine models. In these in vivo studies, it is difficult to dissect out the role of cell mediated (e.g. macrophages) versus cell-autonomous (e.g. PRR-induced activation of non-immune cells such as fibroblasts) innate immunity. Nevertheless, it is quite interesting that

Table 2
Regenerative phenotype of mice deficient of key innate immune signaling molecules.

Mice	Regenerative phenotype
Tlr3 −/−	Delayed skin wound healing [123]
	Decreased angiogenesis and arteriogenesis in hindlimb ischemia model [124]
Tlr4 −/−	Reduced cardiac hypertrophy following pressure overload [126]
	Delayed skin wound healing [125]
Tlr2 −/−	Delayed intestine regeneration [120]
Tlr4 −/− Tlr2 −/−	Impaired lung repair after acute lung injury [127]
MyD88 −/−	Delayed skin wound healing [114]
	Impaired lung repair after acute lung injury [127]
	Delayed liver regeneration [128]
	Delayed intestine regeneration [120]
Ifnar1 −/− MyD88 −/−	Normal liver regeneration [137]
Infγ −/−	Promoted skin wound healing [139]
Il6 −/−	Defective liver regeneration [129]

Tlr3 −/− mice manifest a delayed skin wound closure as characterized by impaired re-epithelialization, granulation formation, and neovascularization together with decreased proinflammatory cytokine expression and less recruitment of myeloid cells [123]. Tlr3 −/− mice also manifest an impairment of blocked angiogenesis and arteriogenesis in hindlimb ischemia [124]. These findings suggest that TLR3 and its downstream signaling facilitates early wound healing and vascular regeneration, possibly through cell-autonomous as well as immune cell-mediated pathways.

Similarly, Tlr4 deficient mice exhibit delayed wound healing, reduced neutrophil infiltration and reduced chemokine levels in the injured tissue [125]. Tlr4 deficient mice also exhibit a reduced cardiac hypertrophy with pressure overload [126]. This phenotype is also associated with a decreased NFκB binding activity. Myd88 KO mice exhibit delayed regeneration in multiple organ systems with impaired wound healing [114], impaired lung repair [127] and reduced liver regeneration [128]. The delayed liver regeneration is associated with reduced serum levels of proinflammatory cytokines such as IL6 and TNFα [128]. Consistent with this observation, there is defective hepatocyte regeneration in IL6 deficient mice [129]. By contrast, knockout of a single TLR such as TLR2, TLR4 or TLR9 did not cause a defect in liver regeneration [130]. Tlr4/Tlr2 double KO mice have impaired lung repair after injury [127]. Deletion of MyD88 or TLR2 in the intestinal epithelium markedly reduces regeneration from colonic injury using dextran sodium sulfate. Breast epithelial cells devoid of TLR2 or MYD88 manifest a reduction in the number of mammary repopulating units, consistent with the idea that cell-autonomous innate immune signaling may also be required for the phenotypic fluidity of metastasizing cancer cells and adaptation to their host [120].

8. Conflicting studies?

Thus, many of the components of innate immune signaling are required for tissue regeneration in mouse models (Table 2). However, not all studies suggest that activation of innate immunity supports regeneration. For example, Tlr3 −/− mice actually manifest early liver regeneration [131] whereas injection of the TLR3 agonist polyI:C inhibits liver regeneration [121]. Furthermore, LPS injection suppresses liver regeneration [132]. Tlr3 −/− protects mice from lethal radiation-induced gastrointestinal syndrome [133]. Trif −/− protects mice from lethal radiation-induced gastrointestinal syndrome [133]. TLR3 deficient airway epithelial cells were protected from hyperoxia induced apoptosis in vitro, and TLR3 −/− mice developed less lung injury and prolonged survival when compared to their wildtype counterparts [134]. Although IRF3 is a major downstream target of TLR3 signaling, Irf3 −/− mice are not protected from lethal radiation-induced gastrointestinal syndrome [133].

Type I and type II IFNs are downstream effector of innate immunity. Type I IFNs mainly signal through their receptor IFNAR1. IFNAR1 blocking antibody promotes arteriogenesis and regeneration in a murine hindlimb ischemia model [135]. Furthermore, loss of IFNAR1 in pancreatic acinar cells ameliorates the disease course of acute pancreatitis [136]. Similarly, Myd88/Ifnar1 double KO mice show normal liver regeneration after hepatectomy [137]. Knockout of Ifnar1 significantly promoted the intestinal hyperplasia in casein kinase 1α deficiency mice [138]. A deficiency of INFγ enhances skin wound healing. This observation suggests a negative role for IFNγ in skin regeneration [139]. IFNγ also inhibits myogenesis [140].

Thus, there seems to be some conflict in the literature regarding the role of innate immune signaling in organ regeneration and tissue repair. Clearly, many studies indicate that innate immune signaling is required for regeneration, whereas some studies suggest that innate immune signaling can impede regeneration. This conflict in the literature may be reconciled if there is a "Goldilock's zone" for cell-mediated as well as cell-autonomous reprogramming and regeneration. Our unpublished data has revealed that *insufficient* activation of innate immune signaling (as when cells are treated with the NFκB decoy oligonucleotide), or *excessive* activation of innate immune signaling (as when cells are exposed to higher concentrations of the TLR3 agonist) each impair nuclear reprogramming at the cellular level. To the extent that nuclear reprogramming and epigenetic plasticity are required for the cellular response to injury, there exists an optimal zone for innate immune signaling. Thus, the conflict in the literature may be more apparent than real, and reflective of the very real phenomenon of an optimal level of innate immune activation for optimal tissue regeneration. For a clinician, this phenomenon makes intuitive sense. Physicians know that wound healing is impaired in patients treated with steroids, in the setting of reduced innate immune signaling, wound healing is impaired, as observed in the patient treated with steroids. Conversely, in the setting of excessive and chronic inflammation, as in the diabetic foot ulcer, tissue regeneration is also deficient.

9. Perspective and significance

To conclude, we have found that cell-autonomous innate immune signaling has global effects on the expression and activity of epigenetic modifiers. Our data suggests that, with optimal activation of innate immunity, there is an increase in epigenetic plasticity that enhances phenotypic fluidity. We have termed this process transflammation. Transflammation is mediated in part by the generation of ROS and RNS. We believe that transflammation is an adaptive response to a cellular challenge, initiated by the activation of pattern recognition receptors that detect DAMPs and/or PAMPs. We are hopeful that a more comprehensive understanding of the role of transflammation and innate immune mechanisms in tissue regeneration and repair, will lead us to new therapeutic avenues for regenerative medicine.

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References

[1] J. Lee, N. Sayed, A. Hunter, K.F. Au, W.H. Wong, E.S. Mocarski, R.R. Pera, E. Yakubov, J.P. Cooke, Activation of innate immunity is required for efficient nuclear reprogramming, Cell 151 (2012) 547–558.
[2] N. Sayed, F. Ospino, F. Himmati, J. Lee, P. Chanda, E.S. Mocarski, JP. Cooke, Retinoic Acid Inducible Gene 1 Protein (RIG1)-Like Receptor Pathway Is Required for Efficient Nuclear Reprogramming, Stem Cells. 35 (5) (2017 May) 1197–1207, https://doi.org/10.1002/stem.2607 Epub 2017 Mar 27. PMID:28276156.
[3] N. Sayed, W.T. Wong, F. Ospino, S. Meng, J. Lee, A. Jha, P. Dexheimer, B.J. Aronow, J.P. Cooke, Transdifferentiation of human fibroblasts to endothelial cells: role of innate immunity, Circulation 131 (2015) 300–309.
[4] G. Zhou, S. Meng, Y. Li, Y.T. Ghebre, J.P. Cooke, Optimal ROS signaling is critical for nuclear reprogramming, Cell Rep. 15 (2016) 919–925.
[5] S. Meng, G. Zhou, Q. Gu, P.K. Chanda, F. Ospino, J.P. Cooke, Transdifferentiation requires iNOS activation: role of RING1A S-nitrosylation, Circ. Res. 119 (2016) e129–e138.
[6] J.B. Gurdon, D.A. Melton, Nuclear reprogramming in cells, Science 322 (2008) 1811–1815.
[7] S. Meng, P. Chanda, J.P. Cooke, Role of innate immune signaling in nuclear reprogramming, in: G. Steinhoff (Ed.), Regenerative Medicine - From Protocol to Patient: 1. Biology of Tissue Regeneration, Springer International Publishing, Cham 2016, pp. 291–305.
[8] K. Takahashi, K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, S. Yamanaka, Induction of pluripotent stem cells from adult human fibroblasts by defined factors, Cell 131 (2007) 861–872.
[9] K. Takahashi, S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, Cell 126 (2006) 663–676.
[10] S.M. Wu, K. Hochedlinger, Harnessing the potential of induced pluripotent stem cells for regenerative medicine, Nat. Cell Biol. 13 (2011) 497–505.
[11] K. Takahashi, S. Yamanaka, A decade of transcription factor-mediated reprogramming to pluripotency, Nat. Rev. Mol. Cell Biol. 17 (2016) 183–193.
[12] J.C. Kovacic, N. Mercader, M. Torres, M. Boehm, V. Fuster, Epithelial-to-mesenchymal and endothelial-to-mesenchymal transition: from cardiovascular development to disease, Circulation 125 (2012) 1795–1808.
[13] E. Ubil, J. Duan, I.C. Pillai, M. Rosa-Garrido, Y. Wu, F. Bargiacchi, Y. Lu, S. Stanbouly, J. Huang, M. Rojas, T.M. Vondriska, E. Stefani, A. Deb, Mesenchymal-endothelial transition contributes to cardiac neovascularization, Nature 514 (2014) 585–590.
[14] D.B. Stairs, H. Nakagawa, A. Klein-Szanto, S.D. Mitchell, D.G. Silberg, J.W. Tobias, J.P. Lynch, A.K. Rustgi, Cdx1 and c-Myc foster the initiation of transdifferentiation of the normal esophageal squamous epithelium toward Barrett's esophagus, PLoS One 3 (2008), e3534.
[15] H. Matsushita, E. Chang, A.J. Glassford, J.P. Cooke, C.P. Chiu, P.S. Tsao, eNOS activity is reduced in senescent human endothelial cells: preservation by hTERT immortalization, Circ. Res. 89 (2001) 793–798.
[16] J.B. Gurdon, V. Uehlinger, "Fertile" intestine nuclei, Nature 210 (1966) 1240–1241.
[17] A.D. Ebert, J. Yu, F.F. Rose Jr., V.B. Mattis, C.L. Lorson, J.A. Thomson, C.N. Svendsen, Induced pluripotent stem cells from a spinal muscular atrophy patient, Nature 457 (2009) 277–280.
[18] H.N. Nguyen, B. Byers, B. Cord, A. Shcheglovitov, J. Byrne, P. Gujar, K. Kee, B. Schule, R.E. Dolmetsch, W. Langston, T.D. Palmer, R.R. Pera, LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress, Cell Stem Cell 8 (2011) 267–280.
[19] P. Reinhardt, B. Schmid, L.F. Burbulla, D.C. Schondorf, L. Wagner, M. Glatza, S. Hoing, G. Hargus, S.A. Heck, A. Dhingra, G. Wu, S. Muller, K. Brockmann, T. Kluba, M. Maisel, R. Kruger, D. Berg, Y. Tsytsyura, C.S. Thiel, O.E. Psathaki, J. Klingauf, T. Kuhlmann, M. Klewin, H. Muller, T. Gasser, H.R. Scholer, J. Sterneckert, Genetic correction of a LRRK2 mutation in human iPSCs links parkinsonian neurodegeneration to ERK-dependent changes in gene expression, Cell Stem Cell 12 (2013) 354–367.
[20] M.A. Israel, S.H. Yuan, C. Bardy, S.M. Reyna, Y. Mu, C. Herrera, M.P. Hefferan, S. Van Gorp, K.L. Nazor, F.S. Boscolo, C.T. Carson, L.C. Laurent, M. Marsala, F.H. Gage, A.M. Remes, E.H. Koo, L.S. Goldstein, Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells, Nature 482 (2012) 216–220.
[21] H. Hiura, M. Toyoda, H. Okae, M. Sakurai, N. Miyauchi, A. Sato, N. Kiyokawa, H. Okita, Y. Miyagawa, H. Akutsu, K. Nishino, A. Umezawa, T. Arima, Stability of genomic imprinting in human induced pluripotent stem cells, BMC Genet. 14 (2013) 32.
[22] J. Yang, J. Cai, Y. Zhang, X. Wang, W. Li, J. Xu, F. Li, X. Guo, K. Deng, M. Zhong, Y. Chen, L. Lai, D. Pei, M.A. Esteban, Induced pluripotent stem cells can be used to model the genomic imprinting disorder Prader-Willi syndrome, J. Biol. Chem. 285 (2010) 40303–40,311.
[23] S.J. Chamberlain, P.F. Chen, K.Y. Ng, F. Bourgois-Rocha, F. Lemtiri-Chlieh, E.S. Levine, M. Lalande, Induced pluripotent stem cell models of the genomic imprinting disorders Angelman and Prader-Willi syndromes, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 17668–17,673.
[24] K. Nishino, M. Toyoda, M. Yamazaki-Inoue, Y. Fukawatase, E. Chikazawa, H. Sakaguchi, H. Akutsu, A. Umezawa, DNA methylation dynamics in human induced pluripotent stem cells over time, PLoS Genet. 7 (2011), e1002085.
[25] K. Martins-Taylor, J.S. Hsiao, P.F. Chen, H. Glatt-Deeley, A.J. De Smith, A.I. Blakemore, M. Lalande, S.J. Chamberlain, Imprinted expression of UBE3A in non-neuronal cells from a Prader-Willi syndrome patient with an atypical deletion, Hum. Mol. Genet. 23 (2014) 2364–2373.
[26] E. Cruvinel, T. Budinetz, N. Germain, S. Chamberlain, M. Lalande, K. Martins-Taylor, Reactivation of maternal SNORD116 cluster via SETDB1 knockdown in Prader-Willi syndrome iPSCs, Hum. Mol. Genet. 23 (2014) 4674–4685.
[27] C. Eguizabal, N. Montserrat, A. Veiga, J.C. Izpisua Belmonte, Dedifferentiation, transdifferentiation, and reprogramming: future directions in regenerative medicine, Semin. Reprod. Med. 31 (2013) 82–94.
[28] K. Okita, N. Nagata, S. Yamanaka, Immunogenicity of induced pluripotent stem cells, Circ. Res. 109 (2011) 720–721.
[29] S. Kaneko, S. Yamanaka, To be immunogenic, or not to be: that's the iPSC question, Cell Stem Cell 12 (2013) 385–386.
[30] S. Yamanaka, H.M. Blau, Nuclear reprogramming to a pluripotent state by three approaches, Nature 465 (2010) 704–712.

[31] H.M. Blau, G.K. Pavlath, E.C. Hardeman, C.P. Chiu, L. Silberstein, S.G. Webster, S.C. Miller, C. Webster, Plasticity of the differentiated state, Science 230 (1985) 758–766.
[32] H. Weintraub, S.J. Tapscott, R.L. Davis, M.J. Thayer, M.A. Adam, A.B. Lassar, A.D. Miller, Activation of muscle-specific genes in pigment, nerve, fat, liver, and fibroblast cell lines by forced expression of MyoD, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 5434–5438.
[33] Y.J. Nam, K. Song, X. Luo, E. Daniel, K. Lambeth, K. West, J.A. Hill, J.M. DiMaio, L.A. Baker, R. Bassel-Duby, E.N. Olson, Reprogramming of human fibroblasts toward a cardiac fate, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 5588–5593.
[34] J. Li, N.F. Huang, J. Zou, T.J. Laurent, J.C. Lee, J. Okogbaa, J.P. Cooke, S. Ding, Conversion of human fibroblasts to functional endothelial cells by defined factors, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 1366–1375.
[35] E.Y. Son, J.K. Ichida, B.J. Wainger, J.S. Toma, V.F. Rafuse, C.J. Woolf, K. Eggan, Conversion of mouse and human fibroblasts into functional spinal motor neurons, Cell Stem Cell 9 (2011) 205–218.
[36] B. Widyantoro, N. Emoto, K. Nakayama, D.W. Anggrahini, S. Adiarto, N. Iwasa, K. Yagi, K. Miyagawa, Y. Rikitake, T. Suzuki, Y.Y. Kisanuki, M. Yanagisawa, K. Hirata, Endothelial cell-derived endothelin-1 promotes cardiac fibrosis in diabetic hearts through stimulation of endothelial-to-mesenchymal transition, Circulation 121 (2010) 2407–2418.
[37] M. Ieda, J.D. Fu, P. Delgado-Olguin, V. Vedantham, Y. Hayashi, B.G. Bruneau, D. Srivastava, Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors, Cell 142 (2010) 375–386.
[38] Q. Zhou, J. Brown, A. Kanarek, J. Rajagopal, D.A. Melton, In vivo reprogramming of adult pancreatic exocrine cells to beta-cells, Nature 455 (2008) 627–632.
[39] J. Ladewig, J. Mertens, J. Kesavan, J. Doerr, D. Poppe, F. Glaue, S. Herms, P. Wernet, G. Kogler, F.J. Muller, P. Koch, O. Brustle, Small molecules enable highly efficient neuronal conversion of human fibroblasts, Nat. Methods 9 (2012) 575–578.
[40] J.A. Efe, S. Hilcove, J. Kim, H. Zhou, K. Ouyang, G. Wang, J. Chen, S. Ding, Conversion of mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy, Nat. Cell Biol. 13 (2011) 215–222.
[41] S. Akira, K. Takeda, T. Kaisho, Toll-like receptors: critical proteins linking innate and acquired immunity, Nat. Immunol. 2 (2001) 675–680.
[42] C.A. Janeway Jr., R. Medzhitov, Innate immune recognition, Annu. Rev. Immunol. 20 (2002) 197–216.
[43] R. Medzhitov, C. Janeway Jr., Innate immunity, N. Engl. J. Med. 343 (2000) 338–344.
[44] T.H. Mogensen, Pathogen recognition and inflammatory signaling in innate immune defenses, Clin. Microbiol. Rev. 22 (2009) 240–273 (Table of Contents).
[45] S. Akira, S. Uematsu, O. Takeuchi, Pathogen recognition and innate immunity, Cell 124 (2006) 783–801.
[46] R. Medzhitov, P. Preston-Hurlburt, C.A. Janeway Jr., A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity, Nature 388 (1997) 394–397.
[47] R. Medzhitov, C.A. Janeway Jr., Innate immunity: impact on the adaptive immune response, Curr. Opin. Immunol. 9 (1997) 4–9.
[48] M. Yoneyama, M. Kikuchi, T. Natsukawa, N. Shinobu, T. Imaizumi, M. Miyagishi, K. Taira, S. Akira, T. Fujita, The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses, Nat. Immunol. 5 (2004) 730–737.
[49] T.D. Kanneganti, M. Lamkanfi, G. Nunez, Intracellular NOD-like receptors in host defense and disease, Immunity 27 (2007) 549–559.
[50] C.J. Desmet, K.J. Ishii, Nucleic acid sensing at the interface between innate and adaptive immunity in vaccination, Nat. Rev. Immunol. 12 (2012) 479–491.
[51] K.P. Mollen, R.J. Anand, A. Tsung, J.M. Prince, R.M. Levy, T.R. Billiar, Emerging paradigm: toll-like receptor 4-sentinel for the detection of tissue damage, Shock 26 (2006) 430–437.
[52] W.G. Land, The role of damage-associated molecular patterns (DAMPs) in human diseases: part II: DAMPs as diagnostics, prognostics and therapeutics in clinical medicine, Sultan Qaboos Univ. Med. J. 15 (2015) e157–170.
[53] W.G. Land, The role of damage-associated molecular patterns in human diseases: part I - promoting inflammation and immunity, Sultan Qaboos Univ. Med. J. 15 (2015) e9–e21.
[54] D.L. Rosin, M.D. Okusa, Dangers within: DAMP responses to damage and cell death in kidney disease, J. Am. Soc. Nephrol. 22 (2011) 416–425.
[55] E. Leclerc, G. Fritz, S.W. Vetter, C.W. Heizmann, Binding of S100 proteins to RAGE: an update, Biochim. Biophys. Acta 1793 (2009) 993–1007.
[56] B. Lemaitre, J.M. Reichhart, J.A. Hoffmann, *Drosophila* host defense: differential induction of antimicrobial peptide genes after infection by various classes of microorganisms, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 14614–14,619.
[57] T. Kawai, S. Akira, The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors, Nat. Immunol. 11 (2010) 373–384.
[58] L. Alexopoulou, A.C. Holt, R. Medzhitov, R.A. Flavell, Recognition of double-stranded RNA and activation of NF-[kappa]B by Toll-like receptor 3, Nature 413 (2001) 732–738.
[59] G. Mancuso, M. Gambuzza, A. Midiri, C. Biondo, S. Papasergi, S. Akira, G. Teti, C. Beninati, Bacterial recognition by TLR7 in the lysosomes of conventional dendritic cells, Nat. Immunol. 10 (2009) 587–594.
[60] C. Guiducci, M. Gong, A.M. Cepika, Z. Xu, C. Tripodo, L. Bennett, C. Crain, P. Quartier, J.J. Cush, V. Pascual, R.L. Coffman, F.J. Barrat, RNA recognition by human TLR8 can lead to autoimmune inflammation, J. Exp. Med. 210 (2013) 2903–2919.
[61] C. Coban, Y. Igari, M. Yagi, T. Reimer, S. Koyama, T. Aoshi, K. Ohata, T. Tsukui, F. Takeshita, K. Sakurai, T. Ikegami, A. Nakagawa, T. Horii, G. Nunez, K.J. Ishii, S. Akira, Immunogenicity of whole-parasite vaccines against *Plasmodium falciparum* involves malarial hemozoin and host TLR9, Cell Host Microbe 7 (2010) 50–61.
[62] H. Kato, O. Takeuchi, S. Sato, M. Yoneyama, M. Yamamoto, K. Matsui, S. Uematsu, A. Jung, T. Kawai, K.J. Ishii, O. Yamaguchi, K. Otsu, T. Tsujimura, C.-S. Koh, C. Reis e Sousa, Y. Matsuura, T. Fujita, S. Akira, Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses, Nature 441 (2006) 101–105.
[63] T. Kawai, S. Akira, TLR signaling, Cell Death Differ. 13 (2006) 816–825.
[64] N.J. Gay, M.F. Symmons, M. Gangloff, C.E. Bryant, Assembly and localization of Toll-like receptor signalling complexes, Nat. Rev. Immunol. 14 (2014) 546–558.
[65] L. Gitlin, W. Barchet, S. Gilfillan, M. Cella, B. Beutler, R.A. Flavell, M.S. Diamond, M. Colonna, Essential role of mda-5 in type I IFN responses to polyriboinosinic: polyribocytidylic acid and encephalomyocarditis picornavirus, Proc. Natl. Acad. Sci. 103 (2006) 8459–8464.
[66] H. Kato, S. Sato, M. Yoneyama, M. Yamamoto, S. Uematsu, K. Matsui, T. Tsujimura, K. Takeda, T. Fujita, O. Takeuchi, S. Akira, Cell type-specific involvement of RIG-I in antiviral response, Immunity 23 (2005) 19–28.
[67] A.G. Bowie, KA. Fitzgerald, "RIG-I: tri-ing to discriminate between self and non-self RNA", Trends Immunol. 28 (4) (Apr 2007) 147–150, https://doi.org/10.1016/j.it.2007.02.002 PMID 17307033.
[68] T. Kawai, K. Takahashi, S. Sato, C. Coban, H. Kumar, H. Kato, K.J. Ishii, O. Takeuchi, S. Akira, IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction, Nat. Immunol. 6 (2005) 981–988.
[69] J.T. Curnutte, D.M. Whitten, B.M. Babior, Defective superoxide production by granulocytes from patients with chronic granulomatous disease, N. Engl. J. Med. 290 (1974) 593–597.
[70] C.F. Nathan, R.K. Root, Hydrogen peroxide release from mouse peritoneal macrophages: dependence on sequential activation and triggering, J. Exp. Med. 146 (1977) 1648–1662.
[71] Y.S. Bae, J.H. Lee, S.H. Choi, S. Kim, F. Almazan, J.L. Witztum, Y.I. Miller, Macrophages generate reactive oxygen species in response to minimally oxidized low-density lipoprotein: toll-like receptor 4- and spleen tyrosine kinase-dependent activation of NADPH oxidase 2, Circ. Res. 104 (2009) 210–218 (221p following 218).
[72] M. Janke, J. Poth, V. Wimmenauer, T. Giese, C. Coch, W. Barchet, M. Schlee, G. Hartmann, Selective and direct activation of human neutrophils but not eosinophils by Toll-like receptor 8, J. Allergy Clin. Immunol. 123 (2009) 1026–1033.
[73] G.Y. Chen, G. Nunez, Sterile inflammation: sensing and reacting to damage, Nat. Rev. Immunol. 10 (2010) 826–837.
[74] C.R. Kliment, J.M. Tobolewski, M.L. Manni, R.J. Tan, J. Enghild, T.D. Oury, Extracellular superoxide dismutase protects against matrix degradation of heparan sulfate in the lung, Antioxid. Redox Signal. 10 (2008) 261–268.
[75] Q. Li, J.F. Engelhardt, Interleukin-1beta induction of NFkappaB is partially regulated by H2O2-mediated activation of NFkappaB-inducing kinase, J. Biol. Chem. 281 (2006) 1495–1505.
[76] G. Bonizzi, J. Piette, S. Schoonbroodt, R. Greimers, L. Havard, M.P. Merville, V. Bours, Reactive oxygen intermediate-dependent NF-kappaB activation by interleukin-1beta requires 5-lipoxygenase or NADPH oxidase activity, Mol. Cell. Biol. 19 (1999) 1950–1960.
[77] N.S. Chandel, P.T. Schumacker, R.H. Arch, Reactive oxygen species are downstream products of TRAF-mediated signal transduction, J. Biol. Chem. 276 (2001) 42728–42,736.
[78] K. Ckless, A. Lampert, J. Reiss, D. Kasahara, M.E. Poynter, C.G. Irvin, L.K. Lundblad, R. Norton, A. van der Vliet, Y.M. Janssen-Heininger, Inhibition of arginase activity enhances inflammation in mice with allergic airway disease, in association with increases in protein S-nitrosylation and tyrosine nitration, J. Immunol. 181 (2008) 4255–4264.
[79] N.L. Reynaert, K. Ckless, S.H. Korn, N. Vos, A.S. Guala, E.F. Wouters, A. van der Vliet, Y.M. Janssen-Heininger, Nitric oxide represses inhibitory kappaB kinase through S-nitrosylation, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 8945–8950.
[80] L.C. Green, K. Ruiz de Luzuriaga, D.A. Wagner, W. Rand, N. Istfan, V.R. Young, S.R. Tannenbaum, Nitrate biosynthesis in man, Proc. Natl. Acad. Sci. U. S. A. 78 (1981) 7764–7768.
[81] J.B. Hibbs Jr., C. Westenfelder, R. Taintor, Z. Vavrin, C. Kablitz, R.L. Baranowski, J.H. Ward, R.L. Menlove, M.P. McMurry, J.P. Kushner, et al., Evidence for cytokine-inducible nitric oxide synthesis from L-arginine in patients receiving interleukin-2 therapy, J. Clin. Invest. 89 (1992) 867–877.
[82] H. Kleinert, A. Pautz, K. Linker, P.M. Schwarz, Regulation of the expression of inducible nitric oxide synthase, Eur. J. Pharmacol. 500 (2004) 255–266.
[83] P.T. Doulias, J.L. Greene, T.M. Greco, M. Tenopoulou, S.H. Seeholzer, R.L. Dunbrack, H. Ischiropoulos, Structural profiling of endogenous S-nitrosocysteine residues reveals unique features that accommodate diverse mechanisms for protein S-nitrosylation, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 16958–16,963.
[84] P. Hernansanz-Agustin, A. Izquierdo-Alvarez, A. Garcia-Ortiz, S. Ibiza, J.M. Serrador, A. Martinez-Ruiz, Nitrosothiols in the immune system: signaling and protection, Antioxid. Redox Signal. 18 (2013) 288–308.
[85] H.S. Park, J.W. Yu, J.H. Cho, M.S. Kim, S.H. Huh, K. Ryoo, E.J. Choi, Inhibition of apoptosis signal-regulating kinase 1 by nitric oxide through a thiol redox mechanism, J. Biol. Chem. 279 (2004) 7584–7590.
[86] A. Matsuzawa, K. Saegusa, T. Noguchi, C. Sadamitsu, H. Nishitoh, S. Nagai, S. Koyasu, K. Matsumoto, K. Takeda, H. Ichijo, ROS-dependent activation of the TRAF6-ASK1-p38 pathway is selectively required for TLR4-mediated innate immunity, Nat. Immunol. 6 (2005) 587–592.
[87] S.M. Miggin, E. Palsson-McDermott, A. Dunne, C. Jefferies, E. Pinteaux, K. Banahan, C. Murphy, P. Moynagh, M. Yamamoto, S. Akira, N. Rothwell, D. Golenbock, K.A. Fitzgerald, L.A. O'Neill, NF-kappaB activation by the Toll-IL-1 receptor domain protein MyD88 adapter-like is regulated by caspase-1, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 3372–3377.

[88] J.B. Mannick, C. Schonhoff, N. Papeta, P. Ghafourifar, M. Szibor, K. Fang, B. Gaston, S-Nitrosylation of mitochondrial caspases, J. Cell Biol. 154 (2001) 1111–1116.
[89] K. Honda, A. Takaoka, T. Taniguchi, Type I interferon [corrected] gene induction by the interferon regulatory factor family of transcription factors, Immunity 25 (2006) 349–360.
[90] N. Yoshioka, E. Gros, H.R. Li, S. Kumar, D.C. Deacon, C. Maron, A.R. Muotri, N.C. Chi, X.D. Fu, B.D. Yu, S.F. Dowdy, Efficient generation of human iPSCs by a synthetic self-replicative RNA, Cell Stem Cell 13 (2013) 246–254.
[91] L. Warren, P.D. Manos, T. Ahfeldt, Y.H. Loh, H. Li, F. Lau, W. Ebina, P.K. Mandal, Z.D. Smith, A. Meissner, G.Q. Daley, A.S. Brack, J.J. Collins, C. Cowan, T.M. Schlaeger, D.J. Rossi, Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA, Cell Stem Cell 7 (2010) 618–630.
[92] S. Palchetti, D. Starace, P. De Cesaris, A. Filippini, E. Ziparo, A. Riccioli, Transfected poly(I:C) activates different dsRNA receptors, leading to apoptosis or immunoadjuvant response in androgen-independent prostate cancer cells, J. Biol. Chem. 290 (2015) 5470–5483.
[93] R.B. Seth, L. Sun, C.K. Ea, ZJ. Chen, "Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3", Cell 122 (5) (Sep 2005) 669–682, https://doi.org/10.1016/j.cell.2005.08.012 PMID 16125763.
[94] S. Wang, P. Xia, B. Ye, G. Huang, J. Liu, Z. Fan, Transient activation of autophagy via Sox2-mediated suppression of mTOR is an important early step in reprogramming to pluripotency, Cell Stem Cell 13 (2013) 617–625.
[95] D. Tang, R. Kang, K.M. Livesey, C.W. Cheh, A. Farkas, P. Loughran, G. Hoppe, M.E. Bianchi, K.J. Tracey, H.J. Zeh III, M.T. Lotze, Endogenous HMGB1 regulates autophagy, J. Cell Biol. 190 (2010) 881–892.
[96] J.R. Klune, R. Dhupar, J. Cardinal, T.R. Billiar, A. Tsung, HMGB1: endogenous danger signaling, Mol. Med. 14 (2008) 476–484.
[97] C.S. Yang, J.J. Kim, S.J. Lee, J.H. Hwang, C.H. Lee, M.S. Lee, E.K. Jo, TLR3-triggered reactive oxygen species contribute to inflammatory responses by activating signal transducer and activator of transcription-1, J. Immunol. 190 (2013) 6368–6377.
[98] J. Escobar, J. Pereda, G. Lopez-Rodas, J. Sastre, Redox signaling and histone acetylation in acute pancreatitis, Free Radic. Biol. Med. 52 (2012) 819–837.
[99] J. Anrather, G. Racchumi, C. Iadecola, NF-kappaB regulates phagocytic NADPH oxidase by inducing the expression of gp91phox, J. Biol. Chem. 281 (2006) 5657–5667.
[100] W. Droge, Free radicals in the physiological control of cell function, Physiol. Rev. 82 (2002) 47–95.
[101] R. Wada, N. Muraoka, K. Inagawa, H. Yamakawa, K. Miyamoto, T. Sadahiro, T. Umei, R. Kaneda, T. Suzuki, K. Kamiya, S. Tohyama, S. Yuasa, K. Kokaji, R. Aeba, R. Yozu, H. Yamagishi, T. Kitamura, K. Fukuda, M. Ieda, Induction of human cardiomyocyte-like cells from fibroblasts by defined factors, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 12667–12,672.
[102] P. Huang, Z. He, S. Ji, H. Sun, D. Xiang, C. Liu, Y. Hu, X. Wang, L. Hui, Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors, Nature 475 (2011) 386–389.
[103] Q.W. Xie, Y. Kashiwabara, C. Nathan, Role of transcription factor NF-kappa B/Rel in induction of nitric oxide synthase, J. Biol. Chem. 269 (1994) 4705–4708.
[104] T. Lawrence, The nuclear factor NF-kappaB pathway in inflammation, Cold Spring Harb. Perspect. Biol. 1 (2009), a001651.
[105] T.W. Lee, G.G. Chen, H. Xu, J.H. Yip, E.C. Chak, T.S. Mok, A.P. Yim, Differential expression of inducible nitric oxide synthase and peroxisome proliferator-activated receptor gamma in non-small cell lung carcinoma, Eur. J. Cancer 39 (2003) 1296–1301.
[106] R.J. Jones, D. Jourd'heuil, J.C. Salerno, S.M. Smith, H.A. Singer, iNOS regulation by calcium/calmodulin-dependent protein kinase II in vascular smooth muscle, Am. J. Physiol. Heart Circ. Physiol. 292 (2007) H2634–2642.
[107] A. Giordano, C. Tonello, A. Bulbarelli, V. Cozzi, S. Cinti, M.O. Carruba, E. Nisoli, Evidence for a functional nitric oxide synthase system in brown adipocyte nucleus, FEBS Lett. 514 (2002) 135–140.
[108] M.T. Forrester, J.W. Thompson, M.W. Foster, L. Nogueira, M.A. Moseley, J.S. Stamler, Proteomic analysis of S-nitrosylation and denitrosylation by resin-assisted capture, Nat. Biotechnol. 27 (2009) 557–559.
[109] Y.I. Lee, D. Giovinazzo, H.C. Kang, Y. Lee, J.S. Jeong, P.T. Doulias, Z. Xie, J. Hu, M. Ghasemi, H. Ischiropoulos, J. Qian, H. Zhu, S. Blackshaw, V.L. Dawson, T.M. Dawson, Protein microarray characterization of the S-nitrosoproteome, Mol. Cell. Proteomics 13 (2014) 63–72.
[110] D. Seth, J.S. Stamler, The SNO-proteome: causation and classifications, Curr. Opin. Chem. Biol. 15 (2011) 129–136.
[111] A. Nott, P.M. Watson, J.D. Robinson, L. Crepaldi, A. Riccio, S-Nitrosylation of histone deacetylase 2 induces chromatin remodelling in neurons, Nature 455 (2008) 411–415.
[112] J.P. Brockes, A. Kumar, Appendage regeneration in adult vertebrates and implications for regenerative medicine, Science 310 (2005) 1919–1923.
[113] R. Zhang, P. Han, H. Yang, K. Ouyang, D. Lee, Y.F. Lin, K. Ocorr, G. Kang, J. Chen, D.Y. Stainier, D. Yelon, N.C. Chi, In vivo cardiac reprogramming contributes to zebrafish heart regeneration, Nature 498 (2013) 497–501.
[114] C.L. Stoick-Cooper, R.T. Moon, G. Weidinger, Advances in signaling in vertebrate regeneration as a prelude to regenerative medicine, Genes Dev. 21 (2007) 1292–1315.
[115] S.J. Forbes, N. Rosenthal, Preparing the ground for tissue regeneration: from mechanism to therapy, Nat. Med. 20 (2014) 857–869.
[116] K.D. Poss, Advances in understanding tissue regenerative capacity and mechanisms in animals, Nat. Rev. Genet. 11 (2010) 710–722.
[117] J.V. Dovi, L.K. He, L.A. DiPietro, Accelerated wound closure in neutrophil-depleted mice, J. Leukoc. Biol. 73 (2003) 448–455.
[118] P. Boros, J.S. Bromberg, New cellular and molecular immune pathways in ischemia/reperfusion injury, Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transplant Surg. 6 (2006) 652–658.
[119] J.G. Tidball, S.A. Villalta, Regulatory interactions between muscle and the immune system during muscle regeneration, Am. J. Physiol. Regul. Integr. Comp. Physiol. 298 (2010) R1173–1187.
[120] F.A. Scheeren, A.H. Kuo, L.J. van Weele, S. Cai, I. Glykofridis, S.S. Sikandar, M. Zabala, D. Qian, J.S. Lam, D. Johnston, J.P. Volkmer, D. Sahoo, M. van de Rijn, F.M. Dirbas, G. Somlo, T. Kalisky, M.E. Rothenberg, S.R. Quake, M.F. Clarke, A cell-intrinsic role for TLR2-MYD88 in intestinal and breast epithelia and oncogenesis, Nat. Cell Biol. 16 (2014) 1238–1248.
[121] R. Sun, B. Gao, Negative regulation of liver regeneration by innate immunity (natural killer cells/interferon-gamma), Gastroenterology 127 (2004) 1525–1539.
[122] N. Kyritsis, C. Kizil, S. Zocher, V. Kroehne, J. Kaslin, D. Freudenreich, A. Iltzsche, M. Brand, Acute inflammation initiates the regenerative response in the adult zebrafish brain, Science 338 (2012) 1353–1356.
[123] Q. Lin, D. Fang, J. Fang, X. Ren, X. Yang, F. Wen, S.B. Su, Impaired wound healing with defective expression of chemokines and recruitment of myeloid cells in TLR3-deficient mice, J. Immunol. 186 (2011) 3710–3717.
[124] J. Holfeld, C. Tepekoylu, C. Reissig, D. Lobenwein, B. Scheller, E. Kirchmair, R. Kozaryn, K. Albrecht-Schgoer, C. Krapf, K. Zins, A. Urbschat, K. Zacharowski, M. Grimm, R. Kirchmair, P. Paulus, Toll-like receptor 3 signalling mediates angiogenic response upon shock wave treatment of ischaemic muscle, Cardiovasc. Res. 109 (2016) 331–343.
[125] L. Chen, S. Guo, M.J. Ranzer, L.A. DiPietro, Toll-like receptor 4 has an essential role in early skin wound healing, J. Invest. Dermatol. 133 (2013) 258–267.
[126] T. Ha, Y. Li, F. Hua, J. Ma, X. Gao, J. Kelley, A. Zhao, G.E. Haddad, D.L. Williams, I. William Browder, R.L. Kao, C. Li, Reduced cardiac hypertrophy in toll-like receptor 4-deficient mice following pressure overload, Cardiovasc. Res. 68 (2005) 224–234.
[127] D. Jiang, J. Liang, J. Fan, S. Yu, S. Chen, Y. Luo, G.D. Prestwich, M.M. Mascarenhas, H.G. Garg, D.A. Quinn, R.J. Homer, D.R. Goldstein, R. Bucala, P.J. Lee, R. Medzhitov, P.W. Noble, Regulation of lung injury and repair by Toll-like receptors and hyaluronan, Nat. Med. 11 (2005) 1173–1179.
[128] E. Seki, H. Tsutsui, Y. Iimuro, T. Naka, G. Son, S. Akira, T. Kishimoto, K. Nakanishi, J. Fujimoto, Contribution of Toll-like receptor/myeloid differentiation factor 88 signaling to murine liver regeneration, Hepatology 41 (2005) 443–450.
[129] D.E. Cressman, L.E. Greenbaum, R.A. DeAngelis, G. Ciliberto, E.E. Furth, V. Poli, R. Taub, Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice, Science 274 (1996) 1379–1383.
[130] J.S. Campbell, K.J. Riehle, J.T. Brooling, R.L. Bauer, C. Mitchell, N. Fausto, Proinflammatory cytokine production in liver regeneration is Myd88-dependent, but independent of Cd14, Tlr2, and Tlr4, J. Immunol. 176 (2006) 2522–2528.
[131] E. Zorde-Khvalevsky, R. Abramovitch, H. Barash, I. Spivak-Pohis, L. Rivkin, J. Rachmilewitz, E. Galun, H. Giladi, Toll-like receptor 3 signaling attenuates liver regeneration, Hepatology 50 (2009) 198–206.
[132] K. Akita, M. Okuno, M. Enya, S. Imai, H. Moriwaki, N. Kawada, Y. Suzuki, S. Kojima, Impaired liver regeneration in mice by lipopolysaccharide via TNF-alpha/kallikrein-mediated activation of latent TGF-beta, Gastroenterology 123 (2002) 352–364.
[133] N. Takemura, T. Kawasaki, J. Kunisawa, S. Sato, A. Lamichhane, K. Kobiyama, T. Aoshi, J. Ito, K. Mizuguchi, T. Karuppuchamy, K. Matsunaga, S. Miyatake, N. Mori, T. Tsujimura, T. Satoh, Y. Kumagai, T. Kawai, D.M. Standley, K.J. Ishii, H. Kiyono, S. Akira, S. Uematsu, Blockade of TLR3 protects mice from lethal radiation-induced gastrointestinal syndrome, Nat. Commun. 5 (2014) 3492.
[134] L.A. Murray, D.A. Knight, L. McAlonan, R. Argentieri, A. Joshi, F. Shaheen, M. Cunningham, L. Alexopolou, R.A. Flavell, R.T. Sarisky, C.M. Hogaboam, Deleterious role of TLR3 during hyperoxia-induced acute lung injury, Am. J. Respir. Crit. Care Med. 178 (2008) 1227–1237.
[135] P.F. Teunissen, M.C. Boshuizen, M.R. Hollander, P.S. Biesbroek, N.W. van der Hoeven, J.Q. Mol, M.J. Gijbels, S. van der Velden, T.C. van der Pouw Kraan, A.J. Horrevoets, M.P. de Winther, N. van Royen, MAb therapy against the IFN-alpha/beta receptor subunit 1 stimulates arteriogenesis in a murine hindlimb ischaemia model without enhancing atherosclerotic burden, Cardiovasc. Res. 107 (2015) 255–266.
[136] K.J. Miller, S. Raulefs, B. Kong, K. Steiger, I. Regel, A. Gewies, J. Kleeff, C.W. Michalski, Loss of Ifnar1 in pancreatic acinar cells ameliorates the disease course of acute pancreatitis, PLoS One 10 (2015), e0143735.
[137] J. Vaquero, K.J. Riehle, N. Fausto, J.S. Campbell, Liver regeneration after partial hepatectomy is not impaired in mice with double deficiency of Myd88 and IFNAR genes, Gastroenterol. Res. Pract. 2011 (2011) 727403.
[138] Y.V. Katlinskaya, K.V. Katlinski, A. Lasri, N. Li, D.P. Beiting, A.C. Durham, T. Yang, E. Pikarsky, C.J. Lengner, F.B. Johnson, Y. Ben-Neriah, S.Y. Fuchs, Type I interferons control proliferation and function of the intestinal epithelium, Mol. Cell. Biol. 36 (2016) 1124–1135.
[139] Y. Ishida, T. Kondo, T. Takayasu, Y. Iwakura, N. Mukaida, The essential involvement of cross-talk between IFN-gamma and TGF-beta in the skin wound-healing process, J. Immunol. 172 (2004) 1848–1855.
[140] P. Londhe, J.K. Davie, Interferon-gamma resets muscle cell fate by stimulating the sequential recruitment of JARID2 and PRC2 to promoters to repress myogenesis, Sci. Signal. 6 (2013), ra107.