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Innate immunity and epigenetic plasticity in cellular reprogramming

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Abstract

Somatic cells can be reprogrammed to express the features of pluripotent cells, in that they can be differentiated into all three germ layers, and that they have the ability to replicate indefinitely.

Recent studies suggest that the efficient induction of pluripotency requires the activation of innate immunity.

The Nobel Prize-winning work of Shinya Yamanaka

Shinya Yamanaka won the Nobel Prize in Physiology or Medicine in November 2012 for his discovery that somatic cells could be induced to form pluripotent stem cells (known as induced pluripotent stem cells, or iPSCs) by forced overexpression of four transcriptional factors (Oct 4, Sox2, KLF4 and cMyc, or OSKM)¹. Yamanaka and others subsequently revealed that these transcriptional factors activated a network of genes required for pluripotency.

This discovery has had a transformational effect on the field of regenerative medicine. The iPSCs may be used as a surrogate for the more controversial embryonic stem cells, to study mechanisms of cell self-renewal and pluripotency. The iPSCs may be differentiated into any somatic cell, so as to study pathobiology of disease, to screen drugs for safety or efficacy, or to be used in cell therapy.

In Yamanaka's original publication, OSKM were overexpressed in somatic cells using retroviral vectors². Since then, many investigators have improved the technology by using nonintegrating methods to overexpress the Yamanaka factors, and by using small molecules (eg. valproic acid, an inhibitor of histone deacetylase) to accelerate the reprogramming progress and to reduce the number of transcriptional factors³. Most recently, a methodology has been reported that uses only small molecules including epigenetic modifiers.⁴ These

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improvements are important for the field, because clinical applications will be more feasible if iPSCs can be generated rapidly and without concerns of integration of foreign DNA into the genome of therapeutic cells.

Nevertheless, the generation of iPSCs remains technically challenging, and requires the activation of an entire network of pluripotency genes, and the parallel suppression of genes enforcing the lineage of the somatic cell that is being reprogrammed. Global changes in the transcriptional profile occur in parallel with genome-wide alterations in histone proteins, noncoding RNAs, and DNA methylation. These genome wide alterations are associated with profound changes in signaling pathways, nuclear structure, metabolism and morphology of the cell that are incompletely characterized⁵. New insights into the mechanisms of nuclear reprogramming to pluripotency may enhance the efficiency and fidelity of the generation of iPSCs.

Role of innate immune signaling in generation of pluripotent stem cells

In 2012 we reported the surprising observation that the Yamanaka method to generate induced pluripotent stem cells (iPSCs) was dependent on activation of innate immunity⁶. In a previously published study, we revealed that the retroviral vectors carrying the Yamanaka factors were more than mere vehicles. Indeed, the retroviral vectors, by activating innate immune signaling (in part through TLR3) were absolutely integral to the reprogramming effort. Specifically, when we exposed human fibroblasts to cell permeant peptides of the Yamanaka factors, we were not able to obtain efficient generation of iPSCs. Furthermore, activation of genes in the downstream core pluripotency network was delayed and depressed. Only after activation of innate immunity, using polyinosinic:polycytidylic acid (Poly I:C, a TLR 3 agonist) could we obtain efficient nuclear reprogramming with cell permeant peptides of the Yamanaka factors. Furthermore, when we knocked down expression of the TLR3 receptor or its adaptor TRIF, iPSC colony formation was reduced and delayed when using the retroviral vectors encoding the Yamanaka factors.

We demonstrated that the same mechanisms were operative in other methods of reprogramming somatic cells to pluripotency. Specifically, we documented the importance of innate immune signaling in nuclear reprogramming when we used modified message RNA to express the Yamanaka factors, or when we employed murine embryonic fibroblasts expressing a doxycycline-inducible cassette of the Yamanaka factors. The generation of iPSCs using these methods could also be reduced by TLR3 knockdown, or by interfering with downstream signaling (ie. knocking down IRF3 or using a decoy oligonucleotide against p65).

The effect of innate immune signaling was mediated by global changes in epigenetic modifiers (eg. downregulation of multiple members of the histone deacetylase family, ie. HDAC 1, 5, 8, 9 and 10; downregulation of Dot1L; upregulation of histone acetyltransferase 1). These changes in the expression of epigenetic modifiers would be expected to increase the open chromatin state. Indeed, we confirmed an increase in H3K4 trimethylation (an activating histone mark) at the Oct 4 and Sox 2 promoters when cell permeant Sox2 was administered with Poly IC, but not in the absence of Poly IC.

It is very important to note that the contribution of innate immune signaling to nuclear reprogramming is not just a feature of the TLR3 receptor. Recently we have shown that activation of Rig-1 is also involved in the efficient reprogramming induced by retroviral vectors or mmRNA encoding the Yamanaka factors (unpublished data). Furthermore, LPS can also induce similar “epigenetic plasticity” through activation of TLR4.

Beyond Yamanaka

These findings are an important insight into the mechanisms of nuclear reprogramming. But we speculate that these observations have wider implications, with respect to the cellular response to a pathogen or to cellular damage. Damage-associated molecular patterns (DAMPs, also known as alarmins) or pathogen associated molecular patterns (PAMPs) can be sensed by an array of receptors such as toll-like receptors (TLRs), RIG-1, receptor for advanced glycosylation endproducts (RAGE), and others. We have unpublished evidence that activation of a wide range of these pattern recognition receptors (PRRs) causes global changes in epigenetic modifiers that increase the probability for an open chromatin state, in a process we term “transflammation”(Figure)⁷. In this state of epigenetic plasticity, the cell is poised to respond to microenvironmental cues that may change its phenotype. This is a heretofore unrecognized response to innate immune activation, which results in greater epigenetic plasticity. This response may permit an adaptive form of phenotypic fluidity so as to better respond to environmental stresses. In brief, our data indicates that when a cell is confronted by a pathogen or tissue damage, activation of receptors for PRRs can markedly increase epigenetic plasticity. In this state of epigenetic plasticity, cell phenotype can be transformed by environmental cues (eg. growth factors, small molecule modulators of cell signaling) so that the cell can adjust its phenotype and functions to respond to the challenge.

The Goldilock’s Zone for Innate Immunity in Nuclear Reprogramming

Because T and B lymphocytes are professional immune cells, they may be poised for epigenetic plasticity. Indeed, the Zambidis group has previously shown that such professional immune cells are easier to reprogram to pluripotency than hematopoietic stem cells, and that this effect is in part dependent upon NFκB expression⁸. Most recently, Guo et al. demonstrated that nuclear reprogramming is accelerated in CD45+ bone marrow derived progenitors of granulocytes and monocytes⁹. This ease of reprogramming in immune cells may be due to a baseline level of innate immune activation. In unpublished studies using murine or human fibroblasts, we have learned that there is an optimal zone of innate immune activation for reprogramming (the ‘Goldilock’s zone’). Below or outside of the zone, reprogramming is minimal or absent. We are attempting to elucidate the biological constraints on the Goldilock’s zone. An understanding of these biological constraints could have therapeutic value; small molecule antagonists that remove such constraints would be expected to increase epigenetic plasticity so as to enhance phenotypic fluidity and regeneration.

Plant biologists have long known that, with extreme environmental stress, mature plant cells can be induced to transdifferentiate into new plants in a process known as somatic embryogenesis¹⁰. We speculate that this process is at least partially recapitulated in higher

organisms, and requires the induction of an epigenetic plasticity that is heavily modulated by innate immune signaling. We suggest that a greater understanding of the mechanisms of transflammation, through careful and rigorous experimentation, will generate scientific insights about regenerative processes that may lead to novel therapeutic avenues.

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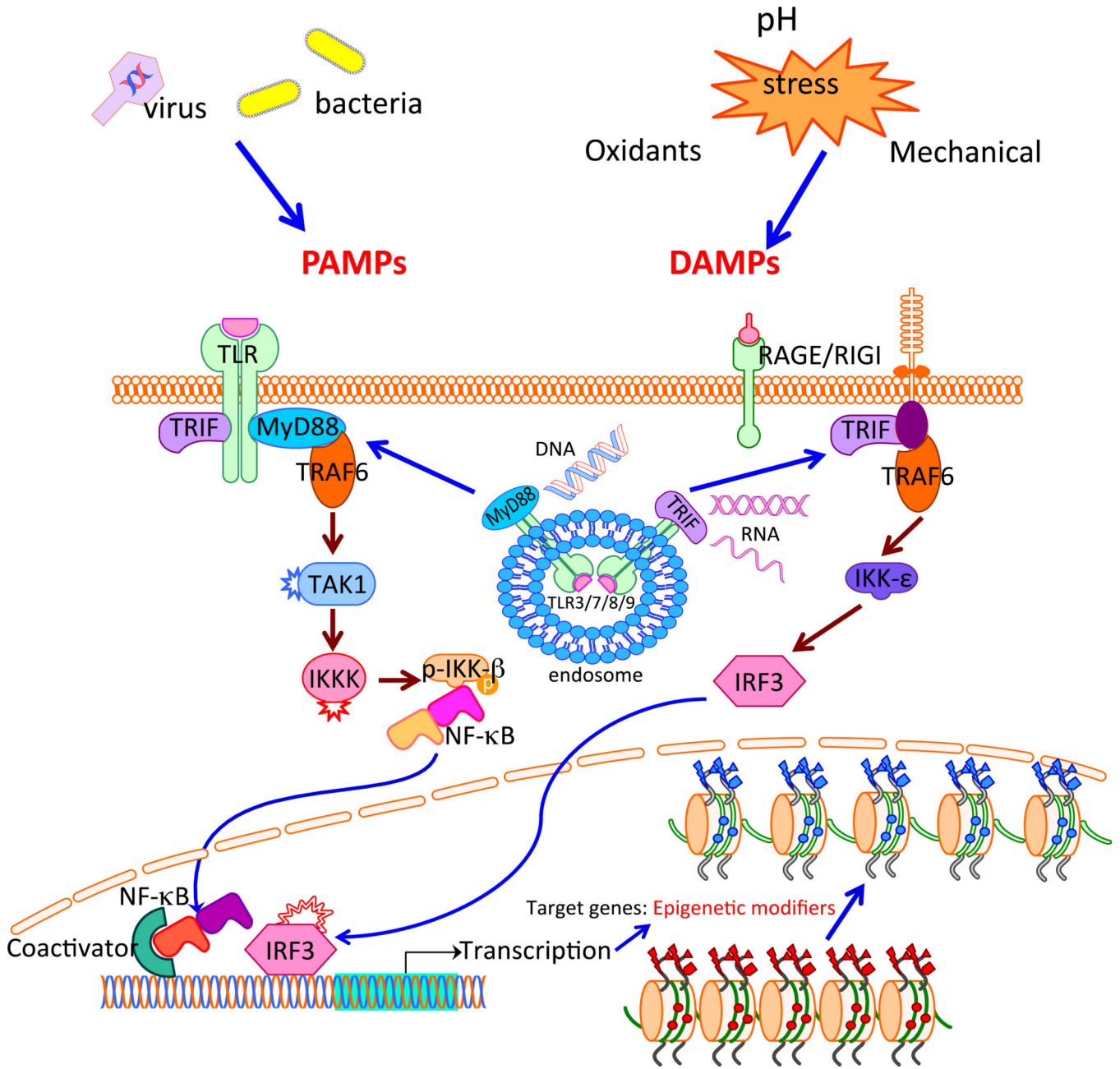


Figure 1. Pathogen associated molecular patterns (PAMPs) or damage associated molecular patterns (DAMPs) are recognized by PRRs such as Toll-like receptors (TLRs), RIG-1 or receptor for advanced glycosylation endproducts (RAGE) on the cell surface or endosomes^{11,12}. Stimulation of PRRs activates innate immune signaling pathways, leading to mobilization of the transcriptional factors NFκB and IRF-3^{11,12}. These transcriptional effects induce changes in epigenetic modifiers (eg. upregulation of the histone acetyltransferases, downregulation of the histone deacetylases), that increases the probability of an open state configuration of the chromatin. The open configuration state increases the epigenetic

plasticity, and thereby the phenotypic fluidity of the cell in an adaptive manner to respond to the stress.