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Utilization of Different Antiviral Mechanisms by Mammalian Embryonic Stem Cells and Differentiated Cells

Yan-Lin Guo

Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, MS, 39406, the United States

Abstract

Embryonic stem cells (ESCs) have received tremendous attention because of their potential applications in regenerative medicine. Over the past two decades, intensive research has not only led to the generation of various types of cells from ESCs that can be potentially used for the treatment of human diseases, but also led to the formation of new concepts and breakthroughs that have significantly impacted our understanding of basic cell biology and developmental biology. Recent studies have revealed that ESCs and other types of pluripotent cells do not have a functional interferon (IFN)-based antiviral mechanism, challenging the idea that the IFN system is developed as the central component of antiviral innate immunity in all types of cells in vertebrates. This finding also provided important insight into a question that has been uncertain for a long time: whether or not the RNA interference (RNAi) antiviral mechanism operates in mammalian cells. An emerging paradigm is that mammals may have adapted distinct antiviral mechanisms at different stages of organismal development; the IFN-based system is mainly utilized by differentiated somatic cells while the RNAi antiviral mechanism may be used in ESCs. This paper discusses the molecular basis and biological implications for mammals to have different antiviral mechanisms during development.

Introduction

The defense response to pathogens is critically important for the growth, development, and survival of all living organisms. Over the course of evolution, different organisms have developed different defense mechanisms. Plants, fungi, and invertebrates defend themselves against viral infection with RNA interference (RNAi)¹ while mammals have developed a protein-based interferon (IFN) system that can mount multiple forms of antiviral activities as a part of their innate immunity.^{2,3} For a long time, it has been uncertain whether the RNAi mechanism is utilized in mammals.^{4–7} Recent studies in mice suggest that RNAi may operate in embryonic stem cells (ESCs) and tissue cells at the early developmental stage.^{8,9} An emerging hypothesis is that mammals appear to have adapted distinct antiviral strategies at different stages of development: differentiated somatic cells mainly use the IFN-based system while ESCs may utilize RNAi.⁴ This hypothesis is in part brought about by the

Correspondence: Yan-Lin Guo, Ph.D., Department of Biological Sciences, University of Southern Mississippi, 118 College Drive 5018, Hattiesburg, MS 39406; Tel: (601) 266-6018; Fax: (601) 266-5797; yanlin.guo@usm.edu.

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recent studies of ESCs and induced pluripotent cells (iPSCs), which somewhat unexpectedly provide important insight for our understanding of the antiviral innate immunity in mammals during development.

Over the past two decades, ESCs have attracted intensive research efforts because of their potential applications in regenerative medicine.^{10–12} Characterized by differentiation potential to various cell lineages (pluripotency) and unlimited proliferation capacity (self-renewal), ESCs can be used as a promising cell source for cell-based therapies. The intensive research has not only led to the development of strategies that can generate various cell types from ESCs, but also led to the formation of new concepts and breakthroughs that have dramatically impacted our understanding of basic cell biology and developmental biology. The best example is the generation of iPSCs which has led to the concept of cell reprogramming and prompted us to rethink the notion of “terminally differentiated cells” defined in cell biology.¹³ The strongest evidence for the existence of the RNAi as an antiviral mechanism in mammals is derived from mouse ESCs (mESCs) and is largely attributed to their lack of IFN response,⁸ a property that has been recently characterized as part of an underdeveloped innate immunity in pluripotent cells.¹⁴ While new investigations have begun to uncover the molecular basis underlying this phenomenon, it is interesting to note that some studies dating back to about 40 years ago have already indicated that IFN-based antiviral mechanism is different in pluripotent cells and differentiated somatic cells.^{15–17} As more and more pieces are being put together, the puzzle of why different antiviral mechanisms are utilized by mammalian cells at different developmental stages begins to make sense.

Recent studies have demonstrated that both human and mouse ESCs (hESCs and mESCs) as well as iPSCs have limited or no response to a wide range of infectious agents and inflammatory cytokines. Accumulating evidence suggests that underdeveloped innate immunity is a common feature of pluripotent cells. In a previous review,¹⁴ we have discussed this subject from the perspective of stem cell biology and regenerative medicine. This paper focuses on the discussion of the molecular basis and the rationale for the selective utilization of different antiviral mechanisms by ESCs and differentiated cells in mammals.

Overview of Different Mechanisms of Antiviral Immunity in Eukaryotes

RNAi antiviral mechanism

Originally discovered in *C. elegans*, the RNAi pathway has been recognized as a major antiviral mechanism in plants, fungi, and invertebrates.^{1,18} The basic process of this mechanism is that invading viral RNA are degraded into ~22-bp dsRNA duplexes (small interfering RNA, siRNA) by a host endoribonuclease known as Dicer. One strand of the siRNA is then loaded into the RNA-induced silencing complex (RISC), where it serves as a guide that directs RISC to the complementary region of invading viral RNA. Once bound, a member of the Argonaute (Ago) family with endonuclease activity (the catalytic component of RISC) cleaves the viral RNA, thereby inhibiting viral replication.¹ This antiviral mechanism is highly specific and efficient to clear viral pathogens from the host without causing the destruction of infected cells. One would assume that the RNAi antiviral

mechanism developed in invertebrates would be conserved in vertebrates over the course of evolution. However, there is no convincing data to support this hypothesis, especially in mammalian species, in which the IFN-based antiviral mechanism has evolved.^{4,19}

IFN-based antiviral innate immunity

Vertebrates have developed sophisticated immune systems that consist of innate and adaptive immunity. Innate immunity, presumably developed in most if not all cell types, is the first line of the organism's defense against a broad range of pathogens in a non-specific manner. Adaptive immunity is the antigen-specific immune response that utilizes highly specialized immune cells (T cells and B cells) and provides the organism with the ability to mount an enhanced immune response to subsequent invasion of the same pathogens.^{20,21}

At the cellular level, the innate immune response is mainly mediated by pattern recognition receptors, including toll-like receptors (TLRs) and retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs). A wide variety of infectious agents, including viral DNA/RNA and bacterial endotoxins, are recognized by different TLRs localized on the cell membrane or in endosomes.²² Viral RNAs in the cytosol are detected by RLRs, such as RIG-I and melanoma differentiation associated factor 5 (MDA5).²³ Recently, cGAS (cGMP-AMP synthase) has been identified as an important intracellular sensor for dsDNA viruses (or dsDNA that is produced by retroviruses). Infection of cells with dsDNA viruses activates cGAS and produces cyclic dinucleotide cGMP-AMP (cGAMP), which binds to and activates stimulator of interferon genes (STING), leading to IFN expression.^{24,25} Although different pathogens are recognized by distinct receptors and the signals are relayed by different molecules, the signal transduction eventually converges at the point of NF κ B (nuclear factor κ B) activation. Together with the other transcription factors, IRF (interferon regulatory factors) and AP1 (activator protein 1), NF κ B activates the transcription of IFN, inflammatory cytokines, and other types of immune modulators (Figure 1).^{22,22,26}

Based on the cellular origin, inducing agents, and cellular receptors, IFN are classified into types I, II, and III. Type I and type III IFN are produced by almost any type of cells upon viral infection, whereas type II IFN is produced mainly by natural killer cells and T lymphocytes.^{2,27,28} However, all three types of IFN share a common function in modulating the activity of the immune system. Through autocrine and paracrine mechanisms, IFN bind to the cell surface receptor complex (IFNAR), which triggers the activation of Janus tyrosine kinases (JAK1/TYK2) that phosphorylate signal transducers and activators of transcription (STAT1/2). Phosphorylated STAT1/2 and IRF3 form an IFN-stimulated gene factor 3 (ISGF3) complex that translocates to the nucleus where it induces the expression of IFN-stimulated genes (ISGs), which participate in antiviral activities via different mechanisms.^{2,27,29} Therefore, the IFN system, consisting of IFN expression and response mechanisms (Figure 1), has evolved as a powerful antiviral mechanism in vertebrates.

Intrinsic antiviral immunity

Intrinsic antiviral immunity refers to the form of innate immunity that directly restricts viral replication and assembly, thereby rendering a cell non-permissive to a specific class or species of viruses.^{30,31} Unlike pattern recognition receptors, which elicit antiviral activity by

inducing IFN, intrinsic immunity is provided by antiviral proteins that are constitutively expressed and ready to act in the cell, although they can be further upregulated by viral infection.³⁰ Several virus-specific intrinsic antiviral factors and their mechanisms of action have been characterized.^{30,31} dsRNA-activated protein kinase R (PKR) and Ribonuclease L (RNase L), which are well known for their roles in the antiviral response,^{2,32} are also considered to be intrinsic antiviral factors with a broad antiviral spectrum. Since their expression is further upregulated by IFN upon viral infection, they are classified as ISGs as a part of IFN responses.² However, differing from other ISGs, which are usually expressed at very low levels in uninfected cells, PKR and RNase L are expressed at high basal levels in uninfected cells and are immediately activated by invading viruses, therefore they can be mechanistically defined as intrinsic antiviral factors.³⁰ Directly activated by viral RNA, PKR causes inhibition of both cellular and viral protein synthesis.³³ RNase L is also known as 2'-5' oligoadenylate synthetase (OAS)-dependent ribonuclease. Activated by viral dsRNA, OAS converts ATP to 2'-5'-linked oligoadenylates (2-5A), which then activate RNase L, leading to both cellular and viral RNA degradation, thereby preventing viral replication³² (Figure 1).

The Potential Function of RNAi Antiviral Mechanism in ESCs

The RNAi mechanism in mammals – a controversial issue in somatic cells and a rational alternate in ESCs

Extensive effort has been made to determine whether or not the RNAi mechanism is used by vertebrate animals, especially mammals. Synthetic siRNA transfected into mammalian cells can effectively inhibit viral replication and has been widely used as a technique for gene silencing, but there is little biochemical evidence that siRNA can be derived from viruses in infected cells.⁵ siRNA in organisms that lack the IFN system can be easily detected and characterized, but this is challenging in mammalian cells where multiple forms of antiviral activity mobilized by the IFN response make it difficult to assess the RNAi effect. In a recent study by Backes et al.,⁷ it was demonstrated that an engineered RNA virus that blocks the function of microRNA (miRNA) and siRNA did not replicate faster than the control virus in either cell culture or in a mouse model.⁷ Furthermore, human and mouse cells with a disrupted *dcr* gene, which are unable to generate siRNA or miRNA, did not show an apparent difference in viral replication from normal cells.³⁴ The lack of biochemical and genetic evidence for the existence of siRNA suggest that the RNAi mechanism seems unlikely to play a role in antiviral immunity in mice or humans. However, studies in pluripotent cells present a different picture.

Using mESCs as a model system, Maillard et al.⁸ demonstrated that siRNA of viral origin with the features of bona fide siRNA were detected in mESCs infected with encephalomyocarditis virus or Nodamura virus, suggesting that the RNAi pathway is functional in mESCs. In a separate study, Li et al.⁹ reported that viral siRNA derived from Nodamura virus, identical to those detected in mESCs, were accumulated in infected newborn mice, supporting the possibility of a functional RNAi mechanism in vivo. Thus, while the physiological significance of RNAi as an antiviral mechanism in ESCs remains to be ascertained, it nonetheless provides a rational alternate for the deficiency of the IFN-based antiviral mechanism in these cells.

The molecular basis for a paradox of RNAi mechanism in mammalian cells

Biologically, the reason why somatic cells do not utilize RNAi can be rationally assumed to be that a powerful IFN-based innate immunity and adaptive immune system can effectively protect the cells from viral infection. As a result, the RNAi mechanism may no longer be necessary. However, at the molecular level, there is evidence indicating that the IFN-based antiviral mechanism and RNAi may have some conflict in their mechanisms of action, which appears to be due to the incompatibility between the IFN system and siRNA/miRNA biogenesis in differentiated cells.

miRNA are small non-coding RNA that regulate gene expression by silencing their target mRNA. In animals, miRNA share similar biogenesis and action model with siRNA, but their processing requires Drosha in addition to Dicer. miRNA or siRNA interacts with RISC, leading to the degradation of cellular mRNA or viral RNA through the catalytic activity of an Ago protein in the RISC complex.³⁵ Multiple members of the Ago protein family with different functions are expressed in different species. The Ago proteins with endonucleolytic activity involved in RNAi in plants, fungi and invertebrates have been well-characterized.¹ Mammals express several Ago proteins, but most of them have lost the endonucleolytic activity, and only Ago2 is responsible for RNA cleavage in mammalian RISC.³⁶ A study by Seo suggested that viral infection inhibited RISC activity via poly-ADP ribosylation of Ago2, indicating that RISC is no longer optimally engaged in RNAi as an antiviral mechanism in differentiated cells.³⁷ The differences in siRNA and miRNA biogenesis in different organisms provide further molecular basis for their altered cellular functions. In insects, two Dicer proteins are expressed for miRNA and siRNA biogenesis, respectively, whereas somatic mammalian cells express a single Dicer that is optimized for miRNA processing, but is inefficient at processing long dsRNAs into siRNA.³⁸ Therefore, current data support a conclusion that RNAi is unlikely to play a significant role in the overall antiviral immunity in developed mammalian species. Although this mechanism might be retained as a functional antiviral mechanism in ESCs in the absence of the IFN-based system,^{8,9} the molecular mechanism responsible for siRNA biogenesis remains to be determined.

IFN-based Antiviral Mechanism in ESCs

ESCs have underdeveloped IFN-based antiviral innate immunity

Based on the principle of the RNAi mechanism, synthetic siRNA have been developed as a powerful tool for gene silencing. In developing this technique, it was noted that long dsRNA can elicit the RNAi effect without adverse effects in the cells of invertebrate organisms, but they cause a global inhibition of translation and cell death in differentiated mammalian cells where long dsRNA are recognized as viral RNA and induce a strong IFN response. However, such cytotoxicity and IFN response were not apparent in ESCs.³⁹ Thus, the lack of an IFN response was previously noted in ESCs, but it was not appreciated within the context of immunology.

One reason that has led to the revisiting of this subject stemmed from the finding that ESC-derived endothelial cells, cardiomyocytes, smooth muscle cells, and osteoblasts have limited

immune responses to a wide range of infectious agents.^{40–43} This is markedly different from their *in vivo* counterparts isolated from tissues. These studies raised questions for the therapeutic application of ESC-derived cells, and also promoted studies seeking answers in the ESCs from which these cells are derived. Indeed, studies have found that both hESCs and mESCs lack immune responses to viral and bacterial pathogens typically seen in somatic cells, although they are susceptible to cytotoxicity of infection.^{41,42,44,44–49} The lack of IFN expression was also noted in mouse and human iPSCs.^{46,50} Together with the early findings,^{15–17} it is apparent that an underdeveloped innate immunity is a common feature of pluripotent cells as we have recently discussed.¹⁴

Significant progress has been made recently in understanding the underdeveloped IFN-based antiviral mechanisms in pluripotent cells. The conclusion for hESCs and hiPSCs is primarily derived from experiments using synthetic RNA, polyinosinic-polycytidylic acid (polyIC), as a viral RNA analog.⁴⁶ In addition to synthetic RNAs (polyIC and long single stranded RNA), our studies with mESCs have used several live viruses, including La Crosse encephalitis virus, Chikungunya virus, West Nile virus, and Sendai virus.^{51–53} In all cases, mESCs were susceptible to viral infection, and none of the tested viruses induced IFN expression. Similarly, a study by Wash et al.⁴⁹ demonstrated that mESCs can be infected with herpes simplex virus type 1 and influenza A virus, a dsRNA and ssRNA virus, respectively, and neither induced IFN expression.⁴⁹

While the lack of IFN expression is strikingly similar in hESCs and mESCs, the IFN response mechanism in hESCs and mESCs is somewhat different. hESCs or hiPSCs fail to express ISGs when exposed to IFN β ,⁵⁴ while mESCs could respond to IFN α and IFN β .^{53,55,56} Furthermore, IFN α , IFN β and IFN ω can protect mESCs from the cytopathic effect of viral infection and inhibit replication of several types of viruses.^{51,53} However, the magnitude of response of mESCs to IFN is much weaker than fibroblasts. Therefore, the IFN response mechanism in mESCs is still significantly underdeveloped in comparison with differentiated cells isolated from mouse tissues^{51,53} (Figure 1).

The biological implications of an underdeveloped IFN-based antiviral mechanism in ESCs

The rationale for ESCs not to have a developed IFN system as in somatic cells is a complex question that we currently do not completely understand, but it can be considered from different perspectives. From the view of developmental biology, ESCs reside in the womb where they have limited exposure to pathogens. The mother's immune system may offer them the necessary protection.⁵⁷ A different hypothesis could be made based on the view that IFN are mainly produced for the purpose of defense with negative effects on proliferation of tissue cells. It would be logical for an early embryo not to produce these cytokines when cell proliferation and differentiation are major events.⁵⁸ It is known that multiple forms of antiviral activities triggered by IFN can cause various adverse effects to the infected cells including cell death.^{2,33} While such effects may not cause much damage to a developed organism, the consequence could be detrimental to ESCs, the progenitors for all ensuing tissues of a developing organism. However, considering the fact that ESCs only exist transiently as part of the inner cell mass during early embryogenesis, whether or not an underdeveloped IFN system in ESCs is necessarily an issue remains an open question.

Further studies of the developmental stage- and tissue-specific expression of IFN, especially more direct in vivo experiments, are needed to elucidate the roles of the IFN system in reproduction and development.

The reciprocal inhibition between pluripotency and the IFN-based innate immunity in ESCs

There is clearly a “reciprocal inhibition” between the IFN system and pluripotency in ESCs that has been illustrated by the processes of in vitro ESC differentiation and reprogramming of fibroblasts to iPSCs. Our recent studies with mESCs have demonstrated that in vitro differentiation is characterized by mESCs losing pluripotency, concurrent with increased expression of viral RNA receptors and the gain of IFN expression capacity in mESC-differentiated fibroblasts.^{51,53} Conversely, primary fibroblasts, which robustly express IFN in response to viral pathogens, lose this capacity after they are reprogrammed into iPSCs, with reduced expression of the signaling molecules essential for the IFN system,^{46,50} meaning that gaining pluripotency is accompanied by the loss of IFN-based innate immunity.

From the perspective of developmental biology, the reciprocal inhibition between pluripotency and IFN-based innate immunity makes sense. It is logical for ESCs to repress the development of the IFN system to avoid potential adverse effects, or it may simply not be needed because of the protection by the mother’s immune system, as previously discussed. However, the results from iPSC reprogramming point to another possibility. Since iPSCs are pluripotent cells that are artificially generated in vitro, losing function of the IFN system is physiologically irrelevant to cellular innate immunity. It seems more likely that the IFN system is repressed during reprogramming because it may somehow conflict with molecular mechanisms that control pluripotency. While we do not fully understand why and how the reciprocal inhibition between the pluripotency and the IFN system is achieved, recent studies have provided convincing data to explain the deficiency of ESCs in expressing IFN and their attenuated response to these cytokines.

The molecular basis for deficient IFN expression in ESCs

The underdevelopment of IFN-based innate immunity is reflected at least at the receptor and transcription level. The major viral RNA receptors (TLR3, RIG-I and MDA5) are expressed at low levels in both hESCs and mESCs.^{46,52,52,59} Similar observations were made in iPSCs,^{46,50} which means that the ability to express IFN in the parental fibroblasts from which iPSCs were derived is reverted to the stem cell state after reprogramming. Therefore, the low expression level of viral RNA receptors in pluripotent cells is at least partially responsible for their lack of IFN expression.

Our recent study in mESCs has further demonstrated that NFκB is not activated by viral infection.⁵¹ This finding provides a direct link to the failure of these cells to express IFN at the transcription level, since NFκB is a key transcription factor that mediates the expression of IFN and inflammatory cytokines.⁶⁰ In mammals, the NFκB family is composed of several transcription factors, but p50 and RelA subunits play a major role in mediating the immune response.⁶¹ In both mESCs and hESCs, RelA and p50 are expressed at low levels but are upregulated upon differentiation.^{62,63} It is particularly interesting to note that Nanog, one of

the key pluripotency genes in ESCs, can bind to and inhibit NF κ B transcriptional activity.⁶⁴ Another study has identified the mRNA of RelA as a target of miR-290 cluster, which belongs to a class of ESC-specific miRNA.⁶⁵ A simplified conclusion from these findings is that NF κ B is repressed in ESCs because its activation promotes differentiation and conflicts with pluripotency. Although these studies were not intended to investigate the role of NF κ B in the immune and inflammatory responses in ESCs, the results in fact provide the molecular basis for the inactive status of NF κ B and point to a new model of NF κ B regulation unique to ESCs.

Since none of the above-mentioned studies were dedicated to investigating the role of NF κ B in antiviral response in ESCs, we used a co-culture system where mESCs and mESC-differentiated fibroblasts were infected with La Crosse encephalitis virus and Chikungunya virus. We demonstrated that NF κ B is exclusively activated in mESC-differentiated fibroblasts, not in mESCs, by viral infection.⁵¹ The same observation was also reported in miPSCs that were infected with baculovirus.⁵⁰ We propose that NF κ B acts as a master switch that controls the antiviral response: it is switched off by the pluripotent state in ESCs and turned on by the process of differentiation.

The molecular basis for attenuated IFN responses in ESCs

While deficiency in the expression of IFN is common to both human and mouse ESCs and iPSCs, the IFN response mechanism in the two species differs to a certain degree. hESCs and hiPSCs fail to express ISGs when exposed to IFN β .⁵⁴ Although the major signaling molecules in the IFN response pathway are expressed at relatively lower levels than in differentiated human cells, the high expression level of suppressor of cytokine signaling 1 (SOCS1, a negative regulator of the JAK/STAT signaling pathway⁶⁶) seems to be the limiting factor for ISG induction in hESCs and hiPSCs.⁵⁴ On the other hand, two early studies have reported that IFN α and IFN β can induce expected responses in mESCs.^{55,56} Our recent studies^{51,53} confirmed this finding in mESCs and provided several lines of evidence that mESCs have a functional IFN response mechanism: 1) the key signaling components that detect and mediate the effects of IFN are expressed, 2) IFN α and IFN β induce ISG expression (ISG15, OAS1, and PKR), and 3) IFN α and IFN β protect mESCs from the cytopathic effect of viral infection and repress replication of several types of viruses.^{51,53} While these results clearly demonstrate that mESCs can detect and respond to IFN, the level of response of mESCs and even mESC-differentiated fibroblasts to IFN is much weaker than that of naturally differentiated mouse fibroblasts, as judged by the levels of ISG induction and antiviral activity elicited by IFN α and IFN β .^{51,53} Therefore, the response of mESCs to IFN is attenuated in comparison with differentiated cells.

Intrinsic antiviral immunity in ESCs

Limited information is available about the specific intrinsic antiviral factors in ESCs as described in somatic cells.^{30,31} However, the common intrinsic antiviral factor PKR is expressed in ESCs.^{46,52,67} In comparison with other viral RNA receptors in hESCs and mESCs, which are expressed at low levels, PKR is readily detected at both mRNA and protein levels. Both synthetic dsRNA (polyIC) and live viral infection activate PKR in mESCs, resulting in inhibition of cell proliferation as in somatic cells.⁵² Intriguingly, PKR is

not activated by polyIC in hESCs and hiPSCs, in which transfected polyIC seems to be sequestered in endosomes.⁴⁶ While the role of PKR in the antiviral response in hESCs remains unclear, a recent study suggests that PKR is activated by cellular dsRNAs during mitosis and acts as a mitotic regulator.⁶⁷

A few studies have investigated OAS and RNase L in ESCs. RNase L is expressed at a lower level in hESCs and hiPSCs than in HeLa cells and fibroblasts, but nevertheless is detectable at the protein level.⁴⁶ Studies in mESCs showed that the mRNA of OAS1 and RNase L are expressed at a moderate level.^{52,55} We have shown that OAS1 was strongly induced by La Crosse encephalitis virus infection in mouse fibroblasts, but not in mESCs. However, OAS1 was induced by exogenously added IFN β in mESCs.⁵³ This is an interesting observation because it is relevant to a finding in somatic cells, in which it is believed that IFN are constitutively secreted at a low basal level as a means of keeping the cells at a “primed” state, so that they can mount a rapid and robust response to invading pathogens.⁶⁸ We speculate that the failure of mESCs to express OAS1 in response to viral infection could be due to their deficiency in producing the basal level of IFN needed to keep them in a “primed” state. However, it is possible that the expression of OAS1 (or other ISGs for that matter) in mESCs could be higher in vivo since they can be “primed” by IFN secreted from other cells (such as trophoblasts of the placenta) via paracrine signaling.⁶⁹ Although the contribution of OAS/RNase L and the PKR pathways to the antiviral activity in ESCs remains to be further investigated, there is a molecular basis that reasonably supports their potential roles in antiviral responses, at least in mESCs.

Differences in Antiviral Innate Immunity between hESCs and mESCs

mESCs and hESCs share fundamental similarities in self-renewal and pluripotency, which are controlled by a transcriptional network consisting of similar core transcription factors,^{10–12} but they nevertheless display a number of differences as in other aspects of the two species.⁷⁰ Most notably, the stem cell state of mESCs is maintained by leukemia inhibitory factor (LIF), whereas FGF2 and activin A, but not LIF, are the primary determinants of hESC self-renewal and pluripotency.^{71,72} mESCs are characterized by a shortened cell cycle, whereas hESCs have a cell cycle time frame similar to that of differentiated cells.^{73,74} PKR activation and response to type I IFN represent the differences that so far have been noted between the two species in terms of innate immunity.^{46,52–54} The mouse models have generated a vast amount of data that remarkably mirrors human biology, but it is not surprising that notable differences in many aspects, including immunity, have been noted between the two species.⁷⁵ Further understanding of the differences between hESCs and mESCs in innate immunity is an important issue not only for understanding basic developmental biology and immunology, but also for of ESC-based regenerative medicine.

Conclusions and Perspective

Along with the remarkable progress that has been made toward the application of ESCs in regenerative medicine, we have also witnessed how the discoveries made from stem cell research have led to a better understanding of many important questions in basic biology. It

has become apparent that ESCs and differentiated cells have distinct antiviral innate immunity. The two antiviral mechanisms established in differentiated cells, the IFN system and the intrinsic antiviral pathway, are both underdeveloped in ESCs. Most notably, the IFN expression and IFN response pathway is either absent or severely attenuated in ESCs (Figure 1). What we have learned from ESCs clearly demonstrated that innate immunity is in fact not “innate” to ESCs; it is rather “acquired” and remodeled during the process of organism development. While it is exciting to see that the findings from pluripotent cell models have already provided new insight into the evolution of RNA-based antiviral mechanisms in different organisms, much more investigation is needed to determine whether or not RNAi makes a physiologically meaningful contribution to the antiviral activity in ESCs and the early stages of organismal development in mammals. Along the line of this direction, it is particularly interesting to note that the recent discovery of miRNA as a new class of immunity modulators adds another layer of complexity to our understanding of the already sophisticated innate immune system.⁷⁶ ESCs and iPSCs could once again be proven to be valuable models for such investigations.

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References

1. Carthew RW, Sontheimer EJ. Origins and Mechanisms of miRNAs and siRNAs. *Cell*. 136:642–655.
2. Samuel CE. Antiviral actions of interferons. *Clin Microbiol Rev*. 2001; 14:778–809. [PubMed: 11585785]
3. Boehm T. Evolution of vertebrate immunity. *Curr Biol*. 2012; 22:R722–R732. [PubMed: 22975003]
4. Pare JM, Sullivan CS. Distinct antiviral responses in pluripotent versus differentiated cells. *PLoS Pathog*. 2014; 10:e1003865. [PubMed: 24516379]
5. Cullen BR, Cherry S, tenOever BR. Is RNA interference a physiologically relevant innate antiviral immune response in mammals? *Cell Host Microbe*. 2013; 14:374–378. [PubMed: 24139396]
6. Jeang KT. RNAi in the regulation of mammalian viral infections. *BMC Biol*. 2012; 10:58. [PubMed: 22734679]
7. Backes S, Langlois R, Schmid S, Varble A, Shim J, Sachs D, et al. The Mammalian response to virus infection is independent of small RNA silencing. *Cell Reports*. 2014; 8:114–125. [PubMed: 24953656]
8. Maillard PV, Ciaudo C, Marchais A, Li Y, Jay F, Ding SW, et al. Antiviral RNA interference in mammalian cells. *Science*. 2013; 342:235–238. [PubMed: 24115438]
9. Li Y, Lu J, Han Y, Fan X, Ding SW. RNA interference functions as an antiviral immunity mechanism in mammals. *Science*. 2013; 342:231–234. [PubMed: 24115437]
10. Wobus AM, Boheler KR. Embryonic stem cells: prospects for developmental biology and cell therapy. *Physiol Rev*. 2005; 85:635–678. [PubMed: 15788707]
11. Keller G. Embryonic stem cell differentiation: emergence of a new era in biology and medicine. *Genes Dev*. 2005; 19:1129–1155. [PubMed: 15905405]
12. Tabar V, Studer L. Pluripotent stem cells in regenerative medicine: challenges and recent progress. *Nat Rev Genet*. 2014; 15:82–92. [PubMed: 24434846]
13. Stadtfeld M, Hochedlinger K. Induced pluripotency: history, mechanisms, and applications. *Gene Dev*. 2010; 24:2239–2263. [PubMed: 20952534]

14. Guo YL, Carmichael GG, Wang R, Hong X, Acharya D, Huang F, et al. Concise Reviews: attenuated innate immunity in embryonic stem cells and its implications in developmental biology and regenerative medicine. *Stem Cells*. 2015; 33:3165–3173. [PubMed: 26086534]
15. Swartzendruber DE, Lehman JM. Neoplastic differentiation: interaction of simian virus 40 and polyoma virus with murine teratocarcinoma cells in vitro. *J Cell Physiol*. 1975; 85:179–187. [PubMed: 164473]
16. Swartzendruber DE, Friedrich TD, Lehman JM. Resistance of teratocarcinoma stem cells to infection with simian virus 40: early events. *J Cell Physiol*. 1977; 93:25–30. [PubMed: 198419]
17. Burke DC, Graham CF, Lehman JM. Appearance of interferon inducibility and sensitivity during differentiation of murine teratocarcinoma cells in vitro. *Cell*. 1978; 13:243–248. [PubMed: 627035]
18. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*. 1998; 391:806–811. [PubMed: 9486653]
19. Cullen BR. Viruses and RNA interference: issues and controversies. *J Virol*. 2014; 88:12934–12936. [PubMed: 25210170]
20. Sen GC. Viruses and infections. *Annu Rev Microbiol*. 2001; 55:255–281. [PubMed: 11544356]
21. Kumar H, Kawai T, Akira S. Toll-like receptors and innate immunity. *Biochem Biophys Res Comm*. 2009; 388:621–625.
22. Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity*. 2011; 34:637–650. [PubMed: 21616434]
23. Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol*. 2004; 5:730–737. [PubMed: 15208624]
24. Xiao TS, Fitzgerald KA. The cGAS-STING pathway for DNA sensing. *Mol Cell*. 2013; 51:135–139. [PubMed: 23870141]
25. Ma Z, Damania B. The cGAS-STING defense pathway and its counteraction by viruses. *Cell Host Microbe*. 2016; 19:150–158. [PubMed: 26867174]
26. Kato H, Takahasi K, Fujita T. RIG-I-like receptors: cytoplasmic sensors for non-self RNA. *Immunol Rev*. 2011; 243:91–98. [PubMed: 21884169]
27. Stetson DB, Medzhitov R. Type I interferons in host defense. *Immunity*. 2006; 25:373–381. [PubMed: 16979569]
28. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon- γ : an overview of signals, mechanisms and functions. *J Leukocyte Biol*. 2004; 75:163–189. [PubMed: 14525967]
29. Schoggins JW, Wilson SJ, Panis M, Murphy MY, Jones CT, Bieniasz P, et al. A diverse range of gene products are effectors of the type I interferon antiviral response. *Nature*. 2011; 472:481–485. [PubMed: 21478870]
30. Yan N, Chen ZJ. Intrinsic antiviral immunity. *Nat Immunol*. 2012; 13:214–222. [PubMed: 22344284]
31. Bieniasz PD. Intrinsic immunity: a front-line defense against viral attack. *Nat Immunol*. 2004; 5:1109–1115. [PubMed: 15496950]
32. Chakrabarti A, Jha BK, Silverman RH. New insights into the role of mda5 in innate immunity. *J Interferon Cytokine Res*. 2011; 31:49–57. [PubMed: 21190483]
33. Garcia MA, Meurs EF, Esteban M. The dsRNA protein kinase PKR: Virus and cell control. *Biochimie*. 2007; 89:799–811. [PubMed: 17451862]
34. Bogerd HP, Skalsky RL, Kennedy EM, Furuse Y, Whisnant AW, Flores O, et al. replication of many human viruses is refractory to inhibition by endogenous cellular microRNAs. *J Virol*. 2014; 88:8065–8076. [PubMed: 24807715]
35. Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol*. 2009; 10:126–139. [PubMed: 19165215]
36. Liu J, Carmell MA, Rivas FV, Marsden CG, Thomson JM, Song JJ, et al. Argonaute2 is the catalytic engine of mammalian RNAi. *Science*. 2004; 305:1437–1441. [PubMed: 15284456]

37. Seo GJ, Kincaid RP, Phanaksri T, Burke JM, Pare JM, Cox JE, et al. Reciprocal inhibition between intracellular antiviral signaling and the RNAi machinery in mammalian cells. *Cell Host Microbe*. 2013; 14:10.
38. Ma E, MacRae IJ, Kirsch JF, Doudna JA. Autoinhibition of human dicer by its internal helicase domain. *J Mol Biol*. 2008; 380:237–243. [PubMed: 18508075]
39. Yang S, Tutton S, Pierce E, Yoon K. Specific double-stranded RNA interference in undifferentiated mouse embryonic stem cells. *Mol Cell Biol*. 2001; 21:7807–7816. [PubMed: 11604515]
40. Rajan R, Ye J, Bai S, Huang F, Guo YL. NF- κ B, but not p38 MAP kinase, is required for TNF- α -induced expression of cell adhesion molecules in endothelial cells. *J Cell Biochem*. 2008; 105:477–486. [PubMed: 18613029]
41. Foldes G, Liu A, Badiger R, Paul-Clark M, Moreno L, Lendvai Z, et al. Innate immunity in human embryonic stem cells: comparison with adult human endothelial cells. *PLoS ONE*. 2010; 5:e10501. [PubMed: 20463927]
42. Zampetaki A, Xiao Q, Zeng L, Hu Y, Xu Q. TLR4 expression in mouse embryonic stem cells and in stem cell-derived vascular cells is regulated by epigenetic modifications. *Biochem Biophys Res Commun*. 2006; 347:89–99. [PubMed: 16814255]
43. Sidney LE, Kirkham GR, Buttery LD. Comparison of osteogenic differentiation of embryonic stem cells and primary osteoblasts revealed by responses to IL-1 β , TNF- α , and IFN- γ . *Stem Cells Dev*. 2014; 23:605–617. [PubMed: 24192281]
44. Yu J, Rossi R, Hale C, Goulding D, Dougan G. Interaction of enteric bacterial pathogens with murine embryonic stem cells. *Infect Immunity*. 2009; 77:585–597. [PubMed: 19029302]
45. Taylor T, Kim YJ, Ou X, Derbigny W, Broxmeyer HE. Toll-like receptor 2 mediates proliferation, survival, NF- κ B translocation, and cytokine mRNA expression in LIF-maintained mouse embryonic stem cells. *Stem Cells Dev*. 2010; 19:1333–1341. [PubMed: 20132051]
46. Chen LL, Yang L, Carmichael GG. Molecular basis for an attenuated cytoplasmic dsRNA response in human embryonic stem cells. *Cell cycle*. 2010; 9:3552–3564. [PubMed: 20814227]
47. Freudenberg MA, Tchaptchet S, Keck S, Fejer Gr, Huber M, Schutze N, et al. Lipopolysaccharide sensing an important factor in the innate immune response to Gram-negative bacterial infections: Benefits and hazards of LPS hypersensitivity. *Immunobiol*. 2008; 213:193–203.
48. Matsumoto M, Seya T. TLR3: Interferon induction by double-stranded RNA including poly(I:C). *Adv Drug Deliv Rev*. 2008; 60:805–812. [PubMed: 18262679]
49. Wash R, Calabressi S, Franz S, Griffiths SJ, Goulding D, Tan E-P, et al. Permissive and restricted virus infection of murine embryonic stem cells. *J Gen Virol*. 2012; 93:2118–2130. [PubMed: 22815272]
50. Chen GY, Hwang SM, Su HJ, Kuo CY, Luo WY, Lo KW, et al. Defective antiviral responses of induced pluripotent stem cells to baculoviral vector transduction. *J Virol*. 2012; 86:8041–8049. [PubMed: 22623765]
51. D'Angelo W, Acharya D, Wang R, Wang J, Gurung C, Chen B, et al. Development of antiviral innate immunity during in vitro differentiation of mouse embryonic stem cells. *Stem Cells Dev*. 2016; 25:648–659. [PubMed: 26906411]
52. Wang R, Wang J, Paul AM, Acharya D, Bai F, Huang F, et al. Mouse embryonic stem cells are deficient in type I interferon expression in response to viral infections and double-stranded RNA. *J Biol Chem*. 2013; 288:15926–15936. [PubMed: 23580653]
53. Wang R, Wang J, Acharya D, Paul AM, Bai F, Huang F, et al. Antiviral responses in mouse embryonic stem cells: differential development of cellular mechanisms in type I interferon production and response. *J Biol Chem*. 2014; 289:25186–25198. [PubMed: 24966329]
54. Hong XX, Carmichael GG. Innate immunity in pluripotent human cells: attenuated response to interferon- β . *J Biol Chem*. 2013; 288:16196–16205. [PubMed: 23599426]
55. Whyatt LM, Duwel A, Smith AG, Rathjen PD. The responsiveness of embryonic stem cells to alpha and beta interferons provides the basis of an inducible expression system for analysis of developmental control genes. *Mol Cell Biol*. 1993; 13:7971–7976. [PubMed: 8247011]
56. Ruffner H, Reis LF, Naf D, Weissmann C. Induction of type I interferon genes and interferon-inducible genes in embryonal stem cells devoid of interferon regulatory factor 1. *Proc Natl Acad Sci USA*. 1993; 90:11503–11507. [PubMed: 8265581]

57. Levy O. Innate immunity of the newborn: basic mechanisms and clinical correlates. *Nat Rev Immunol.* 2007; 7:379–390. [PubMed: 17457344]
58. Hertzog PJ, Hwang SY, Kola I. Role of interferons in the regulation of cell proliferation, differentiation, and development. *Mol Reprod Dev.* 1994; 39:226–232. [PubMed: 7530016]
59. Wang R, Teng C, Spangler J, Wang J, Huang F, Guo Y-L. Mouse embryonic stem cells have underdeveloped antiviral mechanisms that can be exploited for the development of mRNA-mediated gene expression strategy. *Stem Cells Dev.* 2014; 23:594–604. [PubMed: 24219369]
60. Baeuerle PA, Henkel T. Function and activation of NF-kappaB in the immune system. *Annu Rev Immunol.* 1994; 12:141–179. [PubMed: 8011280]
61. Hayden MS, Ghosh S. NF-kB, the first quarter-century: remarkable progress and outstanding questions. *Gene Dev.* 2012; 26:203–234. [PubMed: 22302935]
62. Kim YE, Kang HB, Park JA, Nam KH, Kwon HJ, Lee Y. Upregulation of NF-kappaB upon differentiation of mouse embryonic stem cells. *BMB Rep.* 2008; 41:705–709. [PubMed: 18959816]
63. Kang HB, Kim YE, Kwon HJ, Sok DE, Lee Y. Enhancement of NF-kB expression and activity upon differentiation of human embryonic stem cell line SNUHES3. *Stem Cells Deve.* 2007; 16:615–624.
64. Torres J, Watt FM. Nanog maintains pluripotency of mouse embryonic stem cells by inhibiting NF[kappa]B and cooperating with Stat3. *Nat Cell Biol.* 2008; 10:194–201. [PubMed: 18223644]
65. Luningschror P, Stocker B, Kaltschmidt B, Kaltschmidt C. miR-290 cluster modulates pluripotency by repressing canonical NF-kB Signaling. *Stem Cells.* 2012; 30:655–664. [PubMed: 22232084]
66. Kubo M, Hanada T, Yoshimura A. Suppressors of cytokine signaling and immunity. *Nat Immunol.* 2003; 4:1169–1176. [PubMed: 14639467]
67. Kim Y, Lee JH, Park JE, Cho J, Yi H, Kim VN. PKR is activated by cellular dsRNAs during mitosis and acts as a mitotic regulator. *Gene Deve.* 2014; 28:1310–1322.
68. Gough DJ, Messina NL, Clarke CJ, Johnstone RW, Levy DE. Constitutive type I interferon modulates homeostatic balance through tonic signaling. *Immunity.* 2012; 36:166–174. [PubMed: 22365663]
69. Aikawa H, Tamai M, Mitamura K, Itmainati F, Barber GN, Tagawa Yi. Innate immunity in an in vitro murine blastocyst model using embryonic and trophoblast stem cells. *J Biosci Bioeng.* 2014; 117:358–365. [PubMed: 24113362]
70. Ginis I, Luo Y, Miura T, Thies S, Brandenberger R, Gerecht-Nir S, et al. Differences between human and mouse embryonic stem cells. *Dev Biol.* 2004; 269:360–380. [PubMed: 15110706]
71. Hirai H, Karian P, Kikyo N. Regulation of embryonic stem cell self-renewal and pluripotency by leukaemia inhibitory factor. *Biochem J.* 2011; 438:11–23. [PubMed: 21793804]
72. Humphrey RK, Beattie GM, Lopez AD, Bucay N, King CC, Firpo MT, et al. Maintenance of pluripotency in human embryonic stem cells is STAT3 independent. *Stem Cells.* 2004; 22:522–530. [PubMed: 15277698]
73. Burdon T, Smith A, Savatier P. Signalling, cell cycle and pluripotency in embryonic stem cells. *Trend Cell Biol.* 2002; 12:432–438.
74. Dalton S. Exposing hidden dimensions of embryonic stem cell cycle control. *Cell Stem Cell.* 2009; 4:9–10. [PubMed: 19128789]
75. Mestas J, Hughes CCW. Of Mice and Not Men: Differences between mouse and human immunology. *J Immunol.* 2004; 172:2731–2738. [PubMed: 14978070]
76. Chen CZ, Schaffert S, Fragoso R, Loh C. Regulation of immune responses and tolerance: the microRNA perspective. *Immunol Rev.* 2013; 253:112–128. [PubMed: 23550642]

Figure 1.

Schematic diagram of antiviral mechanisms in mammalian cells: IFN-based antiviral system, including IFN expression pathway (A^E) and IFN response pathway (A^R); the intrinsic antiviral pathway (B); and the RNAi antiviral pathway (C). The functionality of the pathways is denoted by: (++) , fully developed; (+) , partly developed; (-) , not developed or severely attenuated; (?) uncertain or no sufficient data. DC, differentiated cells; ESC refers to both human and mouse ESCs. See the text for other abbreviations and further explanations.