Salmonella-induced miR-155 enhances necroptotic death in macrophage cells via targeting RIP1/3

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Abstract. *Salmonella enterica* serovar Typhimurium (hereafter referred to as *Salmonella*), a virulent pathogen, is known to induce host-cell death. Using reverse transcription-quantitative polymerase chain reaction, a 28-fold increase of microRNA (miR)-155 expression in RAW 264.7 macrophages was observed following infection with *Salmonella* for 24 h. This miR-155 upregulation increased macrophage cell death by up to 40% in 48 h following infection. Western blot analysis revealed that receptor interacting protein 1 (RIP1) and 3 (RIP3) were increased at 18 h following miR-155 transfection to macrophages, similar to *Salmonella* infection. In addition, inhibition of RIP1 by pre-incubating macrophages with necrostatin-1, a RIP1 specific inhibitor, increased the viability of *Salmonella*-infected cells and miR-155-transfected cells by up to 20%. The cleavage of poly (adenosine diphosphate-ribose) polymerase-1 (PARP-1) was also enhanced by miR-155 induction upon *Salmonella* infection. Therefore, it was suggested that RIP1/3-induced necroptosis and PARP-1-mediated necrosis caused by miR-155 induction may represent distinct routes of programmed necrotic cell death of *Salmonella*-infected macrophages.

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Abbreviations: CMPCs, cardiomyocyte progenitor cells; DCs, dendritic cells; IFN, interferon; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; miRNAs, microRNAs; MOI, multiplicity of infection; Nec-1, necrostatin-1; NRU, neutral red uptake; PARP-1, poly (adenosine diphosphate-ribose) polymerase-1; RIP, receptor interacting protein; RIPK, RIP kinase; TNF, tumor-necrosis factor

Key words: miR-155 induction, necroptosis, RIP1/3 activation, *Salmonella* Typhimurium infection

Introduction

Apoptosis and necrosis are two principal mechanisms of eukaryotic cell death that can be distinguished biochemically and morphologically. Apoptosis is a highly regulated and controlled process, requiring ATP and the activation of specific proteases and caspases. Meanwhile, necrosis results from membrane damage either directly or indirectly due to energy depletion. Therefore, necrosis is strongly pro-inflammatory *in vivo*, whereas apoptotic cells are rapidly phagocytosed and thus generate minimal inflammation (1). In addition to apoptosis and necrosis, necroptosis as a programmed necrosis has to come to light. Necroptosis is generally initiated by the engagement of TNFR1, a receptor for tumor-necrosis factor (TNF), which in turn activates receptor interacting protein 1 (RIP1) and 3 (RIP3), and leads to necrotic cell death (2,3).

Salmonella-induced cytotoxicity occurring in murine macrophages had been previously described as apoptosis, because the cells exhibited characteristic features of apoptosis (4). On the other hand, other reports revealed that *Salmonella*-infected macrophages were killed by an unusual caspase-1-dependent mechanism of necrosis (5,6), suggesting that *Salmonella*-induced macrophage death could share features of both apoptosis and necrosis. However, another study showing *Salmonella*-induced necroptosis of macrophages by production of type I interferon (IFN) suggested the importance of necroptosis on macrophage death and pathogenesis of the infection (7,8). Therefore, the precise mechanism of macrophage death by *Salmonella* infection remains to be elucidated.

MicroRNAs (miRNAs) are short (21-25 nt), endogenous, and non-coding RNA molecules, which regulate gene expression post-translationally by imperfect binding to the target sequences. In mammals, miRNAs are estimated to regulate approximately 50% of all protein-coding genes and play important roles in several biological processes, such as cell proliferation and differentiation, signal transduction, metabolism, tumorigenesis, and progression (9,10). Moreover, accumulating evidences have suggested that some miRNAs play an important role in cell survival and death (11,12). During the initial stages of infection, innate immune responses effectively control the replication and survival of *Salmonella*. A microarray study revealed that miRNA-155 (miR-155) was a sole miRNA that was substantially up-regulated by both

polyriboinosinic: polyribocytidylic acid and IFN-γ stimuli, suggesting that miR-155 can act as a component of the primary macrophage response to different types of inflammatory mediators (13). Furthermore, modulation of miR-146a/b, miR-155, and miR-21 was reported in *Salmonella* infection, with NF-κB-dependent miRNAs significantly induced upon infection in mouse macrophages (14,15).

Although miR-155 was first discovered in children with Burkitt lymphoma and further found to act as an oncogene or a tumor suppressor in different types of cancer (16,17), growing evidences have suggested that miR-155 has been considered as an important pleiotropic regulator of cell homeostasis and a typical multifunctional miRNA that regulates multiple pathophysiological pathways (18,19). In 2011, Liu *et al* (20) showed that miR-155 was expressed in growing human cardiomyocyte progenitor cells (CMPCs) and attenuated the CMPC necrosis induced by oxidative stress via targeting RIP1, a death domain protein required for the activation of necroptosis (21). Recently, other reports revealed that lipopolysaccharide (LPS)-induced miR-155 prevented apoptosis through CASP-3 mRNA down-regulation in RAW 264.7 macrophages, as a prerequisite to maintain their crucial function in inflammation (22). It was also revealed that miR-155 was up-regulated in a number of osteosarcoma cell lines and the inhibition of this miRNA led to cancer cell death through MAP3K10 as a target (23). Thus, this suggests that miR-155 can prevent cell death through different targets with cell-type specificity.

In this study, we report the possible role of miR-155 being highly up-regulated in *Salmonella*-infected murine macrophages. Differing from previous studies, our results display that the up-regulation of miR-155 in RAW 264.7 macrophages by *Salmonella* infection enhances cell death by necroptosis via targeting both RIP1/3 and poly(ADP-ribose) polymerase-1 (PARP-1).

Materials and methods

Cell culture and Salmonella infection. Murine Raw 264.7 macrophages were routinely cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin-streptomycin, and maintained at 37˚C in a humidified incubator (5% CO_2). Gram-negative *Salmonella enterica* serovar Typhimurium SL1344 strain (hereafter referred to as *Salmonella*) was cultured in low salt Luria Bertani broth at 37˚C with aeration.

Raw 264.7 cells ($1x10^6$ cells/ml) were infected with *Salmonella* ($3x10^7$ CFU/ml) at a multiplicity of infection (MOI) of 10. Cells were washed with phosphate-buffered saline (PBS) 1 h after infection, followed by the addition of gentamicin (100 µg/ml) for the remainder of the infection. At the indicated times, cells were imaged using microscopy (IX71 instrument, x20; Olympus Corporation, Tokyo, Japan) and were harvested for further analysis.

MiRNA screening and analysis. MiRNA expression in mock infection control and *Salmonella*-infected macrophages was assessed using GeneChip® miRNA 3.0 Array (miRBase v17; Affymetrix; Thermo Fisher Scientific, Inc., Waltham, MA, USA) as previously described (24). The collected data were analyzed by performing Robust MultiArray Average (RMA) and detection above background (DABG) analysis using Affymetrix default analysis settings and were normalized by global scaling. Variations in miRNA expression were analyzed for predicting target miRNAs using TargetScan v7.1 (www.targetscan.org/vert_71/), with a context score percentile of over 90 for determining differentially expressed miRNAs.

MiRNAs identified from primary screening were further analyzed to determine secondary target miRNAs. Firstly, high reproducibility under the repetitive test, secondly, a base log value of >7.0 for both mock-infected and *Salmonella*-infected macrophages, and finally, log ratio of >1.0 between *Salmonella*-infected and mock infection control macrophages were the cut-off values of miRNA used for the selection of secondary target miRNAs.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) for miRNA-155-5p detection. Total DNA-free RNAs were isolated with TRI Reagent (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) according to the manufacturer's instructions. RT-PCR was performed at 37˚C for 1 h then at 85˚C for 5 min to inactivate the enzymes. Expression of miRNAs was analyzed using Mir-X miRNA RT-qPCR SYBR kit (Clontech Laboratories, Inc., Mountainview, CA, USA) according to the manufacturer's protocol. A 5' primer specific for mmu-miR-155-5p detection (accession no. MIMAT0000165, 5'-UUAAUGCUAAUUGUGAUAGGGGU-3') was used in RT-qPCR. The thermocycling conditions for qPCR were as follows: 95˚C for 10 sec followed by 40 cycles of 95˚C for 5 sec and 60˚C for 20 sec. Level of miRNA-155-5p was normalized using the U6 small nuclear RNA (NR_003027; Clontech Laboratories, Inc.) as an internal control and the relative quantity was determined using the $2^{-\Delta\Delta Cq}$ method (25).

MiRNA transfection. Non-targeting control miRNA (nc-miR), anti-miR inhibitor for non-targeting control miRNA (anti-nc-miR), miR-155-5p miRNA (miR-155), and anti-miR inhibitor for miR-155-5p (anti-miR-155) were synthesized by Cosmogenetech (Cosmogenetech Co., Ltd., Seoul, South Korea). Raw 264.7 cells were transfected with 30 nM of individual miRNAs with Lipofectamine 2000 (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Transfection efficiency of the miRNAs was confirmed by RT-PCR.

Cytotoxicity assays. Raw 264.7 macrophages were seeded onto a 96-well plate at a density of $3x10^4$ cells per well and were infected with *Salmonella* at an MOI of 10 (*Salmonella*), along with mock infection (mock). Simultaneously, cells in other wells were transfected with 30 nM of synthetic miRNAs. At the indicated time points (6, 18, 24, and 48 h) after infection or transfection, cell culture supernatants were collected for lactate dehydrogenase (LDH)-release assay, and the remaining cells in each well were used in neutral red uptake (NRU) assay (26). Cell death was quantified by the colorimetric method using LDH Cytotoxicity Detection kit (Takara Bio, Inc., Otsu, Japan). Cell viability was assessed using NRU assay. Briefly, NRU assay consisted of 2 h incubation with neutral red (40 µg/ml) followed by extraction with a mixture of acetic acid, ethanol, and water (1:50:49). Absorbance was measured at 540 nm.

Western blot analysis. Cells were rinsed twice with PBS, and proteins were extracted in cold lysis buffer containing

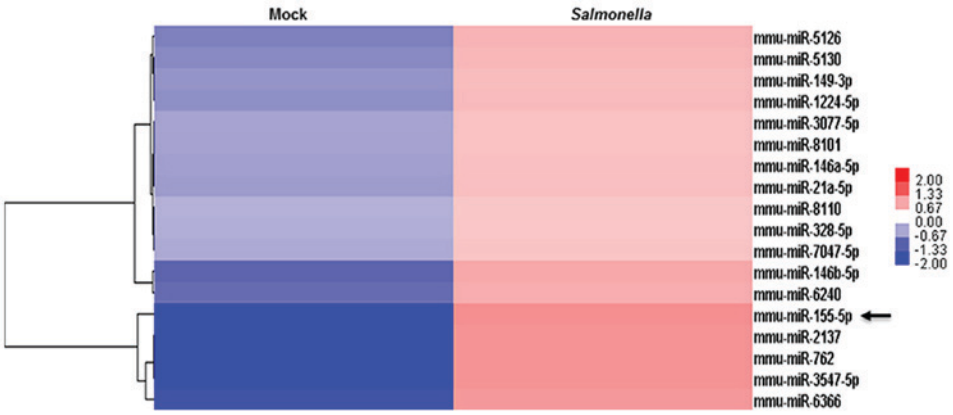

Figure 1. Heat map illustrating the relative expression of a sample of miRNAs in triplicate control or *Salmonella*-treated RAW264.7 cells; red and blue colors represent elevated and decreased expression of miRNAs, respectively. In comparison with miRNAs in mock infection cells, miR-155-5p had the highest expression ratio. miR/miRNA, microRNA.

1% protease inhibitor mixture. Protein concentration was determined by BCA Protein Assay kit (Thermo Fisher Scientific, Inc.). Equal amounts of protein were resolved by SDS-PAGE, and transferred onto a nitrocellulose membrane. The membrane was blocked with 5% non-fat milk in T-PBS (0.1% Tween-20) for 1 h, and then incubated with the indicated primary antibody overnight at 4˚C. After 1 h incubation with horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (cat. no: 7074; Cell Signaling Technology, Inc., Danvers, MA, USA), signals were visualized with Pierce ECL Western blotting substrate (Thermo Fisher Scientific, Inc.). The primary antibodies used were as follows: RIP 1 antibody (1:1,000, rabbit mAb, cat. no: 3493; Cell Signaling Technology, Inc.), RIP3 antibody (1:1,000, rabbit mAb, cat. no: 14401; Cell Signaling Technology, Inc.) and PARP-1 antibody (1:1,000, rabbit polyclonal, sc-7150; Santa Cruz Biotechnology, Inc., Dallas, TX, USA).

Statistical analysis. Statistical analyses were performed with SPSS v24.0 (IBM Corp., Armonk, NY, USA). Measurement data were presented as the mean ± standard deviation. Data were analyzed by a Student's t-test or one-way analysis of variance with Dunnett's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

MiR-155 was highly up-regulated upon Salmonella infection. Secondary target miRNA analysis identified 18 miRNAs which were up-regulated in *Salmonella*-infected murine Raw 264.7 macrophages, compared with mock infection control macrophages (Fig. 1). Of the 18 target miRNAs, miR-155-5p had the highest expression ratio. When miR-155-5p expression rate was determined using RT-qPCR assay in murine macrophages infected with *Salmonella* for 24 h, miR-155-5p levels increased 28-fold (Fig. 2), showing that miR-155 is highly up-regulated upon *Salmonella* infection.

Salmonella induced miR-155-mediated cytotoxicity. Microscopic observations showed that *Salmonella* infection for 48 h resulted in increased macrophage death, when compared to mock infection control. Notably, macrophage transfection

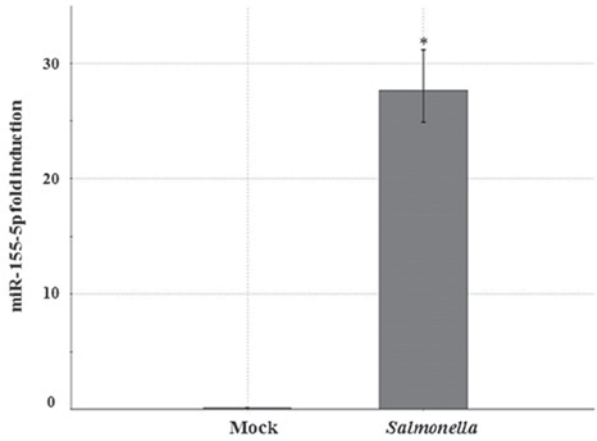

Figure 2. MiR-155-5p expression increases in RAW264.7 macrophages following 24 h of *Salmonella* infection. RAW264.7 cells were infected with *Salmonella* for 24 h, along with mock infection control, and then the miR-155-5p level was measured by reverse transcription-quantitative polymerase chain reaction. Data are presented as the mean ± standard deviation (n=3). *P<0.05 vs. mock control. miR, microRNA.

study with synthetic miRNAs for 48 h revealed that only miR-155 caused remarkable macrophage death, similar to the result of *Salmonella* infection (data not shown). To test whether miR-155 is involved in this *Salmonella* cytotoxicity, macrophages were infected with *Salmonella* or transfected with miRNAs, followed by evaluation of their viability and death rates (%) at 48 h using NRU and LDH-release assays, respectively. The results revealed that cell viability decreased to 50% and cell death ratio increased to 30% at 48 h after *Salmonella* infection, compared to those of mock infection (Fig. 3A and B). Viability of macrophages transfected with miR-155 decreased up to 40% at 48 h post transfection, similar to that of *Salmonella*-infected cells, while either anti-miR-155 or nc-miR and anti-nc-miR as negative controls had a little or no change in cell viability (Fig. 3A). Cell death ratios also showed results similar to those of the viability analysis (Fig. 3B), indicating that miR-155 could enhance death of macrophages. Importantly, pre-transfection of anti-miR-155 into macrophages prior to *Salmonella* infection relieved the *Salmonella* cytotoxicity up to 20% (Fig. 4), suggesting that

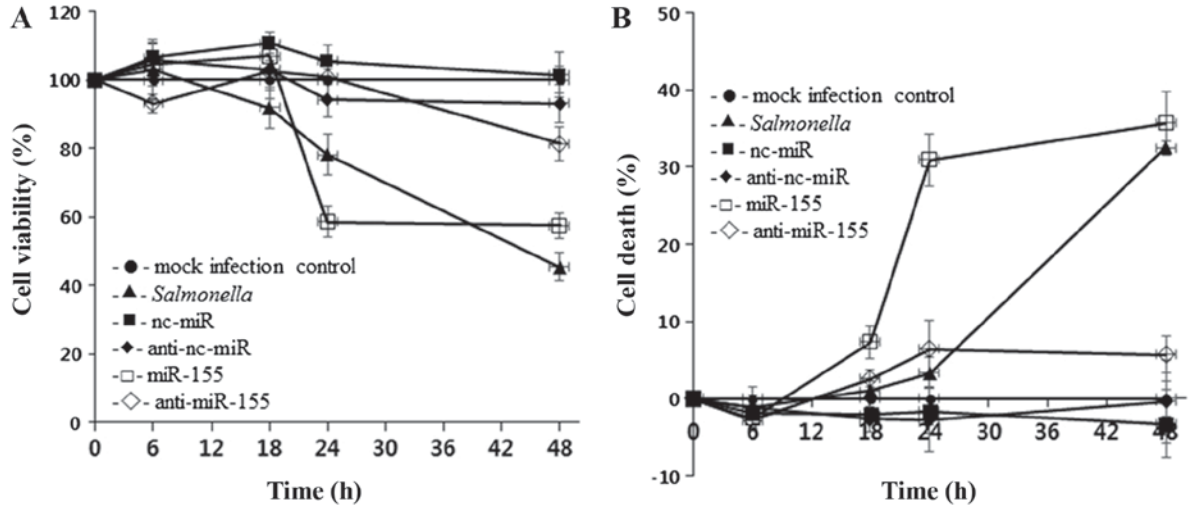

Figure 3. *Salmonella*-induced cytotoxicity is mediated by miR-155. Raw264.7 macrophages in a 96-well plate at density of $3x10^4$ cells per well were infected with *Salmonella*, along with mock infection control, or transfected with nc-miR, anti-nc-miR, miR-155 and anti-miR-155 for 48 h. At the indicated time points, (A) cell viability and (B) cell death were measured. miR, microRNA.

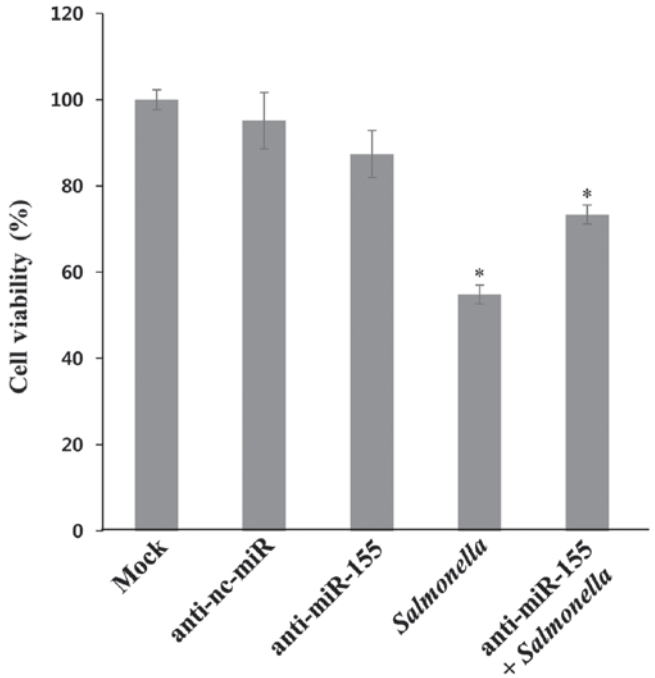

Figure 4. MiR-155 induction by *Salmonella* infection is closely associated with the reduction in macrophage viability. Raw264.7 cells in a 96-well plate at density of $3x10^4$ cells per well were infected with *Salmonella* and mock infection control (Mock), or transfected with anti-nc-miR and anti-miR-155 as experimental controls. To examine the effect of miR-155 on cell death, cells were pre-transfected with 30 nM of anti-miR-155 for 1 h, and then infected with *Salmonella*. Following 48 h, cell viability from each group was measured by neutral red uptake assay. Data are presented as mean ± standard deviation (n=3). *P<0.05 vs. mock control. miR, microRNA; nc, negative control.

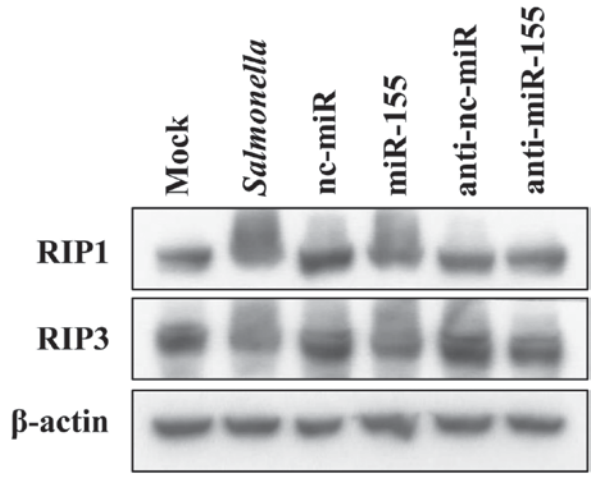

Figure 5. Increased miR-155 induces necrotic cell death via RIP1 and RIP3 activation. Raw264.7 cells in 60 mm dishes at density of $3x10^5$ cells/ml were infected with *Salmonella* and mock infection control (Mock), or transfected with nc-miR, miR-155, anti-nc-miR, and anti-miR-155 for 18 h. Then cells from each group were collected, and western blot analysis of RIP1 and RIP3 was performed. miR, microRNA; nc, negative control; RIP, receptor interacting protein.

miR-155 induction by *Salmonella* infection is largely related to *Salmonella* toxicity on macrophages.

MiR-155 enhanced necrotic cell death by targeting RIP1/3 activation. To test whether miR-155 induction by *Salmonella* infection leads to macrophage death by necroptosis, we examined the activation of RIP1 and RIP3 in macrophages infected with *Salmonella* by Western blot analysis. The activation of RIP1 and RIP3 were not observed at 6 h after *Salmonella* infection (data not shown), consistent with the delayed-death phenotype observed in cell viability and death analysis (Fig. 3A and B). However, upshifted forms representing phosphorylated RIP1 and RIP3 proteins upon activation appeared at 18 h after *Salmonella* infection, indicating necrotic cell death by *Salmonella* infection (Fig. 5). Notably, the upshifts of both RIP1 and RIP3 proteins similarly occurred at 18 h after miR-155 transfection and these forms were not observed by the transfection of anti-miR-155, nc-miR or anti-nc-miR, suggesting that miR-155 up-regulation upon *Salmonella* infection causes necrotic cell death of macrophages through RIP1 and RIP3 activation. Subsequently,

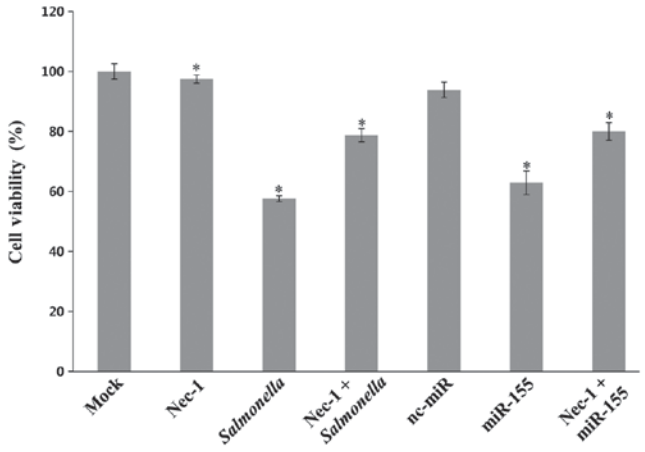

Figure 6. Cell death by miR-155 induction is associated with RIP1. Raw264.7 cells in 96-well plates at density of $3x10^4$ cells per well were infected with *Salmonella* and mock infection control (Mock), or transfected with Nec-1, nc-miR, or miR-155 as experimental control. To examine the role of RIP1 on macrophage death, cells were pretreated with Nec-1 (10 μM) for 1 h and then infected with *Salmonella* or transfected with miR-155. Following 48 h, cell viability from each group was measured by neutral red uptake assay. Data are presented as mean ± standard deviation (n=3). *P<0.05 vs. mock control. miR, microRNA; nc, negative control; RIP, receptor interacting protein; Nec-1, necrostatin-1.

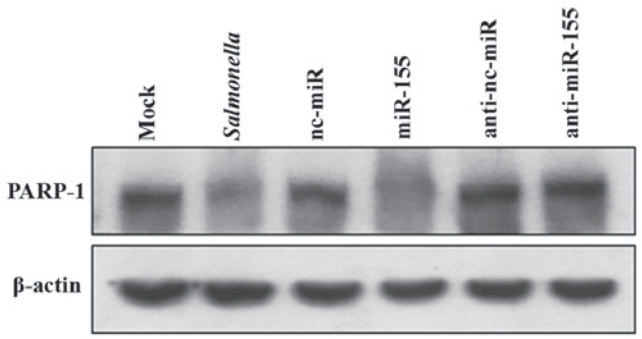

Figure 7. Upregulation of miRNA-155 induces PARP-1 cleavage. Raw264.7 cells in 60 mm dishes at density of $3x10^5$ cells/ml were infected with *Salmonella* and mock infection control (Mock), or transfected with nc-miR, miR-155, anti-nc-miR, and anti-miR-155. Following 24 h, cells from each group were collected, and western blot analysis of PARP-1 was performed. miR, microRNA; nc, negative control; PARP-1, poly (adenosine diphosphate-ribose) polymerase-1.

we tested the effect of necrostatin-1 (Nec-1), a RIP1 specific inhibitor, on cell viability upon *Salmonella* infection. As shown in Fig. 6, macrophages infected with *Salmonella* or transfected with miR-155 showed approximately 40% reduction of cell viability. However, these reductions attenuated up to 20% by the pre-treatment of macrophages with 10 μM of Nec-1 for 24 h, suggesting that Nec-1 partly rescued the macrophage death by either *Salmonella* infection or miR-155 transfection, and that RIP1 is involved in *Salmonella*-induced cytotoxicity mediated by miR-155.

To further test whether this increase in cell death by miR-155 up-regulation is caused by DNA damage, namely apoptosis and necrosis, we scrutinized PARP-1 cleavage in macrophages after *Salmonella* infection or miRNAs transfection by Western blot analysis. In Raw 264.7 cells infected with *Salmonella*, PARP-1 displayed an absence of the mature, uncleaved protein in 24 h (Fig. 7). This cleavage pattern was detected only in miR-155 transfected cells, but not in anti-miR-155, nc-miR, or anti-nc-miR transfected cells, showing that miR-155 induction by *Salmonella* infection could enhance PARP-1 cleavage.

Discussion

Salmonella induces macrophage death to effectively avoid host innate immune responses as an essential strategy for virulence (4,27). Firstly, a 454 deep sequencing analysis revealed that host miRNA expression changed upon *Salmonella* infection in RAW 264.7 macrophages and that NF-kB-associated miRNAs, such as miR-155, miR-146a/b, and miR-21, were shown to be strongly induced (14). Similar results were also observed in human monocytes, suggesting that miRNAs play a first line host defense against bacterial invasion (28,29). However, these miRNAs did not respond to *Salmonella* infection in epithelial HeLa cells, showing that certain miRNA responses to bacterial infection could be cell-type specific (14).

Among miRNAs, miR-155 is one of the best characterized miRNAs. Dysregulation of miR-155 expression has been reported in different types of cells. Firstly, miR-155 is highly expressed in both activated B and T cells (30,31), as well as in activated macrophages and monocytes (28). MiR-155 is also highly up-regulated upon LPS stimulation of human primary dendritic cells (DCs) and during DC maturation (32,33). A microarray analysis in bone marrow-derived macrophages revealed that LPS significantly enhanced miR-155 maturation from its precursors (34). Consistent with other results (14,28), our microarray study also showed that miR-155 was highly induced in RAW 264.7 cells upon *Salmonella* infection. Besides miR-155 induction, we also identified four immensely up-regulated miRNAs (miR-762, miR-2137, miR-3547-5p and miR-6366). The possible roles of these miRNAs induced by *Salmonella* infection are not known yet and remain to be determined. We further validated that *Salmonella* infection resulted in a 28-fold increase of miR-155 level by RT-qPCR. From cell viability and cell death analysis, we established that *Salmonella* infection or miR-155 transfection caused about 30% death of macrophages in 48 h. Furthermore, the pretreatment of anti-miR-155 on macrophages before *Salmonella* infection increased the cell viability up to 20%, indicating that miR-155 may be a key regulator for causing macrophage death by *Salmonella*.

Whether miR-155 is either enhancing or preventing cell death is debatable, depending on the cell type. Evidences for miR-155 anti-apoptotic activity were reported from several cell types, including B lymphocytes (35). When macrophages are tested with various stimuli including cisplatin, *Helicobacter pylori* infection, and LPS, it can be observed that miR-155 enhances macrophage resistance to apoptosis (22,36,37). Targeting miR-155 in acute myelogenous leukemia cell HL-60 lines reveals both cell growth inhibition and apoptosis induction (38). When MG-63 osteosarcoma cells are transfected with anti-miR-155, cell proliferation is significantly reduced, demonstrating that miR-155 deficiency may result in apoptotic cell death (39). However, several reports have contrarily proven that miR-155 could lead to increased apoptosis and cell cytotoxicity, as shown in our study. For example, the

overexpression of miR-155 in DCs results in p27kip1 protein increase and apoptosis in DCs (33). Inhibition of miR-155, which is highly up-regulated in the myocardium of LPS-treated mice, decreases apoptosis in sepsis-induced cardiomyopathy through targeting Pea15a (40).

PARP-1 is known to play two contradictory roles during apoptotic cell death. Its stimulation leads to poly (ADP-ribose) synthesis, whereas caspase activation causes PARP-1 cleavage and inactivation. In apoptotic cells, caspase-3-mediated proteolysis causes DNA fragmentation and nuclear condensation, and PARP-1 cleavage by caspases saves cellular energy levels. Thus, D'Amours *et al* (41) reported a support model in which the inactivation of PARP-1 by caspase cleavage facilitates apoptosis by preventing DNA repair-induced survival and by blocking energy depletion-induced necrosis. However, caspase-3 is not activated during *Salmonella* infection, and PARP-1 remains in its active, uncleaved state, proposing that an unusual caspase-1-dependent mechanism of necrosis is a cause for *Salmonella*-infected macrophage death (5). Since inhibition of PARP-1 fails to protect Jurkat cells from necroptosis, activation of PARP-1 may not be involved in necroptosis (42). Nevertheless, the results showing that Nec-1, a RIP1 specific inhibitor, blocks translocation of apoptosis inducing factor from the mitochondria to the nucleus and inhibits activation of PARP-1 (43,44) suggest that PARP-1 induced necrosis may act downstream of necroptosis (2). Moreover, PARP-1 could be a direct or indirect substrate for RIP kinase1 (RIPK1)- and/or RIPK3-mediated kinase cascade (45), because PARP-1 activity may be regulated by phosphorylation (46). Both RIPK1 and RIPK3 are required for initiation of necroptosis, but it can also occur in the absence of RIPK1 (47,48), suggesting that RIPK3 drives necroptosis whereas RIPK1 is involved in specific cellular contexts.

Here, we found that upshifts of both RIP1 and RIP3 were observed in RAW264.7 macrophages by either *Salmonella* infection or miR-155 transfection, and that pre-treatment of Nec-1 also similarly enhanced the cell viability up to 20% in both *Salmonella*-infected and miR-155-transfected macrophages, suggesting that activation of RIP1 and RIP3 by miR-155 may induce macrophage death by necroptosis. During a previous study on HT-29 cells, a human colorectal adenocarcinoma cell line, RIP3 activation following the induction of necroptosis requires the activity of HSP90 and CDC37 cochaperone complex, and this activation induces upshift of both RIP1 and RIP3 by phosphorylation (49). In addition to RIP1/3 activation by miR-155, we also found that PARP-1 displayed an absence of the mature, uncleaved protein at 24 h in both *Salmonella*-infected and miR-155-transfected cells. This atypical size shift and disappearance of PARP-1 protein was previously reported in L929T cells undergoing TNF-induced necroptosis, and this phenomenon was not due to a general necroptotic destruction of cellular proteins by caspases (50,51). Other reports also showed that the disappearance of PARP-1 signal is an indicator of PARP-1 activation rather than PARP-1 destruction. The disappearance of PARP-1 signal during TNF-induced necroptosis was due to heavy poly ADP-ribosylation, rendering PARP-1 inaccessible for detection by PARP-1 antibodies (3).

Taken together, our results revealed that miR-155 up-regulation in macrophages by *Salmonella* infection causes macrophage death and it may be mediated by both RIP1/3-related necroptosis and PARP-1-mediated necrosis. Differing from our results, however, overexpressing miR-155 in CMPCs revealed that miR-155 attenuated necroptosis by about 40% via targeting RIP1, independent of the activation of Akt pro-survival pathway (20). Hereby, we propose that miR-155 is a potential alternative either for inducing cell death or for improving cell survival by targeting the RIP family. Additionally, it has been observed that it acts with cell-type specificity.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JHL conceived and designed the experiments. YTR, GHJ and SAJ performed the experiments. YTR, EHL, JDS and JHL analyzed the data. YTR wrote the paper. EHL, JDS and JHL revised the manuscript critically for important intellectual content. All of the authors have read and approved the paper.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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