The *Salmonella enterica* serovar Typhimurium sseK3 induce cell apoptosis and enhance glycolysis in macrophages

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Abstract

Background: *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) is an important infectious disease pathogen. Recently, there are increasing researches about the relationship between apoptosis and glycolysis of cells. Previous studies have identified that *S. Typhimurium* secreted effector K3 (*SseK3*) is a novel translated and secreted protein. However, there is no study about the role of *sseK3* in the relationship between apoptosis and glycolysis of cells infected with *S. Typhimurium*. It is unclear whether this protein exerts a significant role in the progress of apoptosis and glycolysis in macrophages. **Results:** The *S. Typhimurium* SL1344 wild-type (WT) group, Δ *sseK3* mutant group and *sseK3*-complemented group were used to infect macrophages and the effects of *sseK3* on apoptosis and glycolysis of macrophages were investigated. The adherence and invasion of Δ *sseK3* mutant group for macrophages were similar to WT group and *sseK3*-complemented group, indicating that *SseK3* did not play an important role in the adherence and invasion of *S. Typhimurium* for macrophages. However, the apoptosis percentage of the Δ *sseK3* mutant group was much lower than WT group and *sseK3*-complemented group using flow cytometry. The caspase-3, caspase-8 and caspase-9 enzyme activity of the Δ *sseK3* mutant group were decreased significantly compared with WT group and *sseK3*-complemented group, which suggested that *sseK3* could improve the activities of caspase-3, caspase-8 and caspase-9 enzyme. We also found that the pyruvic acid level did not significantly change among Δ *sseK3* mutant group, WT group and *sseK3*-complemented group, but the lactic acid level of Δ *sseK3* mutant group was much lower than WT group and *sseK3*-complemented group. The ATP level of Δ *sseK3* mutant group was remarkably higher than WT group and *sseK3*-complemented group. These indicated that the *sseK3* enhanced the level of glycolysis of macrophages infected by *S. Typhimurium*. **Conclusions:** Our data showed that the *sseK3* of *S. Typhimurium* involved in promoting macrophages apoptosis and influencing glycolysis levels of macrophages. These results may give a better clue about the relationship between apoptosis and glycolysis in macrophages induced by *S. Typhimurium sseK3*.

Background

Salmonella enterica serovar Typhimurium (*S. Typhimurium*) is one of the most significant zoonotic pathogens that pose a threat to humans [1]. After being contaminated by *Salmonella*, animal products can be transmitted to humans through the food chain, threatening human health, and potentially leading to deaths [1, 2]. Besides, *S. Typhimurium* can survive and replicate in macrophages. The macrophages or dendritic cells were reported to be able to carry bacteria from the Peyer's patches to adjacent lymph nodes, spleen and liver in mouse models [3].

One of the most essential metabolic pathways in cells is glycolysis [4]. Under anaerobic condition, the pyruvate is eventually converted into lactic acid. However, under aerobic conditions, pyruvic acid enters the tricarboxylic acid cycle (TCA cycle) and eventually is oxidized to CO₂ and H₂O [5]. There are increasing evidences in recent years that the apoptosis is closely related to glycolysis activities [6, 7]. Albina found that the inhibition of the glycolysis with iodoacetate was associated with macrophage apoptosis[8]. Huang found that the genetic silencing of Hypoxia inducible factor-1 α (HIF-1 α) repressed

imiquimod-induced aerobic glycolysis and sensitized cells to imiquimod-induced apoptosis due to faster ATP and Mcl-1 depletion [6]. Kok certificated that Sirtuin 6 modulates hypoxia-induced apoptosis in osteoblasts via inhibition of glycolysis, and the hypoxia-induced apoptosis of osteoblasts is dependent on glycolytic activity [9]. Furthermore, several molecules that function intracellularly as enzymes were involved in glycolysis, which become externalized on the surface of apoptotic cells to be responsible for apoptotic recognition and the triggering of innate apoptotic immunity [10]. Therefore, these studies suggested that the glycolysis is closely related to cell apoptosis.

The immune cells were able to detect the metabolic abnormalities caused by *Salmonella* with the help of inflammatory signals, and glycolysis was essential in *S. Typhimurium* infection with macrophages [11, 12]. Following invasion or phagocytic uptake into the host cell, one of the key virulence determinants is the *Salmonella* pathogenicity island 2 (SPI-2)-encoded type III secretion system 2 (T3SS2), which delivers 28 effector proteins into the host cell [13-15]. Previous studies have identified that *Salmonella* secreted effector K3 (SseK3) is a novel translated and secreted protein of *S. Typhimurium*, which is encoded by *sseK3* gene [16]. SseK3 was a glycosyltransferase, which could transfer an N-acetyl-glucosamine moiety onto the guanidino group of a target arginine, regulating host cell function [17]. The protein belongs to the glycosyltransferase type-A family of glycosyltransferase enzymes and binds the ligand in a metal ion-dependent manner via a DXD motif [17]. SseK3 was co-regulated with the T3SS2 inside host cells and was injected into infected host cells [18]. However, the mechanism of activity of SseK3 during *S. Typhimurium* infection was completely unclear, especially there is no research about *sseK3* affecting macrophages glycolysis.

In this study, we determined that *sseK3* from *S. Typhimurium* induced macrophages apoptosis and glycolysis. Our data showed that the *sseK3* of *S. Typhimurium* could promote macrophage apoptosis and improve glycolysis levels. These results would supply a critical step to provide a better understanding of the relationship between glycolysis and apoptosis in *S. Typhimurium*-infected macrophages.

Results

1. Analysis of adherence and invasion

The invasive and adhesive abilities of the *sseK3* mutant and the complemented strain were similar to those of the WT strain (Supplementary Fig.1A, Fig.1B). There were not remarkable difference among WT, $\Delta sseK3$ mutant and *sseK3*-complemented groups ($P>0.05$). This suggests that *sseK3* does not play an important role in promoting *S. Typhimurium* attachment to and invasion of host macrophages. The same number of bacteria that invade macrophages between different infection groups could ensure the same intracellular load of macrophages to improve the accuracy of later experiments.

2. Analysis of cell apoptosis

The apoptosis assay was performed as previously described [19, 20]. Apoptosis assay showed that the apoptosis percent of $\Delta sseK3$ mutant group was much lower than that of the WT and *sseK3*-

complemented groups. In the $\Delta sseK3$ mutant group, the FITC-Annexin V positive cells were 3.78%, while in the WT group, it was 14.64% (Fig.1), suggesting that SseK3 encoded by *sseK3* plays an important role in the process of inducing macrophages apoptosis by *S. Typhimurium*.

3. Analysis of caspase activity

In the process of apoptosis, the caspase played an essential role [21]. Caspase-3, 8, 9 activities were measured at different time points respectively (Fig.2). The caspase-3, caspase-8 and caspase-9 activities of mock group was much lower than infection groups ($\Delta sseK3$ mutant, WT and *sseK3*-complemented groups), which indicated that the infection groups could stimulate the activities of caspase-3,8,9 in macrophages. However, it could be seen from Fig.2 that the caspase-8 and caspase-9 activities of $\Delta sseK3$ mutant group significantly lower than WT group and *sseK3*-complemented group at 2 h, 4 h, 6 h and 8 h ($***P<0.001$), suggesting that SseK3 could induce the activation of caspase-8 and caspase-9. As for caspase-3, there were no significant difference among $\Delta sseK3$ mutant group ($P>0.05$), WT group and *sseK3*-complemented group at 2 h. But significant difference among infection groups was observed at 4 h, 6 h and 8 h ($***P<0.001$), the caspase-3 activity of $\Delta sseK3$ mutant group significantly lower than WT group and *sseK3*-complemented group. These results illustrated that SseK3 served a pivotal role in the process of inducing macrophage apoptosis by *S. Typhimurium*.

4. Analysis of glycolysis

The analysis of glycolysis was performed as previously described [22-24]. To determine the SseK3 whether can affect the glycolysis of macrophages infected by *S. Typhimurium*, the level of pyruvic acid, lactic acid and ATP were detected respectively (Fig.3). The results showed that the level of pyruvic acid was no significant difference at 2 h, 4 h, 6 h and 8 h ($P>0.05$) among each group. However, as for lactic acid, there was significant difference between mock group and infection group at 2 h, 4 h, 6 h and 8 h ($***P<0.001$). The glycolysis of macrophages of infection group were remarkably increased compared with mock group, and the lactic acid level of $\Delta sseK3$ mutant group was significantly lower than WT and *sseK3*-complemented groups at 4 h, 6 h and 8 h ($***P<0.001$). The investigations of glycolysis in macrophages suggested SseK3 encoded by *sseK3* could boost the lactic acid of macrophages. We also found that the level of ATP in infection groups were significantly lower than in mock group at 4 h, 6 h and 8 h ($***P<0.001$), and the level of ATP in the $\Delta sseK3$ mutant group was much higher than in WT and *sseK3*-complemented groups at 4 h, 6 h and 8 h ($***P<0.001$). These results illustrated that SseK3 encoded by *sseK3* could improve the glycolysis of macrophages through lactic acid and the ATP level changing.

Discussion

Many secreted proteins of *Salmonella* exert different virulence functions in the process of survival and replication in cells [25]. T3SS2 helps *Salmonella* transport virulence (effector) proteins in SCVs into host cells [26]. *S. Typhimurium* possesses the SseK family of proteins, including SseK1, SseK2 and SseK3, which are T3SS effectors [1, 27]. SseK3 is an important protein during *Salmonella* infection of cells and

plays a pivotal role in the natural host immune process [16, 18]. Multiple secretory proteins of *S. Typhimurium* exerted enormous stress during the period of apoptosis of cells and related to cell apoptosis [25, 26, 28, 29]. There were several studies about the function of SseK3. Joshua found that SseK3 target death domain proteins in the TNF and TRAIL signaling pathways and employ the glycosyltransferase effectors to antagonise different components of death receptor signaling [30]. Yang found that SseK3 could bind an E3 ubiquitin ligase (TRIM32) and influence NF- κ B activity [16]. Günster found that SseK3 resulted in weak GlcNAcylation of TNFR1-associated death domain protein (TRADD) and inhibited TNF- α -induced apoptosis in *Salmonella*-infected macrophages. These researches were contradictory [26]. Nonetheless, there was no research about the role of SseK3 in the relationship between apoptosis and glycolysis in *Salmonella*-infected macrophages. Thus, this research is the first to determine the effect of SseK3 encoded by *sseK3* of *S. Typhimurium* on macrophage glycolysis while it induced the macrophage apoptosis.

Santo found that early sipB-dependent and delayed sipB-independent mechanisms could cause the apoptosis induced by *S. Typhimurium* [31]. Kasinskas investigated that the lacking ribose chemoreceptors of *S. Typhimurium* localized in tumor quiescence and induced apoptosis [32]. Besides, there had shown that the cAMP receptor protein of *S. Typhimurium* could induce the apoptosis of macrophages [33]. Therefore, in order to determine the relationship between *S. Typhimurium sseK3* and macrophages apoptosis, we used the WT, $\Delta sseK3$ mutant and *sseK3*-complemented strains to infect the macrophages. In our study, we found that the adherence percentage and invasion percentage of $\Delta sseK3$ mutant group were similar to WT group and *sseK3*-complemented group during the infecting period ($P>0.05$), which ensured that the WT, $\Delta sseK3$ mutant and *sseK3*-complemented strains had the same level going through into the macrophages. Subsequently, the apoptosis in different infection groups were detected by the assay of flow cytometry. The results showed that the apoptosis percentage of $\Delta sseK3$ mutant group (3.78%) was much lower than WT group (14.64%) and *sseK3*-complemented group (11.81%). These indicated that deletion of the *sseK3* gene significantly affected the cell apoptosis.

The caspases activities were further to be detected. The activation of caspases exerts essential role on the cells apoptosis induced by the loss of mitochondria membrane [34]. There had shown that the SopB of *Salmonella* could protect host cell from apoptosis induced by caspase-3 [35]. The activation of caspase-3 and caspase-9 showed that the apoptosis was activated in the macrophages infected by *Escherichia coli* bacteria [36]. Besides, the caspase-8 influenced the synthesis of pro-IL-1 β and was essential for the apoptosis of cells induced by *Salmonella* [37]. As these previously researches, we investigated the caspase-3, caspase-8 and caspase-9 activity during the apoptosis of macrophages infected by *S. Typhimurium*. We found that caspase-3, caspase-8 and caspase-9 activity were remarkably increased in infection groups compared with mock group at 2 h, 4 h, 6 h and 8 h ($***P<0.001$), indicating that the apoptosis were activated in the macrophages apoptosis. Furthermore, the activity of caspase-3, caspase-8 and caspase-9 in $\Delta sseK3$ mutant group were much lower than in WT group and *sseK3*-complemented group, which suggested that SseK3 may be a pivotal component for *S. Typhimurium* activating apoptosis in macrophages.

There were increasing evidences that the apoptosis and glycolysis had closely relationship. Previously, Comin-Anduix found that fermented wheat germ extract (FWGE) inhibited the glycolysis and induced the apoptosis of tumor cells [38]. Jeong found that the modification of glycolysis could change the sensitivity of apoptosis through mitochondria pathway [39]. Recently, There has shown that the glycolysis could be inhibited and the cell apoptosis was further blocked [40]. Li found that methyl jasmonate could inhibit the glycolysis and induce apoptosis of tumor cells [41]. Sumi found that propofol could improve glycolysis of cells and lead to the apoptosis of cells [42]. The pyruvic acid, lactic acid and ATP were important components in the process of cell glycolysis. There have various metabolic pathway to produce pyruvic acid [43]. Pyruvic acid could enter the TCA cycle to be completely oxidized in aerobic condition, whereas it could become lactic acid under anaerobic oxygen condition [44, 45]. HIF-1 α could prohibit the production of ATP and thus regulate the glycolysis of mouse granulosa cells [46]. Thus, WT, Δ *sseK3* mutant and *sseK3*-complemented strains were used to explore the ability of macrophage glycolysis through detecting the levels of pyruvic acid, lactic acid and ATP. We found that the levels of intracellular pyruvic acid did not significantly change among all groups from 2 h to 8 h ($P>0.05$), but the lactic acid and ATP were different, respectively, among these groups. In the beginning stage, compared with WT and *sseK3*-complemented groups, the level of lactic acid of microphages infected by Δ *SseK3* mutant strain did not significantly change at 2 h ($P>0.05$), while there was significant difference between WT and Δ *sseK3* mutant group at 4 h ($***P<0.001$), 6 h ($***P<0.001$) and 8 h ($***P<0.001$), suggesting the deletion of *sseK3* may reduce the glycolysis of macrophages induced by *S. Typhimurium* and SseK3 encoded by *sseK3* may enhance the glycolysis. Furthermore, the levels of lactic acid of WT, Δ *sseK3* mutant and *sseK3*-complemented strains were significantly larger than mock group from 2 h to 8 h ($***P<0.001$), which illustrated that the anaerobic pathway of macrophages was enhanced among infection groups. Besides, as for ATP, there was no significant difference among all groups at 2 h, while the ATP levels of macrophages of Δ *sseK3* mutant group were remarkably larger than that of WT and *sseK3*-complemented groups at 4 h ($***P<0.001$), 6 h ($**P<0.01$) and 8 h ($***P<0.001$), indicating that glycolysis level of WT and *sseK3*-complemented groups were lower than Δ *sseK3* mutant group and SseK3 encoded by *sseK3* may enhance the glycolysis. Moreover, the ATP levels of macrophages of mock group were significantly higher than infections groups at 4 h ($***P<0.001$), 6 h ($***P<0.01$) and 8 h ($***P<0.001$), respectively, which suggested that the mock group may mainly undergoing the aerobic metabolism, for the prominent reason may be that the ATP produced by glycolysis is much lower than ATP produced by aerobic oxidation pathway. Therefore, the deletion of *sseK3* decreased the glycolysis of Δ *sseK3* group compared with WT group and SseK3 could improve the level glycolysis of macrophages infected by *S. Typhimurium*. In a word, the SseK3 of *S. Typhimurium* could induce apoptosis and enhance the glycolysis of macrophages. The conclusion was consistent with Sumi's [42] and Ding's [33] results that both glycolysis and apoptosis of cells could be improved. It was contradict with Li's [41] and Comin-Anduix's [38] results that glycolysis of cells was inhibited and apoptosis of cells was enhanced. There may be the mainly reason is the type of cell. Increased glycolysis is the main source of energy supply in cancer cells that use this metabolic pathway for ATP generation. Altered energy metabolism is a biochemical fingerprint of cancer cells that represents one of the "hallmarks of cancer" [47]. And recently, Ma showed that the Rosmarinic acid exhibits an apoptosis-inducing effect of HepG2 cells, mainly related

to inactivation of the glycolytic pathway of cells [48]. However, macrophages have been shown to demonstrate a high level of plasticity, with the ability to undergo dynamic transition between M1 and M2 polarized phenotypes [49]. Lipopolysaccharide (LPS) of bacteria could induce RAW264.7 macrophages into the classical proinflammatory injury phenotype (M1) [50]. Activation of inflammatory response could lead to the apoptosis of cells by mediating the inactivation of the PI3K/Akt/GSK-3 β signaling pathway [51]. Moreover, the proinflammatory stimulus, LPS, suppresses myelocytomatosis viral oncogene (Myc) expression and cell proliferation and engages a HIF1 α -dependent transcriptional program that is responsible for heightened glycolysis [52]. We speculate that the Ssek3 of *S. Typhimurium* might activate the M1 of macrophages and further wake up HIF1 α , which enhanced the glycolysis and finally resulted in the apoptosis of cells. There may be other different signaling pathways involved in the apoptosis and glycolysis of macrophages effected by Ssek3, which need more experiments to explain this problem. In our future work, we will further clarify the relationship between glycolysis and apoptosis.

Conclusions

In summary, from what has been discussed above, we could conclude that the SseK3 of *S. Typhimurium* could induce cell apoptosis and boost glycolysis in macrophages. This finding may help to illustrate the mechanism that *S. Typhimurium* induces macrophages apoptosis and provide a better understanding of the possible relationship between macrophage apoptosis and glycolysis induced by SseK3.

Methods

Bacterial Strains, cells and culture

S. Typhimurium SL1344, the deletion of *sseK3* *Typhimurium* SL1344 (Δ *sseK3* mutant) and its complemented (*sseK3*-complemented) were used in this study and conserved in the laboratory. The Δ *sseK3* mutant was constructed through use of counterselectable suicide vectors. The *sseK3* gene was cloned into the pBR322 plasmid for complementation studies. Macrophages RAW264.7 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA), and cells were cultured in Dulbecco's Modified Eagle Medium (DMEM)/high-glucose medium (HyClone, USA) containing 10% fetal calf serum (FCS) in an incubator at 37 °C and 5% CO₂.

Adherence and invasion assay

Adhesion and invasion of RAW264.7 cells was assessed as previously described [53, 54]. A 24-well cell culture plate was inoculated with 1 $\times$ 10⁵ RAW264.7 cells per well and incubated for 16 h. WT, Δ *sseK3* mutant and *sseK3*-complemented strains were coincubated with RAW264.7 cells at a multiplicity of infection (MOI) of 100:1, with three replicate wells per strain. To allow the bacteria to fully contact the RAW264.7 cells, the plates were centrifuged and incubated with 5% CO₂ for 2 h at 37 °C. For the adherence assay, the supernatants were aspirated, and the cells were washed three times with PBS. Subsequently, the cells were digested with 0.25% trypsin and plated in a gradient dilution and counted.

For the invasion assay, the supernatants were aspirated, the cells were washed three times with PBS, and gentamicin-containing medium (100 µg/mL) was added and incubated at 37 °C with 5% CO₂. After incubation, the supernatants were aspirated, and the cells were washed three times with PBS. Subsequently, the cells were lysed using 0.1% Triton X-100 and plated with a gradient dilution and counted.

Flow cytometry assay

A 6-well cell culture plate was inoculated with 1×10⁶ RAW264.7 cells per well and incubated for 16 h. WT, Δ *sseK3* mutant and *sseK3*-complemented strains were coincubated with RAW264.7 cells at a multiplicity of infection (MOI) of 100:1, with three replicate wells per strain. To allow the bacteria to fully contact the RAW264.7 cells, the plates were centrifuged with 1000 rpm/min. Then, gentamicin-containing medium (100 µg/mL) was added and incubated at 37 °C with 5% CO₂. After incubation, the supernatants were aspirated, and the cells were washed three times with PBS. The percent of cells undergoing apoptosis was detected by flow cytometry using Annexin V-FITC/PI apoptosis detection kit (KeyGEN BioTECH Jiangsu China). The cells of infected and mock groups were digested with 0.25% trypsin and washed three times with ice-cold phosphate buffered saline (PBS) and suspended in Binding Buffer with 500 µL, followed by adding 5 µL Annexin V-FITC and 5 µL Propidium Iodide (PI). Then the solution was placed in the dark room for 15 min at room temperature followed by immediately analysis using flow cytometry (Beckman Coulter, Inc., Fullerton, CA, US).

Caspase-3, caspase-8 and caspase-9 activity assay

The activity of caspase-3, caspase-8, and caspase-9 was measured by Caspase-3 Assay Kit, Caspase-8 Assay Kit, Caspase-9 Assay Kit (Beyotime, Shanghai, China), respectively. The 6-well cell culture plate was inoculated with 1×10⁶ RAW264.7 cells per well and incubated for 16 h. WT, Δ *sseK3* mutant and *sseK3*-complemented strains were co-incubated with RAW264.7 cells at a MOI of 100:1, with three replicate wells per strain. The plates were centrifuged with 1000 rpm/min and gentamicin-containing medium (100 µg/mL) was added and incubated at 37 °C with 5% CO₂. After that, the supernatants were aspirated, and the cells were washed three times with PBS. Subsequently, the cells of infected and mock groups were digested by trypsinization without EDTA and washed three times with ice-cold lysis buffer 3 times, followed by adding 100µL lysis buffer on ice. After incubated for 15 minutes, the concentration of protein was detected using the Bradford protein assay kit (Beyotime, Shanghai, China). Subsequently, after the cell lysates were incubated with Ac-DEVD-pNA for 4 h at 37°C, the samples were read at 405 nm.

Glycolysis level assay

The glycolysis levels were measured using pyruvic acid analysis kit, lactic acid analysis kit and ATP analysis kit respectively, which were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). WT, Δ *sseK3* mutant and *sseK3*-complemented groups were treated as above similar methods. After incubation with gentamicin-containing medium (100 µg/mL), the supernatants were aspirated, and

the cells were washed three times with PBS. Then they were processed based on manufacturer's instruction at 2 h, 4 h, 6 h and 8 h, respectively. The concentration of protein each group was detected using the Bradford protein assay kit (Beyotime, Shanghai, China). Finally, the absorbance values of pyruvic acid analysis kit, lactic acid analysis kit and ATP analysis kit were read at 505 nm, 530 nm and 636 nm in a microplate spectrophotometer, respectively.

Statistical analysis

The data are presented as the mean $\pm$ standard deviation (SD) of three independent experiments, as based on triplicates assays. Two-way analysis of variance (ANOVA) with a post-hoc test (Bonferroni's multiple-comparison test) was used to compare and assess significance of the differences among all groups. The value of $*P<0.05$, $**P<0.01$ or $***P<0.001$ was considered significant.

Declarations

Ethics approval and consent to participate

Not applicable.

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Availability of data and materials

The data generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Authors' contributions

CY and FD contributed equally to this work. They performed the experiments, analyzed the experimental results and wrote manuscript. FD, CY, and CZ conceived of and designed the experiments. FD, CY, CZ, and YL analyzed experiment performance. CL, LH, and XC analyzed and interpreted all of the data. YL, CL, LH,

and XZ performed the statistical analyse. CY, CZ, YL, and XC did the supervised study. All authors read and approved the manuscript.

Competing interests

The authors declare that they have no competing of interests.

References

1. Zhang X, He L, Zhang C, Yu C, Yang Y, Jia Y, Cheng X, Li Y, Liao C, Li J *et al*: **The impact of sseK2 deletion on Salmonella enterica serovar typhimurium virulence in vivo and in vitro.** *BMC Microbiol* 2019, **19**(1):182.
2. Kalaba V, Golić B, Ž Sladojević, Kalaba D: **Incidence of Salmonella Infantis in poultry meat and products and the resistance of isolates to antimicrobials.** *IOP Conference Series: Earth and Environmental Science* 2017, **85**:012082.
3. Vazquez-Torres A, Jones-Carson J, Bäumler AJ, Falkow S, Valdivia R, Brown W, L M, Berggren R, Parks W T, Fang F C: **Extraintestinal dissemination of Salmonella by CD18-expressing phagocytes.** *Nature* 1999, **401**(6755):804-808.
4. Planqué R, Bruggeman FJ, Teusink B, Hulshof J: **Understanding bistability in yeast glycolysis using general properties of metabolic pathways.** *Math Biosci* 2014, **255**(1):33-42.
5. Phypers B, Pierce JT: **Lactate physiology in health and disease.** *Contin Educ Anaesth Crit Care Pa* 2006, **6**(3):128-132.
6. Shi-Wei H, Jun-Kai K, Chun-Ying W, Sin-Ting W, Hsin-Chen L, Shu-Mei L, Yi-Ju C, Jeng-Jer S: **Targeting aerobic glycolysis and HIF-1alpha expression enhance imiquimod-induced apoptosis in cancer cells.** *Oncotarget* 2014, **5**(5):1363-1381.
7. Liu X, Zhao H, Jin Q, You W, Cheng H, Liu Y, Song E, Liu G, Tan X, Zhang X: **Resveratrol induces apoptosis and inhibits adipogenesis by stimulating the SIRT1-AMPKα-FOXO1 signalling pathway in bovine intramuscular adipocytes.** *Mol Cell Biochem* 2018, **439**(1-2):213-223.
8. Albina JE, Cui S, Mateo RB, JS R: **Nitric oxide-mediated apoptosis in murine peritoneal macrophages.** *J Immunol* 1993, **150**:5080-5085.
9. Kok SH, Hou KL, Hong CY, Chao LH, Lai HH, Wang HW, Yang H, Shun CT, Wang JS, Lin SK: **Sirtuin 6 Modulates Hypoxia-induced Apoptosis inOsteoblasts via Inhibition of Glycolysis: ImplicationforPathogenesis of Periapical Lesions.** *J Endodont* 2015, **41**(10):1631-1637.
10. Ucker DS: **Exploiting death: apoptotic immunity in microbial pathogenesis.** *Cell Death & Differentiation* 2016, **23**:990-996.
11. Sanman LE, Qian Y, Eisele NA, Ng TM, Wa VDL, Monack DM, Weerapana E, Bogyo M: **Disruption of glycolytic flux is a signal for inflammasome signaling and pyroptotic cell death.** *Elife* 2016, **5**:e13663.

12. Bowden SD, Gary R, Hinton JCD, Arthur T: **Glucose and glycolysis are required for the successful infection of macrophages and mice by *Salmonella enterica* serovar typhimurium.** *Infect Immun* 2009, **77**(7):3117-3126.
13. Jennings E, Tlm T, Holden DW: ***Salmonella* SPI-2 Type III Secretion System Effectors: Molecular Mechanisms And Physiological Consequences.** *Cell Host Microbe* 2017, **22**(2):217-231.
14. Ochman H, Soncini F C, Solomon F, Groisman E A: **Identification of a pathogenicity island required for *Salmonella* survival in host cells.** *Proc Natl Acad Sci U S A* 1996, **93**(15):7800-7804.
15. Hensel M, Shea JE, Gleeson C, Jones MD, Dalton E, Holden DW: **Simultaneous identification of bacterial virulence genes by negative selection.** *Science* 1995, **269**(5222):400-403.
16. Yang Z, Soderholm A, Lung TWF, Giogha C, Hill MM, Brown NF, Hartland E, Teasdale RD: **SseK3 Is a *Salmonella* Effector That Binds TRIM32 and Modulates the Host's NF- κ B Signalling Activity.** *PLoS One* 2015, **10**(9):e0138529.
17. Esposito D, Günster RA, Martino L, El KO, Wagner A, Thurston T, Rittinger K: **Structural basis for the glycosyltransferase activity of the *Salmonella* effector SseK3.** *J Biol Chem* 2018, **293**(14):5064-5078.
18. Brown NF, Coombes BK, Bishop JL, Wickham ME, Lowden MJ, Gal-Mor O, Goode DL, Boyle EC, Sanderson KL, Finlay BB: ***Salmonella* Phage ST64B Encodes a Member of the SseK/NleB Effector Family.** *PLoS One* 2011, **6**(3):e17824.
19. Zhang H, Yong H, Qian D, Luo X, Liang Z, Zhao X, Tong D: **Porcine parvovirus infection induces apoptosis in PK-15 cells through activation of p53 and mitochondria-mediated pathway.** *Biochem Bioph Res Co* 2015, **456**(2):649-655.
20. He J, Wang C, Sun Y, Lu B, Cui J, Dong N, Zhang M, Liu Y, Yu B: **Exendin-4 protects bone marrow-derived mesenchymal stem cells against oxygen/glucose and serum deprivation-induced apoptosis through the activation of the cAMP/PKA signaling pathway and the attenuation of ER stress.** *Int J Mol Med* 2016, **37**(4):889-900.
21. Ding L, Huang Y, Du Q, Dong F, Zhao X, Zhang W, Xu X, Tong D: **TGEV nucleocapsid protein induces cell cycle arrest and apoptosis through activation of p53 signaling.** *Biochem Bioph Res Co* 2014, **445**(2):497-503.
22. Wang G, Xu Z, Wang C, Yao F, Li J, Chen C, Sun S: **Differential phosphofructokinase-1 isoenzyme patterns associated with glycolytic efficiency in human breast cancer and paracancer tissues.** *Oncol Lett* 2013, **6**(6):1701-1706.
23. Chen D, Liao Y, Xu Q, Li Y, Wu W, Wu J, Liao W, Xu D, Liu Y, Wu P: **Persistence of systolic and diastolic regional dysfunction after brief episodes of myocardial ischemia evaluated with velocity vector imaging.** *Int j Cardiol* 2013, **167**(3):987-994.
24. Jia Y, Cong R, Li R, Yang X, Sun Q, Parvizi N, Zhao R: **Maternal low-protein diet induces gender-dependent changes in epigenetic regulation of the glucose-6-phosphatase gene in newborn piglet liver.** *J Nutr* 2012, **142**(9):1659-1665.
25. Ibarra JA, Steele-Mortimer O: ***Salmonella* – the ultimate insider. *Salmonella* virulence factors that modulate intracellular survival.** *Cell Microbiol* 2010, **11**(11):1579-1586.

26. Günster RA, Matthews SA, Holden DW, Thurston TL: **SseK1 and SseK3 T3SS effectors inhibit NF-κB signalling and necroptotic cell death in Salmonella-infected macrophages.** *Infect Immun* 2017, **85**(3):e00010-00017.
27. Kujat Choy SL, Boyle EC, Gal-Mor O, Goode DL, Valdez Y, Vallance BA, Finlay BB: **SseK1 and SseK2 are novel translocated proteins of Salmonella enterica serovar Typhimurium.** *Infect Immun* 2004, **72**(9):5115-5125.
28. McIntosh A, Meikle LM, Ormsby MJ, McCormick BA, Christie JM, Brewer JM, Roberts M, Wall DM: **SipA Activation of Caspase-3 Is a Decisive Mediator of Host Cell Survival at Early Stages of Salmonella enterica Serovar Typhimurium Infection.** *Infect Immun* 2017, **85**(9):IAI.00393-00317.
29. He P, Wu S, Chu Y, Yang Y, Li Y, Huang R: **Salmonella enterica serovar Typhi plasmid pR ST98 enhances intracellular bacterial growth and S. typhi -induced macrophage cell death by suppressing autophagy.** *Braz J Infect Dis* 2012, **16**(3):262-266.
30. Newson JPM, Scott NE, Chung IYW, Lung TWF, Giogha C, Gan J, Wang N, Strugnell RA, Brown NF, Cygler M *et al*: **Salmonella Effectors SseK1 and SseK3 Target Death Domain Proteins in the TNF and TRAIL Signaling Pathways.** *Molecular & cellular proteomics : MCP* 2019, **18**(6):1138-1156.
31. Santos RL, Tsolis RM, Bäumlér AJ, Smith R, Adams LG: **Salmonella enterica serovar typhimurium induces cell death in bovine monocyte-derived macrophages by early sipB-dependent and delayed sipB-independent mechanisms.** *Infect Immun* 2001, **69**(4):2293-2301.
32. RW K, NS F: **Salmonella typhimurium lacking ribose chemoreceptors localize in tumor quiescence and induce apoptosis.** *Cancer Res* 2007, **67**(7):3201-3209.
33. Ding K, Zhang C, Li J, Chen S, Liao C, Cheng X, Yu C, Yu Z, Jia Y: **cAMP Receptor Protein of Salmonella enterica Serovar Typhimurium Modulate Glycolysis in Macrophages to Induce Cell Apoptosis.** *Curr Microbiol* 2019, **76**(1):1-6.
34. Wei Y, Kong B, Song K, Qu X, Jin Q, Yang Q: **Involvement of mitochondria-caspase pathway in Hemoporphin-mediated cell death.** *Photochem Photobiol* 2010, **83**(6):1319-1324.
35. Knodler LA, Brett F, Olivia SM: **The Salmonella effector protein SopB protects epithelial cells from apoptosis by sustained activation of Akt.** *J Biol Chem* 2005, **280**(10):9058-9064.
36. Hans HC, Christine F, Hermann W, Georg HC: **Caspase-9/-3 activation and apoptosis are induced in mouse macrophages upon ingestion and digestion of Escherichia coli bacteria.** *J Immunol* 2002, **169**(6):3172-3179.
37. Ming MS, Panagiotis T, Lee H, Monie TP, Fitzgerald KA, Bryant CE: **Salmonella infection induces recruitment of Caspase-8 to the inflammasome to modulate IL-1β production.** *J Immunol* 2013, **191**(10):5239-5246.
38. Comin-Anduix B, Boros LG, Marin S, Boren J, Callol-Massot C, Centelles JJ, Torres JL, Agell N, Bassilian S, Cascante M: **Fermented wheat germ extract inhibits glycolysis/pentose cycle enzymes and induces apoptosis through poly(ADP-ribose) polymerase activation in Jurkat T-cell leukemia tumor cells.** *J Biol Chem* 2002, **277**(48):46408-46414.

39. Jeong DW, Kim TS, Cho IT, Kim IY: **Modification of glycolysis affects cell sensitivity to apoptosis induced by oxidative stress and mediated by mitochondria.** *Biochem Bioph Res Co* 2004, **313**(4):984-991.
40. Zhu Y, Ramos da Silva S, He M, Liang Q, Lu C, Feng P, Jung JU, Gao SJ: **An Oncogenic Virus Promotes Cell Survival and Cellular Transformation by Suppressing Glycolysis.** *PLoS Pathog* 2016, **12**(5):e1005648.
41. Li J, Chen K, Wang F, Dai W, Li S, Feng J, Wu L, Liu T, Xu S, Xia Y: **Methyl jasmonate leads to necrosis and apoptosis in hepatocellular carcinoma cells via inhibition of glycolysis and represses tumor growth in mice.** *Oncotarget* 2017, **8**(28):45965-45980.
42. Sumi C, Okamoto A, Tanaka H, Nishi K, Kusunoki M, Shoji T, Uba T, Matsuo Y, Adachi T, Hayashi JI: **Propofol induces a metabolic switch to glycolysis and cell death in a mitochondrial electron transport chain-dependent manner.** *PLoS One* 2018, **13**(2):e0192796.
43. Merlin TY, Leatham MP, Allen JH, Laux DC, Tyrrell C, Cohen PS: **Role of gluconeogenesis and the tricarboxylic acid cycle in the virulence of Salmonella enterica serovar Typhimurium in BALB/c mice.** *Infect Immun* 2006, **74**(2):1130-1140.
44. Lecarpentier Y, Claes V, Vallée A, Hébert JL: **Thermodynamics in cancers: opposing interactions between PPAR gamma and the canonical WNT/beta-catenin pathway.** *Clin Transl Med*, 2017, **6**(14):1-11.
45. Mickelson MN: **Glucose Degradation, MolarGrowthYields, and Evidence forOxidative Phosphorylation in Streptococcus agalactiae.** *J Bacteriol* 1972, **109**(1):96-105.
46. Zhou J, Li C, Yao W, Alsiddig MC, Huo L, Liu H, Miao YL: **Hypoxia-inducible factor-1 α -dependent autophagy plays a role in glycolysis switch in mouse granulosa cells.** *Biol Reprod* 2018, **99**(2):308-318.
47. Gill K, Fernandes P, O'Donovan T, McKenna S, Doddakula K, Power D, Soden D, Forde P: **Glycolysis inhibition as a cancer treatment and its role in an anti-tumour immune response.** *Biochimica et biophysica acta* 2016, **1866**(1):87-105.
48. Ma Z, Yan H, Wang Y, Yang Y, Li X, Shi A, Jing-Wen X, Yu-Bao L, Li L, Wang X: **Proteomics analysis demonstrating rosmarinic acid suppresses cell growth by blocking the glycolytic pathway in human HepG2 cells.** *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 2018, **105**:334-349.
49. Lin Y, Lee B, Liu P, Wei L: **Receptor-Interacting Protein 140 Orchestrates the Dynamics of Macrophage M1/M2 Polarization.** *Journal of innate immunity* 2016, **8**(1):97-107.
50. Ye Y, Xu Y, Lai Y, He W, Li Y, Wang R, Luo X, Chen R, Chen T: **Long non-coding RNA cox-2 prevents immune evasion and metastasis of hepatocellular carcinoma by altering M1/M2 macrophage polarization.** *J Cell Biochem* 2018, **119**(3):2951-2963.
51. Shi Y, Guo X, Zhang J, Zhou H, Sun B, Feng J: **DNA binding protein HMGB1 secreted by activated microglia promotes the apoptosis of hippocampal neurons in diabetes complicated with OSA.** *Brain, behavior, and immunity* 2018, **73**:482-492.

52. Liu L, Lu Y, Martinez J, Bi Y, Lian G, Wang T, Milasta S, Wang J, Yang M, Liu G *et al*: **Proinflammatory signal suppresses proliferation and shifts macrophage metabolism from Myc-dependent to HIF1 α -dependent.** *Proceedings of the National Academy of Sciences of the United States of America* 2016, **113**(6):1564-1569.
53. Shippy DC, Eakley NM, Bochsler PN, Fadl AA: **Biological and virulence characteristics of *Salmonella enterica* serovar Typhimurium following deletion of glucose-inhibited division (gidA) gene.** *Microb Pathog* 2011, **50**(6):303-313.
54. Yang Y, Yu C, Ding K, Zhang C, Liao C, Jia Y, Li J, Cheng X: **Role of the sseK1 gene in the pathogenicity of *Salmonella enterica* serovar enteritidis in vitro and in vivo.** *Microb Pathog* 2018, **117**(117): 270-275.

Additional File Legends

Supplementary Fig. 1 Adherence and invasion assays for WT, $\Delta sseK3$ mutant and *sseK3*-complemented strains to RAW264.7 cells. There were no significant differences using Bonferroni's multiple-comparison test among all groups ($P>0.05$).

Figures

Figure 1

Flow cytometric analysis of apoptosis in RAW264.1 macrophages infected with WT, Δ sseK3 mutant and its complemented strains. RAW264.1 macrophages were incubated for 4 h with WT, Δ sseK3 mutant and its complemented strains, respectively. And then apoptosis was assessed by using Annexin V-FITC/PI double staining. (A) Representative dot plots of Annexin V-FITC /PI staining. Upper left quadrant Q1 contains the necrotic(Annexin V-FITC-/PI+) population. The upper right quadrant Q2 contains the late apoptotic (Annexin V-FITC+/PI+) population and lower right quadrant Q3 contains the early apoptotic (Annexin V-FITC+/PI-) population. The lower left quadrant Q4 contains the vital (double negative) population. (B)The percentages of the apoptosis of cells exposed to different bacteria were determined by flow cytometric analysis. After 4 h of incubation, the apoptosis rate of Δ sseK3 mutant group was significantly lower than that of the WT and sseK3-complemented groups ($***P<0.001$). Data pooled from three independent experiments show the percentage of apoptotic cells. The error bars indicate the SD from three different experiments.

Figure 2

The activity of caspase-3, caspase-8 and caspase-9 in RAW264.7 cells of all groups. The activity of caspase-3, -8, and -9 was measured by colorimetric assay and analyzed using two-way ANOVA with Bonferroni's multiple-comparison test (* $P<0.05$,** $P<0.01$,*** $P<0.001$). Results are means $\pm$ SD.

Figure 3

The glycolysis of RAW264.7 infected by WT, $\Delta sseK3$ mutant and sseK3-complemented strains. The content of pyruvic acid, lactic acid and ATP were measured by colorimetric assay and analyzed using two-way ANOVA with Bonferroni's multiple-comparison test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Results are means $\pm$ SD.

Supplementary Files

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