(Form 1)

Kyoto University	Doctor of Philosophy in Life Sciences	Name	Zuo Wenjie
Thesis Title	Intracellular dsRNA induces apoptotic cell death via the synergistic activation of PKR and TLR3		

(Thesis Summary)

Upon viral infection, host cells induce antiviral responses and regulate viral growth. The well-known response is production of antiviral cytokine, termed interferon, which promotes antiviral state and directly suppresses viral replication at multiple steps. Also, host cells actively induce apoptosis, which plays an important role in suppressing viral spread in multicellular organism.

This study delineated the mechanism of cell death induced by viral mimetics of double-stranded RNA (dsRNA), poly I:C. The applicant analyzed the roles of dsRNA sensor molecules Retinoic Acid Inducible Gene -I (RIG-I), Protein Kinase activated by RNA (PKR), and Toll-Like Receptor 3 (TLR3) by utilizing microinjection technique to cultured cells and live cell imaging. The analysis revealed that dsRNA stimulates RIG-I to induce efficient interferon production, which induces cell death with slow kinetics in a fraction of cells. In contrast, injection of long dsRNA into cytoplasm induces rapid and efficient cell death. By analyzing gene knockout cell lines, it was revealed that PKR and TLR3 play critical roles. Notably, signaling by TLR3/TIR-domain-containing adapter-inducing interferon-β (TRIF) induced caspase 8 activation and apoptosis. However, TLR3/TRIF signaling alone did not explain the efficient cell death induced by injection of long dsRNA. The cytoplasmic dsRNA sensor, PKR induces a stress response in host cells to halt protein syntheses. Finally, this study demonstrated that PKR promotes apoptosis by TLR3/TRIF/caspase 8 by down-regulating cellular FLICE-like proteins (cFLIPs), the apoptosis inhibitors.

In summary, this study reveals that PKR and TLR3 are both essential for inducing the viral dsRNA-induced apoptosis of infected cells and contribute to the arrest of viral production as a host antiviral response.

(Form 2)

(Thesis Evaluation Summary)

To elucidate molecular mechanism of viral dsRNA-induced cell death, the applicant took strategy to analyze live imaging of cells, which were microinjected with purified dsRNA or proteins. To monitor the activation of antiviral innate immune response in living cells, a cell line expressing fluorescently labeled IRF-3, whose activation is sensed by its nuclear translocation. Other morphological changes linked to cell death were also monitored. Injection of short 5'-ppp dsRNA, a sole ligand of RIG-I, rapidly induced activation of IRF-3 and resulted in induction of interferon. A portion of cells underwent slow death, however, it was indirectly induced by the action of secreted interferon. On the other hand, injection of long dsRNA induced rapid IRF-3 activation and robust caspase mediated apoptosis. Analyzes of knockout HeLa cells (genes for RIG-I, MDA5, IPS-1, IRF-3, IFNAR1, PKR, TRIF, PKR/TRIF) revealed that dsRNA sensors, endosomal TLR3, and cytoplasmic PKR, cooperates the promotion of strong cell death. It has been reported that TLR3/TRIF signaling activates caspase 8, however selective activation of TLR3/TRIF pathway was insufficient for induction of strong cell death induced by injection of dsRNA. The applicant's biochemical analyses delineated that TLR3/TRIF signal induces anti-apoptotic cFLIPs and PKR suppresses its induction through translational arrest. Thus, TLR3/TRIF and PKR cooperate death signaling by distinct mechanisms. Further, blocking of dsRNA-mediated apoptosis increased production of infectious viral particles, indicating that the observed apoptosis is critical as a part of antiviral responses of the host.

This thesis substantiates the candidate's extensive and wide knowledge of life sciences, demonstrates expert research capability in the field of immunology, and presents new discoveries and concepts that contribute to the profound understanding and further development of the candidate's research field. Moreover, the thesis is written logically and coherently, which satisfies the degree requirement that the thesis shall serve as a valuable document for future reference. On August 12th, 2022, the PhD thesis oral examination was held. Pursuant to this oral examination, the thesis examination committee hereby concludes that the candidate has passed all of the requirements for the degree of Doctor of Philosophy in Life Sciences.

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Thesis publication date :