cGAS-STING Pathway in Innate Immune System

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1 Background

As the first line of defense against pathogens, innate immune system is composed of protective barriers (epithelial surface) and **Pathogen Recognition Receptors (PRR)** [1]. PRRs recognize **Pathogen-Associated Molecular Patterns (PAMP)** then activate intracellular signaling pathways.

Abbreviations	Meanings
PAMP	Pathogen-Associated Molecular Patterns
PRR	Pathogen Recognition Receptors
cGAMP	cyclic GMP-AMP
cGAS	cyclic GMP-AMP Synthase
STING	Stimulator of Interferon Genes
PBS	Phosphate-Buffered-Saline
PFA	Paraformaldehyde

Table 1: Terms their Abbreviations

In this context, PAMP refers to cytosolic DNA which is absent in normal cells. Upon DNA recognition, **cGAS** dimerizes and stimulates the formation of **cyclic-GMP-AMP (cGAMP)**. cGAMP then binds directly to **stimulator of interferon genes (STING)** which triggers phosphorylation/activation of the transcription factor IRF3 via TBK1 [2]. The above pathway is visualized in Figure 1.

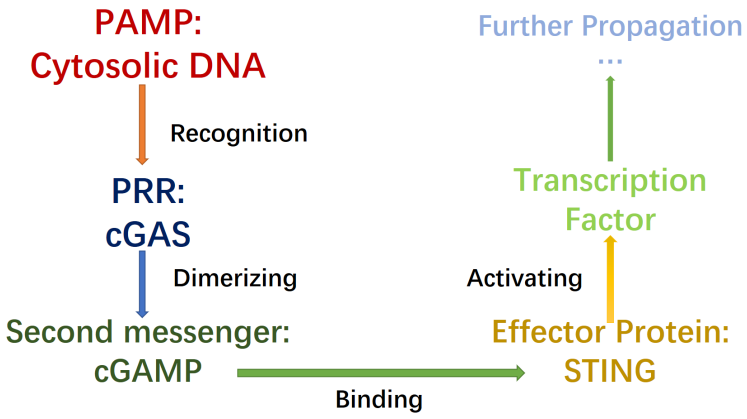


Figure 1: cCAS-STING Pathway in Innate Immune System

The STING is initially confined at Endothelial Reticulum (ER) until activated by cGAMP. With the signal of cGAMP, STING is transported to Golgi Apparatus or vesicular and forming a separated phase ,which helps gathering SING, for operating. In this experiment, we focused on this transportation of STING by observing the different distribution of florescence labeled STING in the cells.

2 Experimental Procedure

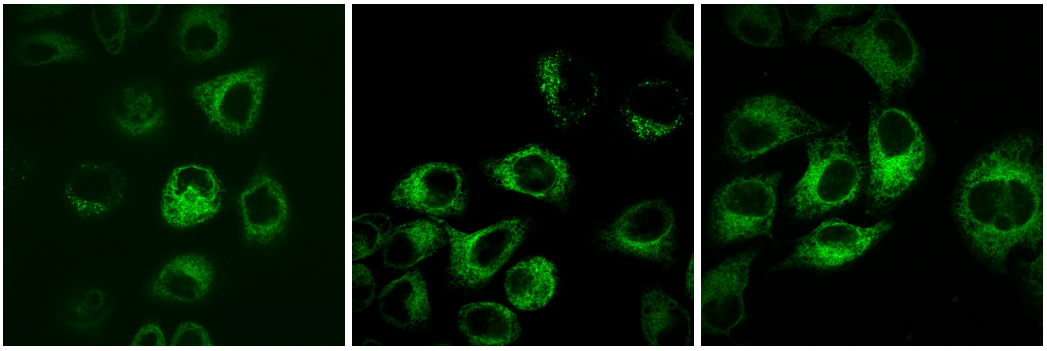
In our experiment, the experimental group was treated by **diABZI (the chemical compound to activate cGAS-STING pathway)**, while the controlled group was not. Hela cell line was used with GFP labelled STING.

We firstly transferred the cultured cells from its culture media to a microscope slide. To achieve this, the following detailed procedure was performed:

- remove liquid culture media by pipette.
- wash the cell by PBS (Phosphate-Buffered-Saline) 3 times, 500 μL in around.
- add 500 μL PFA (Paraformaldehyde) to both controlled group and experimental group.
- wait for 20 minutes for polymerization of PFA.
- add 6 μL sealant to each group to prevent fluorescence quenching.
- seal the specimen by nail polish finally.

3 Observations

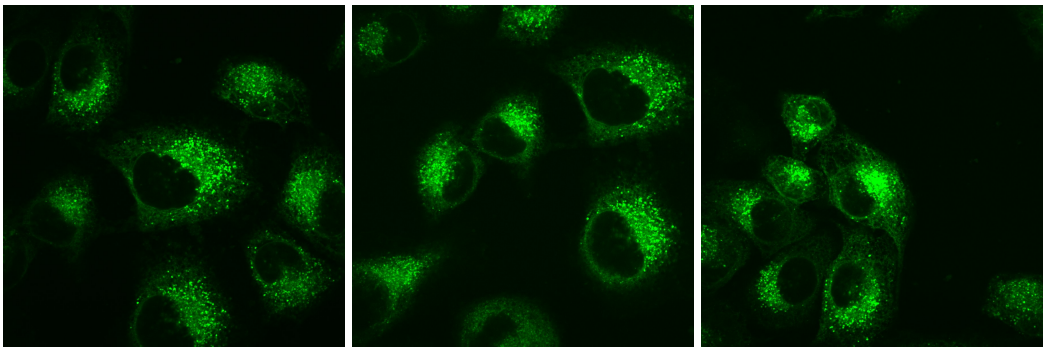
After a week we observed the specimen that we had manipulated using fluorescent light microscope. The pictures captured under microscope are shown in Figure 2 and Figure 3.



(a) capture 1 (b) capture 2 (c) capture 3

Figure 2: Controlled Group

In controlled group, the fluorescence was equally and evenly distributed in cytosol except nucleus. However, a few of unexpected cells (upper right corner of capture 2 in Figure 2) appeared to be activated even without diABZI treatment. Besides, we occasionally discovered several cells in division (may be telophase in mitotic phase).



(a) capture 1 (b) capture 2 (c) capture 3

Figure 3: Experimental Group (Treated by diABZI)

Compared with controlled group, the fluorescence distribution in each cell seemed to be more centered and much more bright forming a series of scatters. This phenomenon was consist with the theory of transportation of STING from ER to Golgi Apparatus or vesicular forming a concentrated separated phase here.

4 Conclusion

In this experiment we verified the transportation of STING protein from ER to Golgi Apparatus or vesicular, which is a critical step in cGAS-STING pathway of innate immune system.

The experiment acquainted us with fluorescence microscope and helped me to understand the importance as well as the mechanisms of innate immune system. Thanks for the instructions from my teacher and teaching assistant.

References

[1] Bruce Alberts, Rebecca Heald, Alexander Johnson, David Morgan, Martin Raff, Keith Roberts, and Peter Walter. *Molecular Biology of the Cell*. W. W. Norton and Company, 7th edition, 2022.

[2] Wikipedia contributors. cgas-sting cytosolic dna sensing pathway. `https://en.wikipedia.org/wiki/CGAS%E2%80%93STING_cytosolic_DNA_sensing_pathway`, 2024. Accessed: 2024-12-20.