Cold-induced FOXO1 nuclear transport aids cold survival and tissue storage

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Presenter: Shia-En Chen **Date/Time:** 2024/10/24, 15:10-16:00

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Background: Organ preservation and transplantation offer hope for patients. However, the more cost-effective 0−4°C static cold storage is still limited by cellular damage caused by cold stress and ischemia-reperfusion injury during rewarming, making it challenging to preserve most organs and tissues long-term at 4°C. For example, pancreatic cells can only be maintained for about 10 hours at 0−4°C in the current gold standard University of Wisconsin (UW) solution. Previous studies on hibernating animals, such as 13-lined ground squirrels (TLGSs), have found that enhanced SUMOylation strengthens covalent protein linkages and improves tolerance to temperature fluctuations. Although non-hibernating, human embryonic stem cells (hESCs) also exhibit good cold tolerance. Identifying common mechanisms of cold adaptation across species could help improve preservation strategies for islets and the pancreas. It could further be applied to long-term static cold storage of donor organs and tissues.

Objective: Investigate the cold adaptation mechanisms in mammals and apply these findings to extend the cold storage of islets and the pancreas.

Results: ATAC-seq revealed that forkhead box protein O1 (FOXO1)-binding motifs are enriched in H1 embryonic stem cells (ESCs) when exposed to 4°C. Immunofluorescence (IF) staining demonstrated that FOXO1 translocates from the cytosol to the nucleus upon 4°C exposure in both H1 ESCs and TLGS-induced pluripotent stem cells (iPSCs). CUT&Tag analysis showed that FOXO1 is more likely to bind to the transcription start site (TSS) at 4°C. To explore the mechanisms of FOXO1 nuclear entry and exit, the interaction between Importin-7 (IPO7) and Exportin-1 (XPO1) was examined. The results indicate that IPO7 facilitates FOXO1's nuclear entry at 4°C, while XPO1 promotes its nuclear exit at 37°C. RAN binding protein 2 (RANBP2), a nuclear pore complex component and a SUMO1 E3 ligase subunit, assists in the SUMOylation of XPO1 and IPO7. Additionally, the disruption of FOXO1's SUMO-interacting motifs (SIM) affects its interaction with XPO1. Administration of the XPO1 inhibitor KPT-330 in mice promotes FOXO1 accumulation in the nucleus and increases survival time at 4°C. To preserve pancreatic islets, a modified hibernation solution (HS) with the addition of KPT-330 and protease effectively preserves cell structure and restores insulin secretion function after warming. Transplantation into diabetic mice also effectively reduces blood glucose levels. This approach could similarly be applied to preserve human islets and pancreatic tissue.

Conclusion: FOXO1 nuclear translocation is regulated by the SUMOylation of IPO7 and XPO1, mediated by RANBP2, and enhancing FOXO1 nuclear accumulation can improve cell survival at low temperatures.