AUTOPHAGY INDUCTION AND AGO2 DEGRADATION DURING GLUTATHIONE DEFICIENCY IN SPERMATOGONIA-TYPE GERM CELL

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INTRODUCTION
Argonaute (Ago) proteins interact with miRNAs that guide translational repression and enhanced degradation of mRNA. Ago2 is degraded as miRNA-free entities by autophagy. This process is the major intracellular degradation system and acts as a pro-survival response during several conditions. The development and survival of male germ cells depend on the antioxidant capacity of the seminiferous tubule. Glutathione (GSH) plays an important role in the antioxidant defenses of the spermatogenic epithelium.

OBJECTIVES
In this work, we evaluated whether autophagy is involved in spermatogonia-type germ cell (GC-1) survival during GSH depletion and if Ago2 is affected during this condition.

MATERIALS AND METHODS
Glutathione content was assayed using GSH/GSSG-Glo Assay. Intracellular ATP content was determined using a CellTiter-Glo Luminescent Cell Viability Assay. Cell viability was assessed by propidium iodide. The level of PI incorporation was quantified in a FACScan flow cytometer. Caspase 3 activity was determined using a Caspase-Glo 3/7 Assay.

DISCUSSION AND RESULTS
We showed that disruption of GSH metabolism with L-buthionine-(S,R)-sulfoximine (BSO) decreased GSH content in GC-1 cells, without altering ROS production and cell viability. Autophagy was assessed by processing the protein LC3I to LC3II and observing its sub-cellular distribution. Immunoblot and immunofluorescence analyses showed a consistent increase in LC3II levels and accumulation of autophagosome under GSH-depletion conditions. This process did not affect the activity of AMP-activated protein kinase (AMPK) or the ATP content. However, inhibition of autophagy resulted in decreased ATP content and increased caspase-3/7 activity in GSH-depleted GC-1 cells. Ago2 protein level decreased during GSH depletion in GC-1 and HeLa cells and when these cells were treated with BSO and chloroquine to inhibit autophagy, accumulation of Ago2 was observed. Finally, GSH depletion and autophagy inhibition affect the interaction of Ago2 with Let7a miRNA.

CONCLUSIONS
These findings suggest that GSH deficiency triggers an AMPK-independent autophagy and that GSH and autophagy machinery are important for the miRNAs function.

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