Glutamine protects mouse spermatogonial stem cells against NOX1-derived ROS for sustaining self-renewal division

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Reactive oxygen species (ROS) are generated from NADPH oxidases and mitochondria; they are generally harmful for stem cells. Spermatogonial stem cells (SSCs) are unique among tissue-stem cells because they undergo ROS-dependent self-renewal via NOX1 activation. However, the mechanism by which SSCs are protected from ROS remains unknown. Here, we demonstrate a crucial role for Gln in ROS protection using cultured SSCs derived from immature testes. Measurements of amino acids required for SSC cultures revealed the indispensable role of Gln in SSC survival. Gln induced *Myc*expression to drive SSC self-renewal *in vitro*, whereas Gln deprivation triggered *Trp53*-dependent apoptosis and impaired SSC activity. However, apoptosis was attenuated in cultured SSCs that lacked NOX1. In contrast, cultured SSCs

lacking *Top1mt* mitochondria-specific topoisomerase exhibited poor mitochondrial ROS production and underwent apoptosis. Gln deprivation reduced glutathione production; supra-molar Asn supplementation allowed offspring production from SSCs cultured without Gln. Therefore, Gln ensures ROS-dependent SSC-self-renewal by providing protection against NOX1 and inducing *Myc*.