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(Phospho-)proteomic Signaling Responses of Human Male Germ Cell Lines to Simulated Microgravity and Hypogravity

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INTRODUCTION & AIM

Over the past century, advancements in space technology and the growing interest in space exploration, including the rise of space tourism, have significantly increased human participation to spaceflight. However, as human exploration extends beyond low Earth orbit (BLEO), with an increase in mission duration and complexity, there is a need for comprehensive research on the mechanisms of adaptation of human physiology to long-term exposure to altered gravitational conditions. Among the organ systems affected by microgravity, the reproductive one has been largely under investigated, despite evidence of disrupted endocrine signalling and altered gonadal cell function during spaceflight. Understanding the effects of microgravity on germ cells is crucial as any alterations could be inherited by offspring.

The present study aims to fill this gap by providing new insights into how altered gravity affects, at the molecular level, male reproductive health . To this end, we exposed two human testicular cancer cell lines to simulated microgravity (SµG) and hypogravity (ShG). Specifically, we selected the TCam-2 cell line, which resembles primordial germ cells/gonocytes, and the NT2D1 cell line, which exhibits pluripotency characteristics. A targeted (phospho-)proteomics approach, namely Reverse-Phase Protein microArrays (RPPAs), was exploited to investigate the molecular mechanisms that determine the cell response to an altered gravitational field.

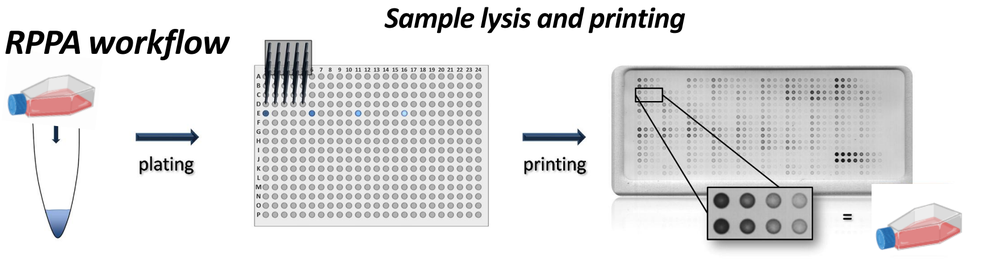
METHODS

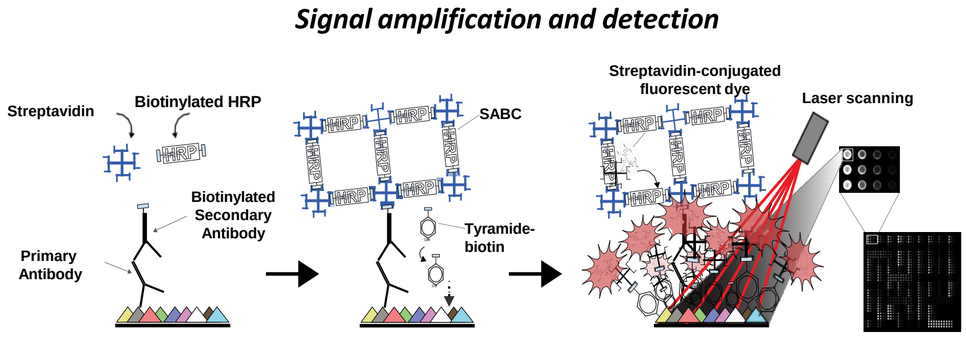
Cell Culture

TCam-2 and NT2D1 cell lines were 2D cultured and exposed for 3, 24, and 72 hours to unitary gravity (“on ground”: OG) or simulated microgravity (SµG) and hypogravity (ShG), using a Random Positioning Machine (RPM). According to the principle of "gravity-vector averaging” the RPM machine mimics the effects of SµG and ShG on standard cell culture flasks.

Reverse-Phase Protein microArrays (RPPA)

RPPA analysis was conducted using a panel of 130 antibody targets, potentially affected by altered gravity conditions, including but not limited to cytoskeleton, cell growth and proliferation as well as cancer-related pathways.

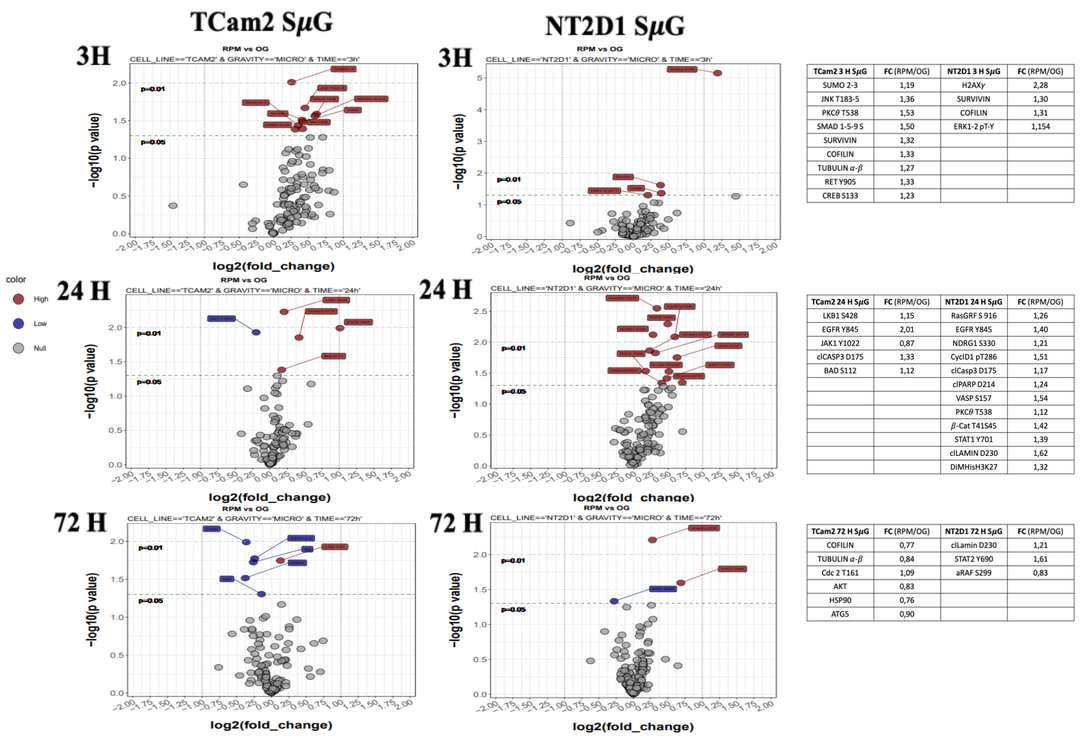




Modified from Signore M. et al. Elsevier Reference Module in Life Sciences. 2017; Akbani R et al. *Mol Cell Proteomics*. 2014.

RESULTS & DISCUSSION

Panel 1. Exposure to SµG induces early upregulation of some phospho-proteomic pathways and late downregulation of some other phospho-proteins.

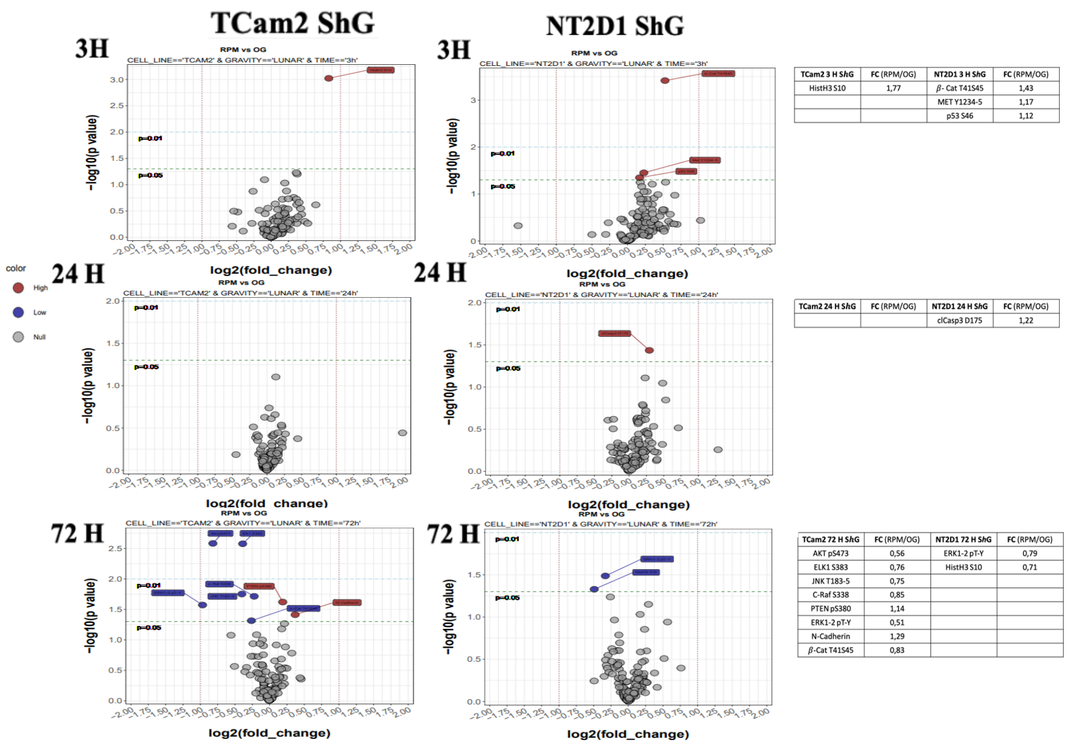


TCam2 3 H SµG	FC (RPM/OG)	NT2D1 3 H SµG	FC (RPM/OG)
SUMO 2-3	1,19	H2AXγ	2,28
JNK T183-5	1,36	SURVIVIN	1,30
PKCθ T538	1,53	COFILIN	1,31
SMAD 1-5-9 S	1,50	ERK1-2 pT-Y	1,154
SURVIVIN	1,32		
COFILIN	1,33		
TUBULIN α-β	1,27		
RET Y905	1,33		
CREB S133	1,23		

TCam2 24 H SµG	FC (RPM/OG)	NT2D1 24 H SµG	FC (RPM/OG)
LKB1 S428	1,15	RasGRF S 916	1,26
EGFR Y845	2,01	EGFR Y845	1,40
JAK1 Y1022	0,87	NDRG1 S330	1,21
clCASP3 D175	1,33	CyclD1 pT286	1,51
BAD S112	1,12	clCasp3 D175	1,17
		clPARP D214	1,24
		VASP S157	1,54
		PKCθ T538	1,12
		β-Cat T41545	1,42
		STAT1 Y701	1,39
		clLAMIN D230	1,62
		DiMHisH3K27	1,32

TCam2 72 H SµG	FC (RPM/OG)	NT2D1 72 H SµG	FC (RPM/OG)
COFILIN	0,77	clLamin D230	1,21
TUBULIN α-β	0,84	STAT2 Y690	1,61
Cdc 2 T161	1,09	aRAF S299	0,83
AKT	0,83		
HSP90	0,76		
ATG5	0,90		

Panel 2. ShG has a limited impact on signaling pathways and mostly leads, in late exposure, to a decrease of some phospho-protein levels



TCam2 3 H ShG	FC (RPM/OG)	NT2D1 3 H ShG	FC (RPM/OG)
HistH3 S10	1,77	β- Cat T41545	1,43
		MET Y1234-5	1,17
		p53 S46	1,12

TCam2 24 H ShG	FC (RPM/OG)	NT2D1 24 H ShG	FC (RPM/OG)
		clCasp3 D175	1,22

TCam2 72 H ShG	FC (RPM/OG)	NT2D1 72 H ShG	FC (RPM/OG)
AKT pS473	0,56	ERK1-2 pT-Y	0,79
ELK1 S383	0,76	HistH3 S10	0,71
JNK T183-5	0,75		
C-Raf S338	0,85		
PTEN pS380	1,14		
ERK1-2 pT-Y	0,51		
N-Cadherin	1,29		
β-Cat T41545	0,83		

The figure shows volcano plots of TCam-2 and NT2D1 exposed to SµG (panel 1) or ShG (panel 2) for the indicated timepoints. Dots represent the log2 of RPM to OG ratio (x axis) versus negative log10 of p value (y axis) for each individual antibody analyzed. Statistical significance was set to 0.05 (n=3). Statistically significant results (RPM versus OG) are labeled and colored as blue and red dots, respectively for down- and upregulated antibodies in the volcano plots and listed in a tabular format on the right of both Panel 1 and 2.

CONCLUSION

This study investigated the (phospho-)proteomic responses of TCam2 and NT2D1 germ cells to altered gravity conditions (simulated micro- and hypo-gravity) using RPPA. Overall, we found that modifications of the gravity vector result in a limited but significant number of phospho-protein changes (29/130 for TCam2 and 25/130 for NT2D1, respectively). These findings demonstrate the sensitivity of these cell lines to altered gravity confirming previous studies of our group on TCam-2 cells [Ferranti F. et al., Biomed Res Int. 2014; Berardini M. et al., Cells 2023]. Further investigations are needed to disclose whether such molecular changes are intrinsic features of the selected cancer cell lines or are a shared biological property of germ cells. Moreover, the transient nature of these molecular changes, and their difference over the exposure time, leaves an encouraging margin for cellular adaptation to altered gravitational force.

FUTURE WORK / REFERENCES

Confirmation of significant results via orthogonal techniques and complementation of RPPA data with other molecular analyses, in order to obtain a comprehensive scenario of the cellular responses to SµG and ShG.