Supplementary data for "Intraovarian transplantation of primordial follicles fails to rescue chemotherapy injured ovaries"

Mi-Ryung Park[1], Yun-Jung Choi[1], Deug-Nam Kwon[1], Chankyu Park[1], Hong-Thuy Bui[1], Sangiliyandi Gurunathan[1], Ssang-Goo Cho[1], Hyuk Song[2], Han Geuk Seo[1], Gyesik Min[3*], and Jin-Hoi Kim[1*]

[1]Department of Animal Biotechnology, College of Animal Bioscience & Technology, KonKuk University, Seoul 143-701, Republic of Korea.

[2]Department of Animal Science, College of Natural Science, Konkuk University, Chung-ju 380-701, Korea.

[3]Department of Pharmaceutical Engineering, College of Bioscience, Gyeongnam National University of Science and Technology

Supplementary data include 2 Tables and 3 Figure

sTable 1. Ratio of ovarian follicle disrupted mouse at 5 weeks after busulfan treatment.

	% of ovarian follicle disrupted mouse					
	100%	**90%**	**80%**	**70%**	**60%**	**50%**
No. of mice	**13/28**	**11/28**	**3/28**	**0/28**	**0/28**	**1/28**

sTable 2. Primers and conditions used for semi-quantitative RT-PCR.

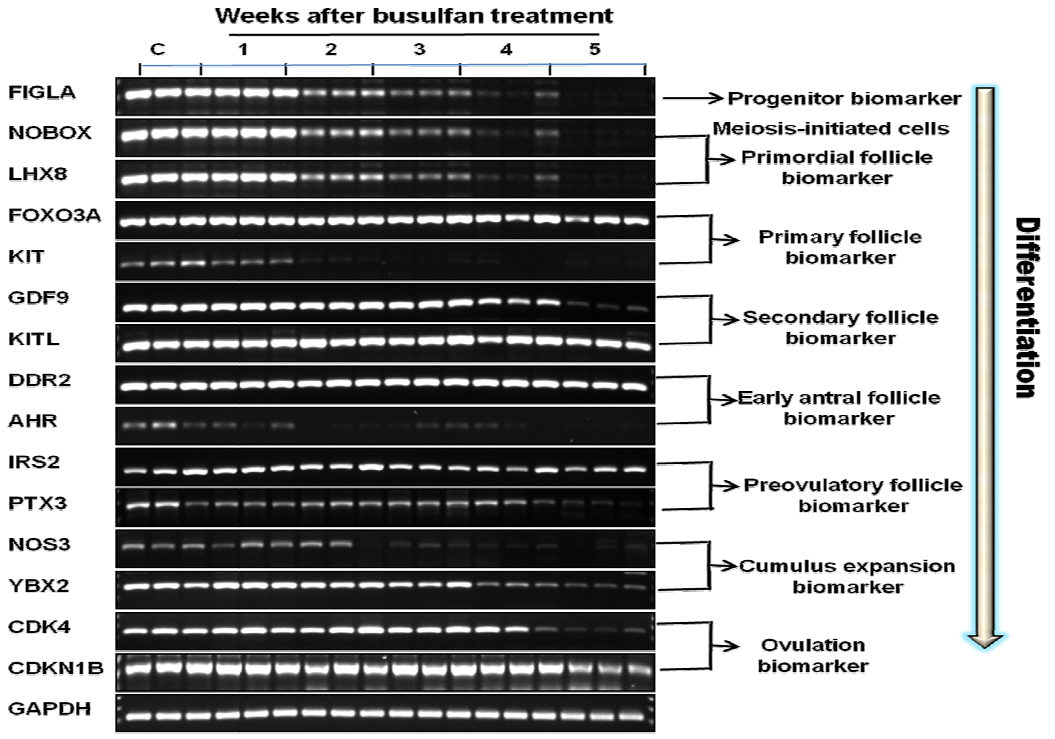
Stage	Gene	Primer sequence	TM	Product size
Progenitor	**FIGLA**	F: ACAGAGCAGGAAGCCCAGTA R: ACTCGCACAGCTGGTAGGTT	63ºC	205 bp
Primordial follicle	**NOBOX**	F: ACAAACGCCATGAGATTTCC R: AACAGGGCCAGGTTCTAGGT	63 ºC	215 bp
	LHX8	F: CAGTTCGCTCAGGACAACAA R: AGCCATTTCTTCCAACATGG	58 ºC	207 bp
Primary follicle	**FOXO3A**	F: GATGATGATGGACCCCTGTC R: TCTTGGCGGTATATGGGAAG	56 ºC	247 bp
	KIT	F: GTGGAGTGTAAGGCCTCCAA R: CCTCGACAACCTTCCATTGT	55 ºC	220 bp
Secondary follicle	**GDF9**	F: CCCCAAAACGAGTGTGAACT R: CACACTCAGGGGGCTGTACT	63 ºC	243 bp
	KITL	F: TCCGAAGAGGCCAGAAACTA R: TGCGGCTTTCCTATTACTGC	55 ºC	238 bp
Early antral follicle	**DDR2**	F: CCGAAAGCTTCCAGAGTTTG R: TCTCCCAGCTTCTCCTTGAA	55 ºC	249 bp
	AHR	F: ATGGTTCCTGTGTGCCCTACC	63 ºC	465 bp

		R: GCTGCCTATAGTCGGGCTCTG		
Preovulatory follicle	**SOX3**	F: GTCGTATTGCTTCTGTTTTCCTCT R: AATGCGTACACGAAATAGCAAATA	60 ℃	218 bp
	IRS2	F: ACAACCTATCGTGGCACCTC R: GGTGGTGGTAGAGGA AA	62 ℃	223 bp
Cumulus expansion	**TNFAIP6**	F: ATTTGAAGGTGGTCGTCTCG R: TGTGCCAGTAGCAGACCTGG	61 ℃	317 bp
	PTX3	F: GTGGGTGGAAAGGAGAACAA R: CCCAGATATTGAAGCCTGTGAT	56 ℃	274 bp
Ovulation	**NANOS3**	F: CTGACAAGGCAAAGACACAGGA R: AGGCAACACCACAAGTAGGG	62 ℃	281 bp
	YBX2	F: ATGGGGTAGAACCCAAGGAG R: GGAGGTCTCTGCGGCTATAG	58 ℃	281 bp
Luteal	**CDK4**	F: GAAACTCTGAAGCCGACCAG R: TTGTGCAGGTAGGAGTGCTG	57 ℃	244 bp
	CDKN1B	F: CAGAATCATAAGCCCCTGGA R: TCTGACGAGTCAGGCATTTG	59 ℃	224 bp
Apoptosis-related	**P53**	F: GGAGTATTTGGACGACCG R: TCAGTCTGAGTCAGGCCC	56 ℃	350 bp
	BCL-2	F: TAAGCTGTCACAGAGGGGCT R: TGAAGAGTTCCTCCACCACC	58 ℃	344 bp
	BAX	F: CGAGCTGATCAGAACCATCA R: GAAAAATGCCTTTCCCCTTC	55 ℃	283 bp
	FAS	F:GAGAATTGCTGAAGACATGACAATCC R: GTAGTTTTCACTCCAGACATTGTCC	60 ℃	314 bp
	FASL	F:TTAGCTTCTCTGGAGCAGTCAGCGTC R:CCTTCTTCTTTAGAGGGGTCAGTGGC	62 ℃	320 bp
	TNFaR55	F: TGCTGCACCAAGTGCCACAAAG R: CACACGGTGTTCTGAGTCTCC	60 ℃	325 bp
c-Kit signaling	**AKT**	F: TCAAGAGGCAGGAAGAAGAGAC R: AAGGAAGGGATGCCTAGAGTTC	65 ℃	302 bp
	MEK	F: TACTCTGTGCAGTCGGACATCT R: TGCTTCAGATCTGCTCTCTCTG	62 ℃	344 bp
	mTOR	F: CTCCGATTGTGAAATTGTTTGA R: TGCTGGTAAATCAAAGGGTCTT	62 ℃	355 bp
	SRC	F: TTGTCCTGATCATTTCAACACC R: AGATGCCACAAATCATCAACTG	62 ℃	377 bp
Autophagy-related	**Atg4A**	F: CCCTCACACAACCCAGACTT R: CCCCTGTGGTTGTCACTTCT	55 ℃	287 bp
	Atg4B	F: TGCTTTGAGAACCCAGACCT R: CTCCTGACCCACTGCTCTTC	55 ℃	250 bp
	Atg5	F: GGAGAGAAGAGGAGCCAGGT R: TGTTGCCTCCACTGAACTTG	55 ℃	227 bp
	Atg6	F: GGCCAATAAGATGGGTCTGA R: GCTTTTGTCCACTGCTCCTC	55 ℃	333 bp
	LC3	F: TTCTTCCTCCTGGTGAATGG R: GTGGGTGCCTACGTTCTCAT	55 ℃	251 bp
	Gaparap	F: CAGCAGGAGGGGTAATGGTA R: CCAATGTCAATCCCTTCCAC	55 ℃	226 bp
Housekeeping gene	**GAPDH**	F: AGGTCGGTGTGAACGGATTTG R: TGTAGACCATGTAGTTGAGGTCA	57 ℃	123 bp

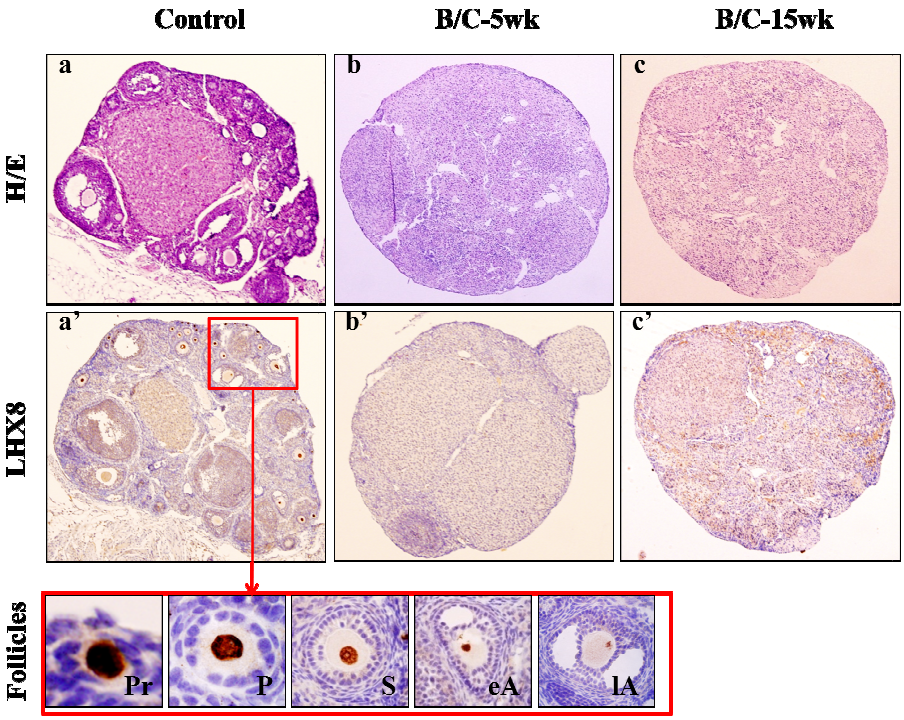
sFigure 1. Female germ cell-specific gene expression levels in control and busulfan-treated mouse ovaries. FIGLA is expressed mainly from progenitor cells to primordial follicles, whereas NOBOX and LHX8 are

expressed mainly in primordial follicles. Both FOXO3A and c-kit are expressed in primary follicles and during the transition from primary to secondary follicles. GDF 9 and KITL expression is limited to secondary follicles. DDR2 and AHR are expressed primarily in early antral follicles, and PTX3 and IRS2 are expressed primarily in preovulatory follicles. YBX2 and CDK2 are involved in cumulus cell expansion, and CDK4 and CDKN1B are involved in ovulation. Time points after busulfan treatment are indicated above each panel.

sFigure 2. Expression of Lhx8 in control (upper left panel) and busulfan-treated mouse ovaries [5 (upper middle panel) and 15 (upper right panel) weeks]. Expression and localization of LHX8 protein were detected immunohistochemically using a rabbit anti-LHX8 polyclonal antibody. LHX8 protein was located in the nucleus of oocytes, and increasingly expressed during primordial (Pr), primary (P), secondary (S), early antrum (eA), and late antrum (lA) follicles in control ovary. However, any positive signal was not detected in both of 5 and 15 weeks ovaries after busulfan treatment. **Method.** Ovaries were fixed in 10% formaldehyde for 12–24 h before paraffin embedding. The samples were serially sectioned at 5μm and mounted on glass slides. Antigen retrieval was performed using 0.01M sodium citrate. The sections were blocked by BDT (3% BSA, 10% normal goat serum in TBS) for 30 min and incubated with rabbit anti-LHX8 polyclonal antibody at a dilution of 1: 200 (Abcam, Ab45118) overnight at 4°C. After rinsing thoroughly with TBS, the sections were incubated with HRP-conjugated goat anti-rabbit secondary antibody at a dilution of 1: 50 (Beyotime, A0208) for 30 min at 37°C. LHX8 expression in sectionswas detected by the reaction of peroxidase with 3,3′-diaminobenzidine tetrahydrochloride (DAB) and analyzed under Olympus BX51 fluorescence microscope.


Weeks after busulfan treatment
C
1
2
3
4
5
FIGLA
NOBOX
LHX8
FOXO3A
KIT
GDF9
KITL
DDR2
AHR
IRS2
PTX3
NOS3
YBX2
CDK4
CDKN1B
GAPDH
Progenitor biomarker
Meiosis-initiated cells
Primordial follicle biomarker
Primary follicle biomarker
Secondary follicle biomarker
Early antral follicle biomarker
Preovulatory follicle biomarker
Cumulus expansion biomarker
Ovulation biomarker
Differentiation

sFig. 1


Control
B/C-5wk
B/C-15wk
H/E
LHX8
Follicles
a
b
c
a'
b'
c'
Pr
P
S
eA
lA

sFig. 2