Redox Biology 15 (2018) 125–134

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Research Paper

Small Maf functions in the maintenance of germline stem cells in the *Drosophila* testis

Sharon Wui Sing Tan[a], George W. Yip[a], Toshio Suda[b], Gyeong Hun Baeg[a,*]

[a] Department of Anatomy, Yong Loo Lin School of Medicine, National University of Singapore, MD10, 4 Medical Drive, Singapore 117594, Singapore
[b] Cancer Science Institute of Singapore, National University of Singapore, MD6, 14 Medical Drive, Singapore 117599, Singapore

ARTICLE INFO

Keywords:
Reactive oxygen species
Drosophila
Germline stem cell
Small Maf
Keap1/Nrf2
Microarray

ABSTRACT

Reactive oxygen species (ROS) are byproducts generated during normal cellular metabolism, and redox states have been shown to influence stem cell self-renewal and lineage commitment across phyla. However, the downstream effectors of ROS signaling that control stem cell behavior remain largely unexplored. Here, we used the *Drosophila* testis as an *in vivo* model to identify ROS-induced effectors that are involved in the differentiation process of germline stem cells (GSCs). In the Affymetrix microarray analysis, 152 genes were either upregulated or downregulated during GSC differentiation induced by elevated levels of ROS, and a follow-up validation of the gene expression by qRT-PCR showed a Spearman's rho of 0.9173 ($P < 0.0001$). Notably, 47 (31%) of the identified genes had no predicted molecular function or recognizable protein domain. These suggest the robustness of this microarray analysis, which identified many uncharacterized genes, possibly with an essential role in ROS-induced GSC differentiation. We also showed that *maf-S* is transcriptionally downregulated by oxidative stress, and that *maf-S* knockdown promotes GSC differentiation but Maf-S overexpression conversely results in an over-growth of GSC-like cells by promoting the mitotic activity of germ cell lineage. Together with the facts that Maf-S regulates ROS levels and genetically interacts with Keap1/Nrf2 in GSC maintenance, our study suggests that Maf-S plays an important role in the *Drosophila* testis GSC maintenance by participating in the regulation of redox homeostasis.

1. Introduction

Reactive oxygen species (ROS), including superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl free radical ($HO\cdot$), are the byproducts of cellular metabolism and homeostasis. The reactive oxygen anion $O_2^{\cdot -}$ is converted throughout a series of enzymatic reactions to H_2O_2, which is then further catalyzed to release highly reactive $HO\cdot$. On the other hand, the scavenger antioxidative molecules such as superoxide dismutase, catalase, glutathione peroxidase and peroxiredoxin convert $O_2^{\cdot -}$ to H_2O_2, and subsequently convert H_2O_2 to H_2O and dioxide (O_2) [1]. ROS were initially considered as a destructive byproduct of metabolism that are highly associated with a wide variety of human diseases such as cancer and neurodegenerative disorders [2–4]. However, increasing evidence has shown that ROS can be generated on purpose to benefit various physiological and biological processes such as cell growth, survival, signal transduction and protein-folding [5–7].

Stem cells are essential for the development of an organism, as well as to replenish damaged tissue lost throughout life. The characteristics of stem cells include the ability to self-renew and differentiate into specialised cell types. Importantly, stem cell homeostasis must be tightly regulated to prevent unfavourable balance between stem cells and differentiated cells, as dysregulated stem cell behavior is closely associated with various human diseases such as cancers, neurological diseases and irritable bowel syndrome (IBS) [8–10]. Intriguingly, intracellular ROS levels have been reported to play important roles in balancing self-renewal and differentiation of various stem cell populations across phyla. Normal stem cells are known to reside in the stem cell niches characterized by a low ROS environment so that stem cells remain in a quiescent state, a property that is essential for their self-renewal capacity [11]. Consistently, it was shown that low levels of ROS in stem cell niches are of importance to maintain the stem identity of hematopoietic stem cells [12], which were reported to lose their stem identity when ROS levels become excessive [13]. Embryonic stem cells (ESCs) are also known to maintain their stemness and pluripotency under low levels of ROS, but they undergo apoptosis or senescence when exposed to prolonged ROS [14,15]. On the other hand, elevated ROS levels were detected during the differentiation process of human adipose tissue-derived multipotent adult stem cells into a neural

* Corresponding author.
E-mail address: antbgh@nus.edu.sg (G.H. Baeg).

https://doi.org/10.1016/j.redox.2017.12.002
Received 29 November 2017; Accepted 5 December 2017
Available online 08 December 2017

phenotype [16]. However, despite the essential role of redox states in stem cell homeostasis, ROS-induced downstream effectors that regulate stem cell behavior are not fully characterized.

The *Drosophila* testis germline stem cell (GSC) system is one of the best understood adult stem cell models for studying and understanding the fundamental cellular mechanisms of stem cell behavior, as stem cells and their progenies can be easily identified, traced, imaged and genetically manipulated *in vivo* [17]. Importantly, we previously showed that high levels of ROS facilitate GSC differentiation through the activation of EGFR signaling, whereas decreased ROS levels conversely promote the proliferation of GSC-like cells in the *Drosophila* testes [18]. In this study, we performed Affymetrix microarray analysis using the *Drosophila* testes to identify the downstream effectors of ROS-mediated GSC differentiation. 152 genes were found to be differentially expressed during GSC differentiation. Several genes such as *maf-S* (*small maf*) and *lox2* (*lipoxygenases 2*), whose products are implicated in redox signaling, were identified, suggesting the validity of this genome-wide approach. Notably, many of the identified genes have not yet been characterized. Lastly, our genetic analyses revealed that *maf-S* knockdown promotes GSC differentiation, but Maf-S overexpression conversely facilitates the proliferation of GSC-like early-stage germ cells. Since Maf-S genetically interacted with Keap1/Nrf2 in GSC homeostasis, our study suggests that Maf-S functions in the regulation of ROS-associated stem cell behavior in the *Drosophila* testis.

2. Material and methods

2.1. Fly strains and fly husbandry

UAS-Maf-S (II), *UAS-Keap1*^{RNAi}, *UAS-CncC, UAS-CncC*^{RNAi}, *keap1*^{036}, *keap1*^{EY5} and *GstD1-GFP* fly lines were obtained from D. Bohmann [19]. *UAS-ND75*^{RNAi} and *UAS-ND42*^{RNAi} were obtained from the NIG-FLY Stock Center. *Nanos (nos)-Gal4, esg*$^{M5-4}$*-lacZ* was obtained from S. Dinardo [20], and *bam-GFP* transgenic line was obtained from D.M. Mckearin [21]. *UAS-maf-S*^{RNAi} (BL#40853) was obtained from the Bloomington *Drosophila* Stock Center. For UAS/Gal4 experiments, eclosed F1 adult male flies were incubated at 30 °C to maximize the Gal4 activity. All fly stocks were maintained on a standard diet at room temperature.

2.2. Immunohistochemistry

The *Drosophila* testes were dissected on a glass slide with dissection buffer at pH 7.2 (130 mM NaCl; 1.9 mM $CaCl_2$; 4.7 mM KCl; 10 mM HEPES) and fixed with 4% paraformaldehyde for 20 min, followed by washing with PBST (1XPBS with 0.3% Triton-X) for three times, 20 min each. The testes were then incubated with primary antibodies at 4 °C for overnight. Testes were washed and subsequently incubated with secondary antibodies at room temperature for 2 h. Images were taken using the Olympus FluoView ™ FV1000 Confocal Laser Microscope. ImajeJ was used to measure the distance of cells. Primary antibodies used were: rat anti-Vasa (Developmental Studies Hybridoma Bank [DSHB], 1:100), mouse anti-Fasciclin III (DSHB, 1:100), rabbit anti-GFP Alexa Fluor® 488 conjugate (Molecular Probes®, 1:500), mouse anti-1B1 (DSHB, 1:150), mouse anti-β-gal (β-galactosidase) (Sigma Aldrich [SA] #G4644, 1:200) and rabbit anti-pH3 (Cell Signaling Technology #9701, 1:200). Secondary antibodies used were: Alexa Fluor® 488-AffiniPure Donkey Anti-Mouse IgG (Jackson ImmunoResearch Laboratories Inc. [JIR] #715-545-150, 1:300), Alexa Fluor® 594-AffiniPure Goat Anti-Rat IgG (JIR #112-585-003, 1:300) and Goat anti-rabbit Alexa Fluor® 488 (ThermoFisher Scientific [TFS] #R37116, 1:200).

2.3. DHE assay

Testes were dissected into 1 ml of Schneider media with 10% FBS. 1ul of reconstituted DHE dye (TFS) was added and allowed to rock for 5 min in the dark. Testes were then washed three times with Schneider media for 5 min each, followed by fixation with 4% paraformaldehyde for 10 min. Testes were mounted and immediately viewed under the Olympus FluoView™ FV1000 Confocal Laser Scanning Biological Microscope. The intensity of DHE staining was quantified using ImageJ.

2.4. Sample preparation for microarray

Testes expressing *ND75*^{RNAi} under the control of *nanos*-Gal4 driver were used as an experimental group (high ROS levels), whereas testes carrying the driver alone served as a control group (physiological/moderate ROS levels). Three biological replicates for each group were prepared. For each replicate, approximately 200 adult male flies were dissected in ice cold Schneider media, and the resulting testes were washed with ice cold 1XPBS for three times. 800μl of a solution containing 0.25% collagenase (TFS) and 0.5% trypsin (TFS) prepared in 1XPBS were used to dissociate the testes by rocking for 15 min. The solution was then filtered through a 40 μm mesh, and the reaction of cell dissociation was stopped by the addition of 500μl Schneider media. Cell pellet was then obtained by centrifugation at 600*g* for 10 min at 4 °C, followed by RNA extraction by RNeasy microarray tissue Kit (Qiagen). The quality of the RNA was determined using an Agilent Bioanalyzer.

2.5. Microarray

100 ng of RNA were converted into double-stranded cDNA, which was then amplified to cRNA by *in vitro* transcription. The cRNA was purified and subjected to 2nd-cycle single-stranded sense cDNA synthesis, followed by fragmentation and terminal labelling (Affymetrix GeneChip WT PLUS Reagent Kit) before hybridization to Affymetrix *Drosophila* Gene 1.0 ST array. The arrays were washed and stained using GeneChip Hybridization, Wash and Stain Kit (Affymetrix), and subsequently scanned by Affymetrix 3000 7 G scanner. The differentially expressed genes between control and experimental group were determined using the Transcriptome Analysis Console 3.0 software (Affymetrix), with the criteria of at least a 1.5- or −1.5-fold change, and FDR *p-value* < 0.05.

2.6. Quantitative RT-PCR

RNA was extracted from testes with different genotypes, using TRIzol Reagent (TFS). 1 μg of RNA was converted to cDNA using the RevertAid First Strand cDNA Synthesis Kit (TFS). RT-qPCR analysis was then performed using FAST SYBR Green Master Mix (TFS) and the AB7900HT Fast Real Time PCR system (Applied Biosystems). The list of primer sets used is described in the Supplementary Table 1.

2.7. Statistical analysis

Data was presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using the GraphPad Prism 6.0 software (La Jolla, CA, USA). The Student's *t*-test was used to compare between two groups. For comparisons among three or more groups, one-way ANOVA was carried out followed by post-hoc Bonferroni test. Association between two variables was evaluated using Spearman rank correlation. P-values below 0.05 were deemed to be statistically significant.

3. Results and discussion

3.1. Inhibition of ND75 promotes GSC differentiation by increasing ROS levels in the Drosophila testis

Oxidative stress has been shown to influence stem cell behavior by

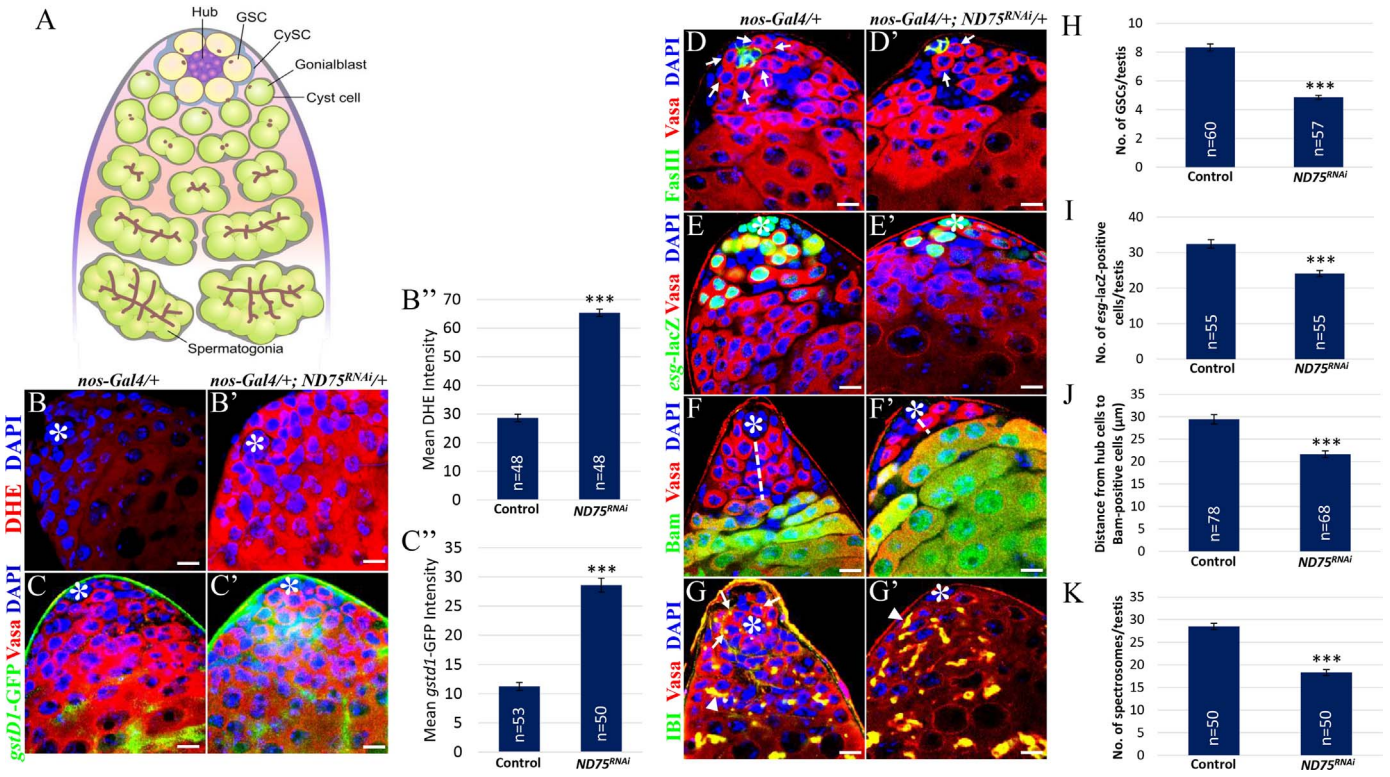

Fig. 1. *ND75* knockdown increases ROS levels and promotes GSC differentiation in the *Drosophila* testis. (A) Schematic of the *Drosophila* testis. (B, B' C and C') ROS levels were monitored by using DHE probe and the *in vivo* ROS reporter *GstD1-GFP* in (B and C) control testes and (B' and C') testes expressing $ND75^{RNAi}$. (B" and C") The intensity of DHE staining and GFP was quantified using the ImageJ software. (D, E, F and G) Control and (D', E', F' and G') *ND75* knockdown testes. (D and D') Testes stained for FasIII and Vasa, which mark hub cells and germ cell lineage, respectively (arrows indicate GSCs attached to hub cells). (E and E') Testes stained for *esg*-lacZ, which marks early-stage germ cells. (F and F') Testes stained for the differentiation marker Bam. Dashed lines indicate the distance between hub cells and differentiating germ cells. (G and G') Testes stained with 1B1, which stains spectrosomes and branching fusomes. Arrows indicate spectrosomes and arrowheads indicate branched fusomes. Quantification of (H) GSCs, (I) *esg*-lacZ-positive cells, (J) the distance between hub cells and Bam-positive germ cells, and (K) spectrosomes. Error bar is SEM from three independent experiments. Significance was assessed using unpaired *t*-test (***$p < 0.001$). GSC: germline stem cell. CySC: (somatic) cyst stem cell. Scale bar, 10 μM. *hub cells.

facilitating the proliferation, differentiation or apoptosis of stem cell populations [1]. In the *Drosophila* testis, redox states regulated by Keap1/Nrf2 activity play important roles in GSC maintenance [18]. In an attempt to identify molecules that are associated with GSC differentiation induced by high levels of ROS, we first confirmed the effects of increased intracellular ROS levels by knocking down *ND75* on GSC homeostasis. The mitochondrial electron transport chain (ETC) is the major consumer of O_2 and thus serves as a key contributor to ROS generation in mammalian cells [22]. ND75 is the *Drosophila* homolog of human mitochondrial complex I subunit NDUFS1 (NADH: Ubiquinone Oxidoreductase Core Subunit S1), which has NADH (nicotinamide adenine dinucleotide, reduced) dehydrogenase and oxidoreductase activities, and whose inhibition was shown to increase ROS levels [23–25]. To assess if altered ND75 activity in the *Drosophila* testis can affect ROS levels and thus disrupt GSC behavior, testes expressing $ND75^{RNAi}$ under the control of *nos*-Gal4, which is expressed in early-stage germ cells, were stained with DHE dye to monitor $O_2^{\cdot -}$ levels. In the *Drosophila* testis, 6–12 GSCs are present, and each is enclosed by a pair of somatic cyst stem cells (CySCs); both GSCs and CySCs attach and surround 10–15 post-mitotic hub cells, forming the stem cell niche (Fig. 1A). GSCs undergo asymmetric division to produce one daughter cell that remains as a stem cell, and another differentiating daughter cell called gonialblast that is displaced away from hub cells. The gonialblast then proceeds with the four rounds of synchronous transit-amplifying (TA) divisions, giving rise to a cyst of 16 spermatogonia which go on to eventually differentiate into mature sperms [17]. Notably, testes expressing $ND75^{RNAi}$ showed a higher intensity of DHE staining as compared to control testes (Fig. 1B, B' and B"), suggesting that *ND75* knockdown can cause an increase in ROS levels. This finding was further validated by using transgenic flies carrying an independent oxidative stress reporter gene *gstD1-GFP* [19]. While GFP expression was barely detected at the apical tip of control testes in which GSCs and early-stage germ cells reside, an intense GFP expression was observed at the apical tip of *ND75* knockdown testes (Fig. 1C, C' and C").

We next examined whether high levels of ROS induced by $ND75^{RNAi}$ can affect GSC maintenance. Testes were stained with Vasa antibody, which marks germ cell lineage. As compared to control testes, *ND75* knockdown testes showed a reduced number of GSCs, which are Vasa-positive cells directly attached to hub cells and are arranged in a rosette pattern (Fig. 1D and D', arrows). While testes expressing $ND75^{RNAi}$ had an average of 4.9 GSCs per testis, control testes had an average of 8.3 GSCs (Fig. 1H). Consistently, in testes expressing $ND75^{RNAi}$ much less Vasa-positive cells were found to be positive for *esg* (*escargot*)-lacZ, which marks only early-stage germ cells such as GSCs and gonialblasts, as compared to control (Fig. 1E, E' and I). To further confirm the finding that ND75 inhibition decreases GSC number, we next examined the expression pattern of the differentiation marker Bam (Bag of marble), which is normally expressed in 4- to early 16-germ cells located several cell diameters away from the hub cells. In testes expressing $ND75^{RNAi}$, Bam was detected much closer to the hub cells as compared to control (Fig. 1F, F' and J), indicative of premature GSC differentiation. The inhibitory effects of ND75 on GSC differentiation was further confirmed by the decreased number of spectrosomes, which appear a small dot in GSCs and GSC/gonialblast pairs, in testes expressing $ND75^{RNAi}$ as compared to control testes (Fig. 1G, G' and K, arrows). All these findings are in accordance with our previous observation that high levels of ROS induced by the oxidant paraquat promote GSC differentiation [18], and suggest that testes with elevated ROS by *ND75* knockdown can be

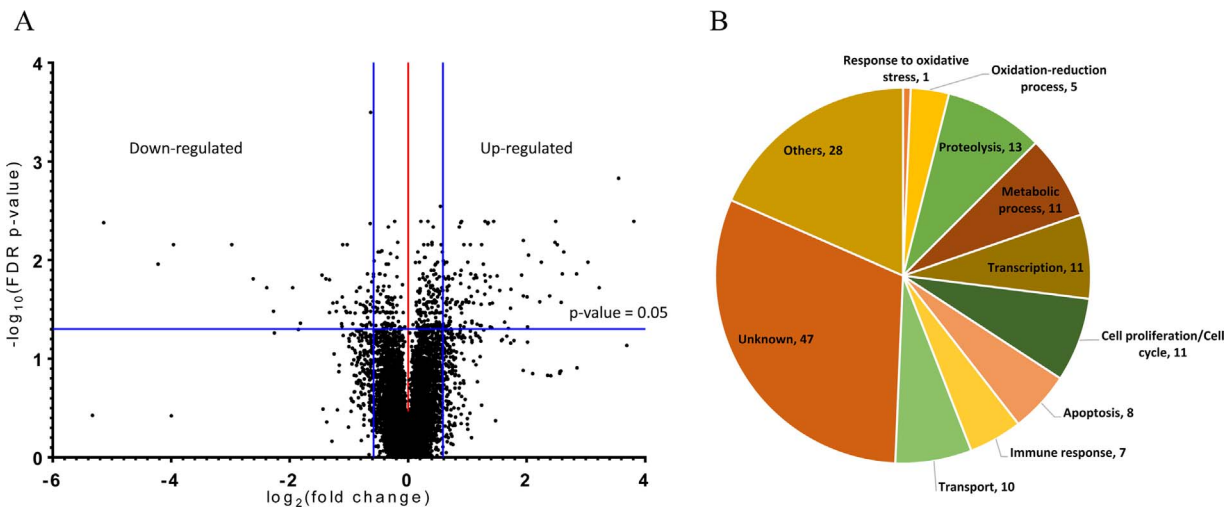

Fig. 2. Affymetrix microarray data analysis. (A) A volcano plot. 152 genes were found to be differentially expressed by more than 1.5 folds in testes with high levels of ROS as compared to control testes. (B) The identified genes were assigned to categories based on their predicted molecular functions, protein domains, and reports from the ontology database in the Flybase.

utilized for genome-wide studies to identify the downstream effectors of high ROS-induced GSC differentiation.

3.2. Microarray and data analysis

The regulators of redox homeostasis within stem cells remain largely unexplored. In particular, ROS-induced effectors that control stem cell behavior are not fully understood. To identify those that are involved in GSC differentiation facilitated by high levels of ROS, we performed Affymetrix genechip microarray analyses to establish gene expression profiling of testes with moderate (control) and high (knockdown of *ND75*) ROS levels. 152 genes were found to be differentially expressed in testes with *ND75* knockdown as compared to control testes; 96 genes were upregulated and 56 genes were downregulated by more than 1.5 folds (Fig. 2A, Table 1). We identified several genes, including *maf-S* (*small maf*), *GstE1* (*Glutathione S transferase E1*) and *lox2* (*lipoxygenases 2*), whose products have yet been implicated in redox homeostasis as a candidate. In an attempt to predict functions and generate testable hypotheses for the characterization of the 152 genes, we have assigned these genes to categories based on their predicted molecular functions, protein domains, and reports from the ontology database in the Flybase (http://flybase.org/). These categories include (1) oxidation-reduction process, (2) metabolic process, (3) transcription, and (4) cell proliferation (Fig. 2B). Notably, 31% of the identified genes showed no predicted molecular function and/or recognizable protein domain, suggesting that this genome-wide microarray analysis identified many uncharacterized genes, possibly with an essential role in ROS-induced GSC differentiation. Importantly, we randomly selected 20 genes among the candidates for further validation by quantitative RT-PCR analyses, and found that expression pattern of the 20 genes tested was highly correlated to that obtained from microarray analysis, with the Spearman's rho of 0.9173 ($P < 0.0001$) (Fig. 3A and B).

Interestingly, *mal-A6* (*maltase A6*), the *Drosophila* homolog of the cysteine transporter gene *Slc3a1* (*solute carrier family* 3 *member A1*), was identified whose product has been reported to function in carbohydrate process (Table 1). Notably, high SLC3A1 expression was shown to promote the cysteine uptake and the accumulation of reductive glutathione (GSH), leading to a decrease in ROS levels and an activation of Akt signaling [26]. By contrast, knockdown of *Slc3a1* was reported to decrease intracellular GSH and thus increase intracellular ROS activity [27]. These may suggest the potential role of Akt signaling in ROS-associated GSC behavior, and the presence of a feedback loop between ROS signaling and Mal-A6, in the *Drosophila* testis. We also found *wts*, the *Drosophila* homolog of *Lats* (*Large tumor suppressor kinase*), to be transcriptionally regulated upon high levels of ROS (Table 1). LATS is a central component of the Hippo pathway that negatively regulates the activity of the transcriptional co-factor YAP (Yes-associated protein) [28]. Notably, YAP has been reported to form a heterodimer with FoxO1, binding to the promoters of the *catalase* and *manganese superoxide dismutase* (*MnSod*) antioxidant genes and activating their transcription [29], suggesting the potential role of the Hippo signaling pathway in redox homeostasis and ROS-mediated biological processes such as stem cell maintenance. All these observations suggest the validity and reliability of our microarray analysis to identify ROS-associated effectors that may regulate GSC behavior in the *Drosophila* testis.

3.3. The gene maf-S is transcriptionally downregulated by high levels of ROS

Keap1 (Kelch-like ECH-associated protein 1)/Nrf2 (NF-E2-related factor 2) signaling acts as a key regulator of redox homeostasis. Upon electrophilic and oxidative stress, Keap1/Cul3 E3 ubiquitin ligase-mediated proteosomal degradation of Nrf2 becomes disrupted, allowing Nrf2 to be stable and translocate into the nucleus to form a heterodimerization with the basic region leucine zipper (bZIP)-type transcription factor Maf for the transcriptional activation of many phase II detoxification enzyme genes such as *thioredoxin reductase* and *glutathione reductase* [30–35]. The small Mafs (sMafs) comprise of MafF, MafG and MafK. They form heterodimers with cap 'n' collar (CNC) proteins such as Nrf1 and Nrf2, and bind to the antioxidant/electrophile response element (ARE/EpRE), indicating the role of sMafs in redox balance. However, despite their putative role in the regulation of antioxidant gene expression, their function in stem cell homeostasis is not fully characterized.

Keap1/Nrf2 signaling is evolutionarily well conserved in *Drosophila* [19]. In this study, Maf-S, a sole small Maf family member in *Drosophila*, was found to be downregulated in testes with high levels of ROS. To determine whether Maf-S functions in GSC maintenance, we further confirmed the transcription of *maf-S* by qRT-PCR using RNA extracted from testes with increased ROS levels induced by the suppression of ND75 or ND42 (the *Drosophila* homolog of NDUFA10, a mitochondria complex 1 subunit), and testes with decreased ROS levels induced by *keap1* knockdown ($keap^{RNAi}$). Consistent with the microarray data, we found that *maf-S* mRNA levels are significantly reduced in testes with high levels of ROS (Fig. 4A). On the other hand, we found that

Table 1
A list of genes transcriptionally modulated during GSC differentiation.

Up-regulated genes					Down-regulated genes				
Transcript Cluster ID	Fly Gene Symbol	Human Ortholog	Fold Change	FDR p-value	Transcript Cluster ID	Fly Gene Symbol	Human Ortholog	Fold Change	FDR p-value
18197227	CG14369	N/A	14.03	0.004053	18143346	Ref2	ALYREF	−1.5	0.013804
18200889	Gr93d	N/A	9.34	0.019055	18198373	CG17186	N/A	−1.5	0.014708
18164680	CG43110	GZMH	7.17	0.013804	18133084	CG5440	UBE2E3	−1.51	0.030002
18199988	CG10000	GALNT14	6.17	0.008274	18194131	wts	LATS2	−1.51	0.02603
18145258	CG33307	N/A	5.99	0.0268	18188101	sosie; CR45216	N/A	−1.53	0.016607
18141312	CG3528	C4orf22	5.62	0.004053	18171734	CG33217	PELP1	−1.54	0.023726
18162277	CG2736	SCARB2	5.57	0.006617	18188937	CG11897	ABCC4	−1.54	0.045327
18138530	CG43402	N/A	4.75	0.010525	18162274	Phk-3	N/A	−1.55	0.033
18156218	gsb-n	PAX3	4.66	0.02644	18194371	mia	TAF6L	−1.56	0.028082
18134734	dpr19	USH2A	4.09	0.008924	18169211	Tgi	VGLL4	−1.56	0.004262
18144843	CG31777	N/A	4.04	0.047532	18145722	del	N/A	−1.57	0.042923
18185346	CG18473	PTER	4.02	0.023605	18189333	CG11333	ISOC1	−1.6	0.020327
18136286	Gr32a	GZMH	3.85	0.006331	18145631	CG34367	SHOX2	−1.6	0.020466
18151208	CG10764	N/A	3.85	0.023075	18142936	CG5676	FUNDC1	−1.61	0.038272
18180564	CG34025	N/A	3.31	0.030731	18151843	maf-S	MAFK	−1.62	0.032486
18132093	Acyp	ACYP2	3.23	0.049199	18146894	a	PDZD2	−1.62	0.045041
18197785	CG14905	CCDC63	3.09	0.042576	18155416	mi	N/A	−1.62	0.02644
18141034	CG11912	PRTN3	2.92	0.020284	18133793	CG18269	MCMBP	−1.63	0.049199
18136753	CG31776	GALNTL6	2.83	0.01447	18134015	CG3430	N/A	−1.63	0.047739
18157091	imd	PIDD1	2.72	0.004053	18135993	DCTN5-p25	DCTN5	−1.63	0.030878
18139932	cutlet	CHTF18	2.68	0.027276	18161200	Or56a	N/A	−1.64	0.046053
18163569	sprt	PARD3B	2.66	0.048525	18151370	CG14500	N/A	−1.64	0.031712
18177597	CG10361	GCAT	2.61	0.018769	18165004	CG43668	N/A	−1.65	0.029659
18135200	CG16820	N/A	2.61	0.047532	18202690	CG42487; CG4884	C6orf203	−1.65	0.034006
18154275	Idgf5	CHIT1	2.6	0.027711	18187489	Fadd	PIDD1	−1.68	0.034006
18143767	Cyp310a1	TBXAS1	2.55	0.004184	18168671	CG3335	RBM19	−1.69	0.028633
18135886	CG1421	N/A	2.54	0.004262	18173465	Mocs1	MOCS1	−1.7	0.046053
18156154	Cyp6a2	TBXAS1	2.47	0.016296	18202203	Nlg3	NLGN4Y	−1.73	0.045041
18141305	CG18641	LIPI	2.45	0.022122	18183067	GstD5	VARS	−1.75	0.028009
18168480	CG13309	N/A	2.45	0.01418	18151405	GstE1	GSTT2B	−1.75	0.018895
18201360	CG31174	DCTN3	2.36	0.006981	18178062	CG18649	N/A	−1.77	0.019055
18152278	CG13527	N/A	2.35	0.028082	18137896	gkt	TDP1	−1.83	0.020721
18165049	CG43742	N/A	2.35	0.046053	18191472	tal-AA; tal-1A; tal-2A; tal-3A	N/A	−1.84	0.0268
18188796	CG1894	KAT8	2.32	0.04604	18161799	RpL37b	RPL37	−1.91	0.044144
18197860	CG17477	KLK14	2.24	0.044589	18179059	CG7298	N/A	−1.97	0.034066
18136253	Tep3	CD109	2.23	0.015042	18195064	Aats-met-m	MARS2	−2.04	0.006981
18202255	CG34283	GTSF1L	2.2	0.01418	18132382	CG17834	N/A	−2.05	0.034006
18191104	CG34436	N/A	2.16	0.015331	18199863	CG5402	N/A	−2.07	0.033
18131563	Ance	ACE	2.15	0.02719	18139318	prd	PAX3	−2.11	0.02995
18130728	salr	SALL4	2.11	0.043396	18141809	Vps52	VPS52	−2.15	0.006981
18179578	CG13053	N/A	2.11	0.041534	18138505	Phae1	PRSS53	−2.16	0.023724
18141944	CG9107	RRP7A	2.09	0.023266	18193273	Blm	BLM	−2.17	0.047191
18144661	CG31639	UCHL5	2.08	0.013804	18187075	CG16727	SLC22A24	−2.18	0.044165
18195851	CG11286	LAPTM5	2.07	0.006981	18133474	CG2772	LIPN	−2.3	0.0268
18180053	CG32232	TUBGCP5	2.04	0.018899	18190914	CG34278	N/A	−2.32	0.033063
18201456	CG31244	TACO1	2.04	0.026546	18160388	CG8195	SLC35F5	−2.41	0.027265
18186923	CG7694	RNF181	2.03	0.0268	18185768	CG14715	FKBP2	−2.51	0.034006
18201815	CG32945	N/A	2.01	0.010525	18199680	TwdlP	N/A	−2.51	0.015833
18200489	CG15563	N/A	1.97	0.025277	18206397	Cp7Fb	N/A	−2.62	0.015445
18146064	CG42399	FAM179B	1.97	0.02644	18152912	CG3829	SCARB2	−2.74	0.01418
18185352	CG8129	SRR	1.95	0.042771	18178266	CG13047	N/A	−3.53	0.043603
18136832	Qtzl	SCLY	1.92	0.047532	18186763	CG17283	REN	−4.83	0.033
18167562	CG12017	N/A	1.92	0.006981	18170763	CG11458	N/A	−5.23	0.019055
18142751	CG4438	PDAP1	1.89	0.0268	18169512	CG13445	N/A	−6.13	0.015445
18175784	CG2211	JMJD6	1.88	0.027711	18169271	CG8100	N/A	−15.57	0.006981
18190415	CG31465	N/A	1.87	0.004053	18171820	CG33500	N/A	−18.66	0.010997
18186284	CG3259	TRAF3IP1	1.86	0.01269					
18138632	CG43707	RHPN2	1.86	0.030878					
18136825	ZnT33D	SLC30A3	1.85	0.017284					
18133131	Der-1	DERL1	1.85	0.004184					
18164674	CG43108	N/A	1.84	0.046581					
18131918	SA	STAG1	1.84	0.046053					
18131649	Try29F	PRSS3	1.84	0.01418					
18204185	CG32006	FOXJ1	1.83	0.029567					
18143367	CG17036	SLC19A2	1.82	0.004625					
18183737	Gcn2	EIF2AK4	1.78	0.02644					
18158424	CG14591	TMEM164	1.75	0.02669					
18153402	CG30056	N/A	1.67	0.025134					
18181298	nbs	NBN	1.65	0.049927					
18199613	CG4960	REEP5	1.64	0.041962					

(*continued on next page*)

Table 1 (*continued*)

Up-regulated genes					Down-regulated genes				
Transcript Cluster ID	Fly Gene Symbol	Human Ortholog	Fold Change	FDR p-value	Transcript Cluster ID	Fly Gene Symbol	Human Ortholog	Fold Change	FDR p-value
18198659	CG6475	UGT2B10	1.63	0.038221					
18145247	Muc30E	MPV17L2	1.62	0.041534					
18180076	CG32263	N/A	1.62	0.045041					
18142588	Ostgamma	TUSC3	1.61	0.015445					
18177436	Ufd1-like	UFD1L	1.61	0.018179					
18203120	CG42778	N/A	1.61	0.043368					
18192979	Dip-C	PEPD	1.6	0.046053					
18160269	Adgf-E	CECR1	1.58	0.020284					
18138273	CG43050	MAIP1	1.58	0.018766					
18152420	CG9863	N/A	1.58	0.046581					
18134839	CG6094	MRPL58	1.57	0.028082					
18164795	CG43326; CG43325	N/A	1.57	0.03253					
18149666	Cyp4p3	CYP4Z1	1.57	0.010525					
18177352	CG6674	TSSC4	1.56	0.046581					
18161537	lox2	LOXL3	1.56	0.026405					
18185958	Gnmt	GNMT	1.55	0.0365					
18134385	CG13090	MOCS3	1.55	0.027298					
18175967	CG5687	SLC5A6	1.55	0.015445					
18199611	CG4956	ZDHHC24	1.54	0.04802					
18153798	Mal-A6	SLC3A1	1.53	0.010525					
18196850	CG3313	DCAF12	1.52	0.010997					
18153174	Obp56i	N/A	1.51	0.044165					
18163701	CG34216	N/A	1.51	0.014708					
18142869	CG5846	SMOX	1.5	0.046053					
18177135	CG5653	RFXANK	1.5	0.020284					
18162351	CG16926	N/A	1.5	0.048525					

decreased levels of ROS induced by *keap1* knockdown do not cause any significant alterations in *maf-S* transcription (Fig. 4A), suggesting that Maf-S expression is responsive to oxidative stress in the *Drosophila* testis. Interestingly, we found that the expression of the oxidative stress response gene *GstE1* is also downregulated upon high ROS (Table 1, Fig. 4B), whereas the expression of other oxidative stress response genes such as *GstE9*, *Gclc* (*Glutamate-cysteine ligase catalytic subunit*) and *Gclm* (*Glutamate-cysteine ligase modifier subunit*) showed a trend of increase upon high ROS in our microarray experiment (data not shown). In an attempt to show that *GstE1* expression is related to Maf-S, we assessed whether overexpression of Maf-S can restore the decreased expression of *GstE1* caused by *ND75* knockdown. We found that ectopic expression of Maf-S efficiently restores *GstE1* expression, suggesting that *GstE1* expression is associated with Maf-S (Fig. 4C). It has been reported that many stimuli such as H_2O_2, cadmium and zinc, which can induce Nrf2 activity, enhance the expression of MafG [36–38]. On the other hand, exogenous H_2O_2 decreased c-Maf expression in human adipose tissue-derived mesenchymal stem cells (hAMSCs) [39]. These observations may suggest that *maf* genes are transcriptionally modulated upon redox states in a cell, tissue and/or context-specific manner.

3.4. Knockdown of maf-S promotes GSC differentiation

To examine the potential role of Maf-S in GSC maintenance, we first assessed if Maf-S can function in redox homeostasis by quantifying DHE staining in testes ectopically expressing *maf-S*^{RNAi} or Maf-S under the control of *nos*-Gal4. While *maf-S* knockdown significantly increased ROS levels, ectopic expression of Maf-S decreased ROS levels, suggesting that Maf-S plays a role in the maintenance of redox homeostasis (Supplementary Fig. 1). Since high levels of ROS downregulate *maf-S* transcription and decrease GSC number by promoting GSC differentiation, we asked whether knockdown of *maf-S* is indeed associated

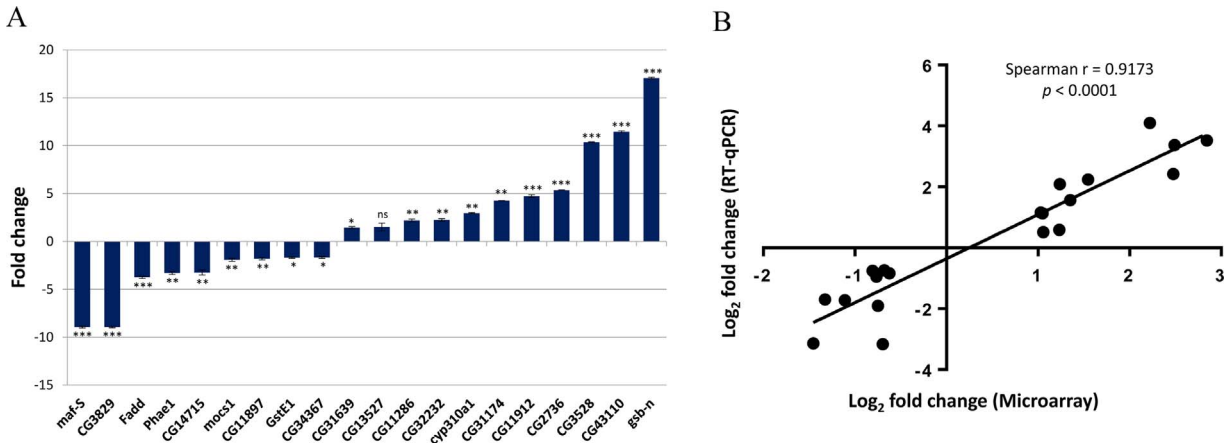

Fig. 3. Validation of microarray data by qRT-PCR analysis. (A) qRT-PCR analysis. Randomly selected 20 genes were validated by qRT-PCR analyses using the same RNA extracted for microarray analysis. Error bar is SEM from three independent experiments. Significance was assessed using unpaired *t*-test (*$p < 0.05$, ***$p < 0.01$, ***$p < 0.001$). (B) Spearman's rho. Expression pattern of the 20 genes tested by qRT-PCR is highly correlated to that observed in microarray analysis, with a Spearman's correlation coefficient value of 0.9173 ($P < 0.0001$).

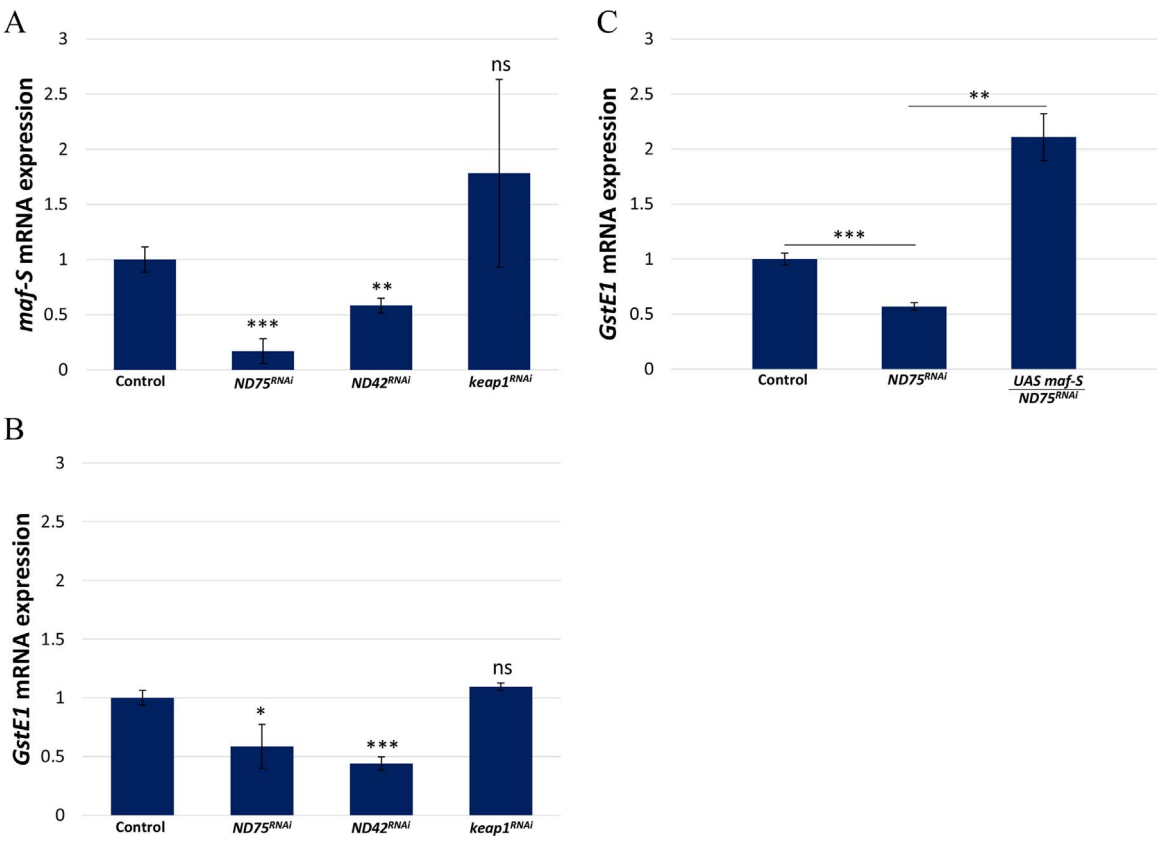

Fig. 4. The gene *maf-S* is transcriptionally downregulated upon oxidative stress. The mRNA levels of (A) *maf-S* and (B) *GstE1* are downregulated upon increased levels of ROS induced by knockdown of *ND75* or *ND42*, but are not affected upon decreased levels of ROS induced by knockdown of *keap1*. (C) Overexpression of Maf-S restores the decreased expression of *GstE1* caused by *ND75* knockdown. Error bar is SEM from three independent experiments. Significance was assessed using unpaired *t*-test (*$p < 0.05$, ***$p < 0.01$, ***$p < 0.001$).

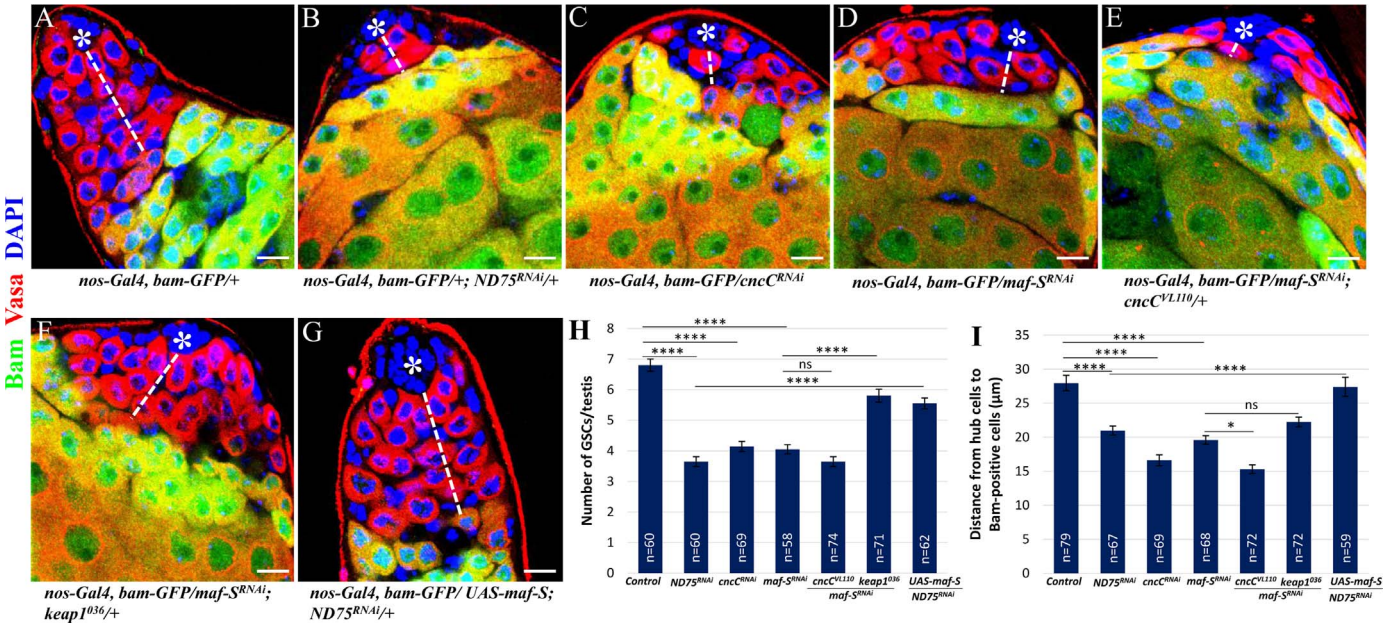

Fig. 5. Maf-S inhibition causes a decrease in GSC number by promoting GSC differentiation. (A, B, C and H) GSC number in testes with *ND75* or *CncC* knockdown decreases as compared to that in control testes. (D and H) *maf-S* knockdown testes also show decreased number of GSCs, suggesting that Maf-S is required for GSC homeostasis. (E, F and H) Inhibitory effects of Maf-S on GSC number in $cncC^{+/-}$ and in $keap1^{+/-}$ testes. (G and H) Ectopic expression of Maf-S rescues the inhibitory effects of ND75 on GSC number, suggesting that Maf-S acts as a downstream effector of high ROS-associated GSC differentiation. (A, B, C, D, I) Bam-positive germ cells in testes expressing $ND75^{RNAi}$, $cncC^{RNAi}$ or $maf\text{-}S^{RNAi}$ are detected closer to hub cells as compared to those in control testes. (E, F and I) Inhibitory effects of Maf-S on GSC differentiation in $cncC^{+/-}$ and in $keap1^{+/-}$ testes. (G and I) Ectopic expression of Maf-S rescues the $ND75^{RNAi}$ phenotype of GSC differentiation. Error bar is SEM from more than three independent experiments. Significance was assessed using one-way ANOVA with post-hoc Bonferroni test (*$p < 0.05$, ****$p < 0.0001$). Scale bar, 10 µM. *hub cells.

with the decrease in GSCs. As a positive control, we inhibited ND75 under the control of *nos*-Gal4 in testes to show that high levels of ROS decrease GSC number as compared to control (Fig. 5A, B and H). To confirm the effects of high ROS on GSC number, we disrupted the activity of CncC, the *Drosophila* homolog of Nrf2, by expressing $cncC^{RNAi}$, and found that CncC inhibition also results in a decrease in GSC number as compared to control (Fig. 5A, C and H). Interestingly, *maf-S* knockdown also led to a significant decrease in GSC number, indicating that Maf-S is a downstream effector of ROS-induced GSC differentiation and is required for GSC maintenance (Fig. 5A, D and H). To examine if Maf-S genetically interacts with Keap1/Nrf2 (CncC) in GSC homeostasis, Maf-S was inhibited in testes heterozygous for *cncC* ($cncC^{VL110}$) or *keap1* ($keap1^{036}$). The inhibitory effect of Maf-S on GSC number was not enhanced in $cncC^{+/-}$ testes, but significantly suppressed in $keap1^{+/-}$ testes (Fig. D, E, F and H). Since Maf-S is a downstream effector of ROS signaling, we hypothesized that ectopic expression of Maf-S would suppress the phenotype of GSC loss induced by oxidative stress. As expected, the effect of ND75 inhibition on GSC number was greatly bypassed by Maf-S overexpression (Fig. 5B, G and H).

We next examined the expression of Bam to confirm the above mentioned findings. Bam expression was detected much closer to hub cells in testes expressing either $ND75^{RNAi}$, $cncC^{RNAi}$ or $maf\text{-}S^{RNAi}$, as compared to control, suggesting that elevated ROS levels decreased GSC number by promoting their differentiation (Fig. 5A, B, C, D and I). Furthermore, we found that Maf-S inhibition-promoted GSC differentiation was further enhanced by removing one copy of *cncC* alleles, but was not significantly suppressed by removing one copy of *keap1* alleles (Fig. 5D, E, F and I). Importantly, Bam-positive cells were detected further away from hub cells in testes co-expressing $ND75^{RNAi}$ and *Maf-S*, as compared to those in testes expressing $ND75^{RNAi}$ alone (Fig. 5B, G and I). It is worth to note that knockout of all three sMafs in mouse embryonic fibroblasts was shown to interfere with the induction of antioxidant genes such as *thioredoxin reductase 1*, suggesting the essential role of sMafs in scavenging excessive ROS [38]. The oxidative stress-sensitive c-Maf has also been shown to be downregulated by exogenous H_2O_2 which is associated with H_2O_2-mediated reduced pluripotency and adipogenic differentiation of hAMSCs [39]. Together with the facts that Maf-S functions in the maintenance of redox homeostasis and genetically interacts with Keap1/CncC in GSC behavior, our data suggests that Maf-S plays an important role in GSC maintenance by engaging in the regulation of redox balance in the *Drosophila* testis.

3.5. Ectopic expression of Maf-S causes an over-growth of GSC-like cells

Since loss of function of Maf-S promoted GSC differentiation, we hypothesized that ectopic expression of Maf-S may conversely promote GSC growth by decreasing intracellular ROS levels. We previously showed that germ cells mutant for *cncC* exhibit higher ROS levels than neighbouring control cells, suggesting that ectopic expression of CncC decreases ROS levels [18]. Interestingly, testes overexpressing CncC contained a dramatically increased number of Vasa- and *esg*-LacZ-positive cells, which are distributed widely throughout the testes and appear as single cells, whereas *esg*-LacZ-positive cells were found to be restricted to the apical tip of control testes (Fig. 6A and B). Indeed, 22.7% of testes (n = 132) overexpressing CncC showed the phenotype of GSC-like cell proliferation (Fig. 6F). We also observed the similar phenotype in 9.1% of testes (n = 109) overexpressing Maf-S, suggesting that Maf-S overexpression can promote the proliferation of GSC-like cells, possibly through lowering ROS levels *via* activating the transcription of antioxidant genes (Fig. 6C and F). We next overexpressed Maf-S in $cncC^{+/-}$ testes to examine their genetic interaction in the growth of GSC-like cells, and found a trend of almost two-fold decrease in the penetrance by removing one copy of *cncC* alleles, although the result did not achieve statistical significance at the 0.05 level (Fig. 6C, D and F). In a *keap1*-null background, many of the ARE-dependent genes have been shown to be persistently activated in an Nrf2-dependent manner [38]. Furthermore, we previously showed that decreased levels of ROS by Keap1 inhibition causes an over-growth of GSC-like cells [18]. Thus, we next overexpressed Maf-S in testes heterozygous for *keap1* ($keap1^{EY5}$), expecting that more ARE-dependent gene products become available. As expected, the penetrance was drastically elevated upon removing one copy of *keap1* alleles as compared to that observed in testes expressing Maf-S alone (9.1% *vs*. 18.8%) (Fig. 6C, E and F), suggesting that the over-growth of GSC-like cells observed in testes expressing Maf-S is associated with the downregulation of ROS levels.

In an attempt to demonstrate that the over-growth phenotype of GSC-like cells is a cause of increased mitotic activity of germ cells, we next examined the expression of phospho-Histone H3 (pH3), a mitotic marker detected only in actively dividing cells [40]. In control testes, pH3 staining is detected only in self-renewing GSCs directly attached to hub cells, as well as in transit-amplifying spermatogonia (Fig. 6A'). However, testes overexpressing CncC or Maf-S showed a significantly increased number of both Vasa- and pH3-positive cells throughout the testes (Fig. 6B' and C'), suggesting that germ cell lineage underwent abnormal cell division. Notably, the over-growth of germ cells caused by Maf-S overexpression was not significantly affected in $cncC^{+/-}$ testes, but greatly enhanced in $keap1^{+/-}$ testes (Fig. 6C', D', E' and G).

In mammals, sMafs form heterodimers with other specific bZIP transcription factors, such as CNC and Bach family members. Notably, sMafs were reported to be an indispensable partner of Nrf2 in various cellular processes such as the transcriptional activation of antioxidant enzymes and keratinocyte differentiation [38,41]. In *Drosophila,* Maf-S was shown to interact both physically and genetically with CncC [42,43]. Overexpression of Maf-S or its dimerization partner, CncC, could restore the locomotor activity in the *Drosophila* model of Parkinson's disease, which is the most common neurodegenerative movement disorder highly associated with oxidative stress [44]. Furthermore, it was reported that ectopic expression of Maf-S can restore the age-associated decline in the oxidative stress resistance and can upregulate the expression of the CncC-target antioxidant genes such as *gstD1* (*Glutathione S transferase D1*), *gclc and gclm* [43]. Importantly, these observations are in accordance with our finding that Maf-S genetically interacts with Keap1/CncC in GSC homeostasis, and ectopic expression of Maf-S or CncC results in the similar phenotype of GCS-like cell proliferation in the *Drosophila* testis.

4. Conclusion

The disruption of redox balance interferes with the maintenance and self-renewal of various stem cell populations by affecting their proliferation, differentiation and senescence. Furthermore, oxidative stress has been implicated in a wide variety of human diseases, including neurodegenerative diseases, cardiovascular diseases, diabetes mellitus type 2 and cancers. Hence, it is essential to elucidate molecular mechanisms by which altered intracellular ROS levels induce these biological processes. Here, we performed Affymetrix microarray analysis to identify the downstream effectors of ROS signaling that may control GSC differentiation in the *Drosophila* testis. We demonstrated that 1) 152 genes are transcriptionally modulated during GSC differentiation, and 31% of the identified genes have no predicted molecular function and protein domains, suggesting that our analysis identified many uncharacterized genes, possibly with essential roles in ROS-associated stem cell homeostasis; 2) *maf-S* transcription is downregulated upon oxidative stress; 3) Maf-S functions in the maintenance of redox homeostasis; 4) *maf-S* knockdown and Maf-S overexpression promotes premature GSC differentiation and the over-growth of GSC-like cells, respectively; 5) premature GSC differentiation induced by oxidative stress is suppressed by the ectopic expression of Maf-S, indicating that Maf-S acts as a downstream effector of ROS signaling; 6) Maf-S genetically interacts with Keap1/CncC in GSC homeostasis. Taken altogether, our study reveals that Maf-S is one of the key downstream

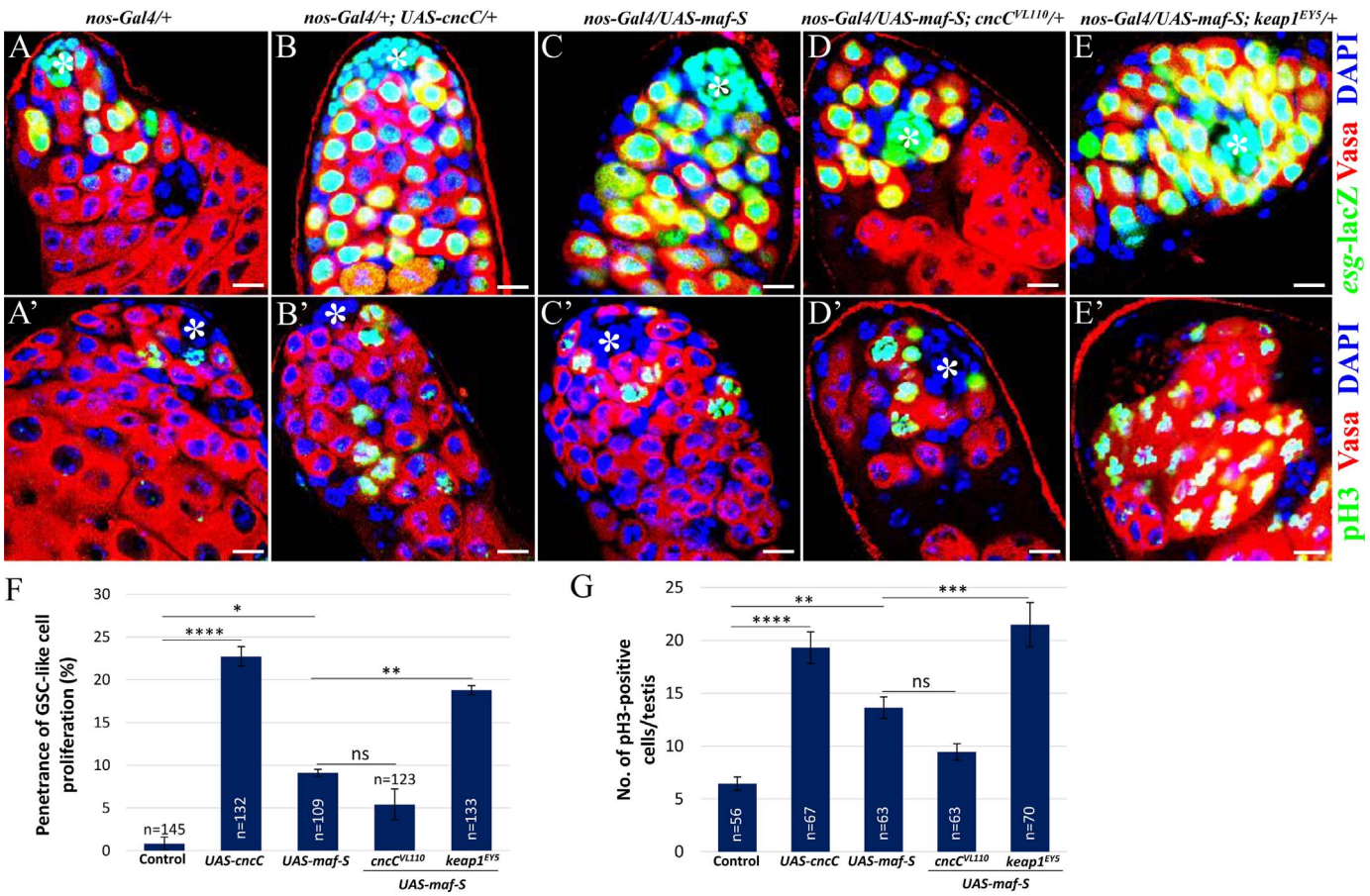

Fig. 6. Ectopic expression of Maf-S causes an over-growth of GSC-like cells. (A, B, C, D and E) Testes stained for *esg*-lacZ. As compared to (A) control, ectopic expression of (B) CncC or (C) Maf-S causes a drastic increase in the number of *esg*-lacZ-positive early-stage germ cells. (D and E) Effects of Maf-S overexpression on GSC-like cells in $cncC^{+/-}$ and in $keap1^{+/-}$ testes. (F) Quantification of the penetrance of GSC-like cell proliferation. (A', B', C', D' and E') Testes stained for pH3, which acts as a mitotic marker. As compared to (A') control, ectopic expression of (B') CncC or (C') Maf-S significantly increases the number of pH3-positive germ cells. (D' and E') Effects of Maf-S overexpression on pH3-positive cell number in $cncC^{+/-}$ and in $keap1^{+/-}$ testes. (G) Quantification of pH3-postive cell number. Error bar is SEM from more than three independent experiments. Significance was assessed using one-way ANOVA with post-hoc Bonferroni test ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$ and $****p < 0.0001$). Scale bar, 10 μM. *hub cells.

effectors of redox signaling that controls GSC behavior in the *Drosophila* testis.

Acknowledgements

We thanks D. Bohmann, L. Liu, S. Dinardo, D.M. Mckearin, H. Jasper, NIG-FLY Stock Center and BDSC for fly stocks; DSHB for antibodies. We also thanks S.L. Bay for her assistance in preparing the illustration. This work was supported by MOE TIER1 (R-181-000-172-112).

Author contribution

W.S.T. performed the experiments and wrote the manuscript. G.W.Y. analysed the microarray data. T.S. proofread the manuscript. G.H.B designed the experiments and wrote the manuscript.

Conflict of interest statement

The authors have no conflict of interest to declare.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.redox.2017.12.002.

References

[1] C.L. Bigarella, R. Liang, S. Ghaffari, Stem cells and the impact of ROS signaling, Development 141 (22) (2014) 4206–4218.
[2] G.Y. Liou, P. Storz, Reactive oxygen species in cancer, Free Radic. Res. 44 (5) (2010) 479–496.
[3] D. Trachootham, J. Alexandre, P. Huang, Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? Nat. Rev. Drug Discov. 8 (7) (2009) 579–591.
[4] V. Shukla, S.K. Mishra, H.C. Pant, Oxidative stress in neurodegeneration, Adv. Pharmacol. Sci. 2011 (2011) 572634.
[5] D. Trachootham, W. Lu, M.A. Ogasawara, R.D. Nilsa, P. Huang, Redox regulation of cell survival, Antioxid. Redox Signal. 10 (8) (2008) 1343–1374.
[6] T. Finkel, Signal transduction by reactive oxygen species, J. Cell Biol. 194 (1) (2011) 7–15.
[7] Y.M. Janssen-Heininger, B.T. Mossman, N.H. Heintz, H.J. Forman, B. Kalyanaraman, T. Finkel, et al., Redox-based regulation of signal transduction: principles, pitfalls, and promises, Free Radic. Biol. Med. 45 (1) (2008) 1–17.
[8] T. Reya, S.J. Morrison, M.F. Clarke, I.L. Weissman, Stem cells, cancer, and cancer stem cells, Nature 414 (6859) (2001) 105–111.
[9] R.R. Ager, J.L. Davis, A. Agazaryan, F. Benavente, W.W. Poon, F.M. LaFerla, et al., Human neural stem cells improve cognition and promote synaptic growth in two complementary transgenic models of Alzheimer's disease and neuronal loss, Hippocampus 25 (7) (2015) 813–826.
[10] S. Ratanasirintrawoot, N. Israsena, Stem Cells in the Intestine: Possible Roles in Pathogenesis of Irritable Bowel Syndrome, J. Neurogastroenterol. Motil. 22 (3) (2016) 367–382.
[11] R. Liang, S. Ghaffari, Stem cells, redox signaling, and stem cell aging, Antioxid. Redox Signal. 20 (12) (2014) 1902–1916.
[12] Y.Y. Jang, S.J. Sharkis, A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche, Blood 110 (8) (2007) 3056–3063.
[13] K. Naka, T. Muraguchi, T. Hoshii, A. Hirao, Regulation of reactive oxygen species and genomic stability in hematopoietic stem cells, Antioxid. Redox Signal. 10 (11)

(2008) 1883–1894.
[14] N.R. Forsyth, A. Musio, P. Vezzoni, A.H. Simpson, B.S. Noble, J. McWhir, Physiologic oxygen enhances human embryonic stem cell clonal recovery and reduces chromosomal abnormalities, Cloning Stem Cells 8 (1) (2006) 16–23.
[15] Y.L. Guo, S. Chakraborty, S.S. Rajan, R. Wang, F. Huang, Effects of oxidative stress on mouse embryonic stem cell proliferation, apoptosis, senescence, and self-renewal, Stem Cells Dev. 19 (9) (2010) 1321–1331.
[16] R. Domenis, N. Bergamin, G. Gianfranceschi, C. Vascotto, M. Romanello, S. Rigo, et al., The redox function of APE1 is involved in the differentiation process of stem cells toward a neuronal cell fate, PLoS One 9 (2) (2014) e89232.
[17] M. de Cuevas, E.L. Matunis, The stem cell niche: lessons from the Drosophila testis, Development 138 (14) (2011) 2861–2869.
[18] S.W.S. Tan, Q.Y. Lee, B.S.E. Wong, Y. Cai, G.H. Baeg, Redox homeostasis plays important roles in the maintenance of the Drosophila testis germline stem cells, Stem Cell Rep. 9 (1) (2017) 342–354.
[19] G.P. Sykiotis, D. Bohmann, Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in Drosophila, Dev. Cell 14 (1) (2008) 76–85.
[20] N.A. Terry, N. Tulina, E. Matunis, S. DiNardo, Novel regulators revealed by profiling Drosophila testis stem cells within their niche, Dev. Biol. 294 (1) (2006) 246–257.
[21] D. Chen, D. McKearin, Dpp signaling silences bam transcription directly to establish asymmetric divisions of germline stem cells, Curr. Biol. 13 (20) (2003) 1786–1791.
[22] E.B. Tahara, F.D. Navarete, A.J. Kowaltowski, Tissue-, substrate-, and site-specific characteristics of mitochondrial reactive oxygen species generation, Free Radic. Biol. Med. 46 (9) (2009) 1283–1297.
[23] A. Iuso, S. Scacco, C. Piccoli, F. Bellomo, V. Petruzzella, R. Trentadue, et al., Dysfunctions of cellular oxidative metabolism in patients with mutations in the NDUFS1 and NDUFS4 genes of complex I, J. Biol. Chem. 281 (15) (2006) 10374–10380.
[24] S.A. Sinenko, J. Shim, U. Banerjee, Oxidative stress in the haematopoietic niche regulates the cellular immune response in Drosophila, EMBO Rep. 13 (1) (2011) 83–89.
[25] I. Lopez-Fabuel, J. Le Douce, A. Logan, A.M. James, G. Bonvento, M.P. Murphy, et al., Complex I assembly into supercomplexes determines differential mitochondrial ROS production in neurons and astrocytes, Proc. Natl. Acad. Sci. USA 113 (46) (2016) 13063–13068.
[26] Y. Jiang, Y. Cao, Y. Wang, W. Li, X. Liu, Y. Lv, et al., Cysteine transporter SLC3A1 promotes breast cancer tumorigenesis, Theranostics 7 (4) (2017) 1036–1046.
[27] Y. Amano, M. Mandai, K. Yamaguchi, N. Matsumura, B. Kharma, T. Baba, et al., Metabolic alterations caused by HNF1beta expression in ovarian clear cell carcinoma contribute to cell survival, Oncotarget 6 (28) (2015) 26002–26017.
[28] K.F. Harvey, X. Zhang, D.M. Thomas, The Hippo pathway and human cancer, Nat. Rev. Cancer 13 (4) (2013) 246–257.
[29] D. Shao, P. Zhai, D.P. Del Re, S. Sciarretta, N. Yabuta, H. Nojima, et al., A functional interaction between Hippo-YAP signalling and FoxO1 mediates the oxidative stress response, Nat. Commun. 5 (2014) 3315.
[30] R.S. Friling, A. Bensimon, Y. Tichauer, V. Daniel, Xenobiotic-inducible expression of murine glutathione S-transferase Ya subunit gene is controlled by an electrophile-responsive element, Proc. Natl. Acad. Sci. USA 87 (16) (1990) 6258–6262.
[31] T.H. Rushmore, M.R. Morton, C.B. Pickett, The antioxidant responsive element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity, J. Biol. Chem. 266 (18) (1991) 11632–11639.
[32] H. Motohashi, T. O'Connor, F. Katsuoka, J.D. Engel, M. Yamamoto, Integration and diversity of the regulatory network composed of Maf and CNC families of transcription factors, Gene 294 (1–2) (2002) 1–12.
[33] A. Kobayashi, M.I. Kang, H. Okawa, M. Ohtsuji, Y. Zenke, T. Chiba, et al., Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2, Mol. Cell Biol. 24 (16) (2004) 7130–7139.
[34] Y. Liu, J.T. Kern, J.R. Walker, J.A. Johnson, P.G. Schultz, H. Luesch, A genomic screen for activators of the antioxidant response element, Proc. Natl. Acad. Sci. USA 104 (12) (2007) 5205–5210.
[35] T.W. Kensler, N. Wakabayashi, S. Biswal, Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway, Annu Rev. Pharmacol. Toxicol. 47 (2007) 89–116.
[36] D.R. Crawford, K.P. Leahy, Y. Wang, G.P. Schools, J.C. Kochheiser, K.J. Davies, Oxidative stress induces the levels of a MafG homolog in hamster HA-1 cells, Free Radic. Biol. Med. 21 (4) (1996) 521–525.
[37] T. Suzuki, V. Blank, J.S. Sesay, D.R. Crawford, Maf genes are involved in multiple stress response in human, Biochem. Biophys. Res. Commun. 280 (1) (2001) 4–8.
[38] F. Katsuoka, H. Motohashi, T. Ishii, H. Aburatani, J.D. Engel, M. Yamamoto, Genetic evidence that small maf proteins are essential for the activation of antioxidant response element-dependent genes, Mol. Cell Biol. 25 (18) (2005) 8044–8051.
[39] P.M. Chen, C.H. Lin, N.T. Li, Y.M. Wu, M.T. Lin, S.C. Hung, et al., c-Maf regulates pluripotency genes, proliferation/self-renewal, and lineage commitment in ROS-mediated senescence of human mesenchymal stem cells, Oncotarget 6 (34) (2015) 35404–35418.
[40] C. Tapia, H. Kutzner, T. Mentzel, S. Savic, D. Baumhoer, K. Glatz, Two mitosis-specific antibodies, MPM-2 and phospho-histone H3 (Ser28), allow rapid and precise determination of mitotic activity, Am. J. Surg. Pathol. 30 (1) (2006) 83–89.
[41] H. Motohashi, M. Yamamoto, Nrf2–Keap1 defines a physiologically important stress response mechanism, Trends Mol. Med. 10 (11) (2004) 549–557.
[42] A. Veraksa, N. McGinnis, X. Li, J. Mohler, W. McGinnis, Cap 'n' collar B cooperates with a small Maf subunit to specify pharyngeal development and suppress deformed homeotic function in the Drosophila head, Development 127 (18) (2000) 4023–4037.
[43] M.M. Rahman, G.P. Sykiotis, M. Nishimura, R. Bodmer, D. Bohmann, Declining signal dependence of Nrf2-MafS-regulated gene expression correlates with aging phenotypes, Aging Cell 12 (4) (2013) 554–562.
[44] M.C. Barone, G.P. Sykiotis, D. Bohmann, Genetic activation of Nrf2 signaling is sufficient to ameliorate neurodegenerative phenotypes in a Drosophila model of Parkinson's disease, Dis. Model Mech. 4 (5) (2011) 701–707.