Higher gene expression stability during aging in long-lived giant molerats than in short-lived rats

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ABSTRACT

Many aging-associated physiological changes are known to occur in short- and long-lived species with different trajectories. Emerging evidence suggests that numerous life history trait differences between species are based on interspecies variations in gene expression. Little information is available, however, about differences in transcriptome changes during aging between mammals with diverging lifespans. For this reason, we studied the transcriptomes of five tissue types and two age cohorts of two similarly sized rodent species with very different lifespans: laboratory rats (*Rattus norvegicus*) and giant mole-rats (*Fukomys mechowii*), with maximum lifespans of 3.8 and more than 20 years, respectively. Our findings show that giant mole-rats exhibit higher gene expression stability during aging than rats. Although well-known aging signatures were detected in all tissue types of rats, they were found in only one tissue type of giant mole-rats. Furthermore, many differentially expressed genes that were found in both species were regulated in opposite directions during aging. This suggests that expression changes which cause aging in short-lived species are counteracted in long-lived species. Taken together, we conclude that expression stability in giant mole rats (and potentially in African mole-rats in general) may be one key factor for their long and healthy life.

INTRODUCTION

Compared to short-lived mammals, long-lived mammals have repeatedly been shown to exhibit fewer age-associated changes in numerous physiological parameters related to the functional decline during aging [1-4]. Recent RNA-seq studies have suggested that much of the remarkable lifespan diversity among mammals is based on interspecies differences in gene expression [5, 6]. However, those studies focused on identifying particular genes and pathways that are

differentially expressed between species with divergent longevities. Whether short- and long-lived species differ at the transcript level with respect to their amount of differentially expressed genes (DEGs) during aging (hereinafter referred to as "gene expression stability") has, to the best of our knowledge, not been explored yet.

Here, we examined age associated transcriptome changes in two similarly sized rodent species with different longevities: the laboratory rat (*Rattus norvegicus*), which has a maximum lifespan of 3.8 years



Figure 1. Comparative transcriptomics in giant mole-rats and laboratory rats of elderly vs. young individuals. (A) Counts of differentially expressed genes (DEGs) during aging in five tissue types from laboratory rats (*Rattus norvegicus*) and giant mole-rat (*Fukomys mechowii*). Only orthologous genes in both transcript catalogs were counted (n=14,062). (B) Numbers of biological processes (Gene Ontology) enriched for DEGs during aging in five tissue types from laboratory rats (*R. norvegicus*) and giant mole-rats (*F. mechowii*).

[7], and the giant mole-rat (Fukomys mechowii), which has a maximum lifespan of more than 20 years ([8] and own unpublished data). In giant mole-rats, longevity is significantly correlated with the reproductive status. Breeding animals outlive non-breeders by far [8]. In the current study, we examined only non-breeding males. Male non-breeding giant mole-rats have a maximum lifespan of approximately 10 years and an average lifespan of approximately 6 years, still clearly exceeding the life expectancy of the laboratory rat [8]. For both species, we performed RNA-seq on tissue samples from five organs (blood, heart, kidney, liver, and skin; hereinafter called simply tissues) of young and elderly adults. The tissues were collected from young and elderly cohorts of laboratory rats (0.5 and 2.0 years) and giant mole-rats (young: approximately 1.5 years at average; elderly: approximately 6.8 years at average; see Tables S1-S3 for details). For both species, the first time points were chosen to sample young, sexually mature adults. The second time points correspond to an age-associated survival rate of less than 40% in rats and giant mole-rats (Tables S1-S3) [8, 9]. For each species, we determined DEGs between the two respective time points and searched for enriched functional categories.

RESULTS

The giant mole-rat transcriptomes changed much less during aging (Tables S4-S13). In four of five tissue types, the number of orthologous DEGs in the giant mole-rats was only a fraction of the respective number in the laboratory rats (0.6%-19.0\%; Fig. 1a). The number of DEGs was similar only in the blood of both species but still was 40% lower in blood from the giant mole-rats than in blood from the laboratory rats. Across tissues, the giant mole-rat transcriptomes contained significantly fewer DEGs during aging than did the laboratory rats (P = 0.016, Wilcoxon signed-rank test).

To ensure that the low number of identified DEGs in the giant mole-rat was not caused by a low statistical power compared to the rat, we examined the statistical power



Figure 2. REVIGO treemap summary of gene ontology processes that are significantly enriched (false discovery rate [FDR] < 0.05) for differentially expressed genes during aging. For each species and tissue, the superclusters, *i.e.*, the highest summarization level of gene ontology processes, as identified by REVIGO [13] are shown. Each rectangle painted with a unique color represents a supercluster. The colors only serve to distinguish superclusters. The size of the rectangles represents their p-value, *i.e.*, largest rectangles represent the most significant superclusters. For giant mole-rat skin, no treemap could be generated since no gene ontology process was significantly enriched (Fig. 1b). Corresponding REVIGO treemap summarizations are provided as high-resolution Figures S1-S9, showing also the clusters within the superclusters.

per species and tissue using the RNA-seq data dispersion [10]. We estimated a statistical power of 83-95% and 80-90% in giant mole-rat and rat, respectively, depending on the tissue (Table S14). Thus, this finding corroborates our evidence that the lower number of detected DEGs in the giant mole-rat might indeed reflect a greater expression stability during aging. Furthermore, we ensured that there was no relevant difference in the measured gene expression levels between the examined species (Fig. S10, all DEGs). Those genes, however, that were found to be differentially expressed in both species (Fig. S10, overlapping DEGs), tended to be lower expressed in the rat and higher expressed in the giant mole-rat, compared to median across all DEGs.

Upon Gene Ontology [11] analysis of the differentially expressed genes, we found typical molecular aging signatures across all examined tissues in the rat (Fig. 1b). For instance, altered expression levels of immune response genes (Gene Ontology [GO]:0006955; Tables S15-S24) and inflammatory response genes (GO:0006954) are known to be hallmarks of aging [12]. These, as well as many related processes, such as response to cytokine (GO:0034097) and leukocyte aggregation (GO:0070486), were consistently enriched for DEGs in all examined laboratory rat tissues. In the giant mole-rat, on the other hand, we found these signatures only in blood.

The clustering of DEG enriched biological processes with REVIGO [13] revealed that immune-related functions, immune process or regulation of immune process, determine the largest superclusters in four of five rat tissues (Fig. 2, Fig. S1-S9). Additional agingrelevant clusters found across rat tissues were apoptotic process (GO:0006915; all tissues except heart), coagulation (GO:0050817; all tissues) and oxidationreduction process (GO:0055114; all tissues except liver). Except in blood, the giant mole-rat did not exhibit the same (or similar) DEG enriched biological processes. These findings indicate typical agingdependent gene expression alterations are slowed down in several vital tissues of giant mole-rats.

On the single gene level, there was a modest but still statistically significant (P < 0.05; Fisher's exact test) overlap between the DEGs of laboratory rats and those of giant mole-rats in blood, heart, and skin tissues (Fig. 1a; Tables S25-S29). Common DEGs in the blood of laboratory rat and giant mole-rat were often regulated in the same direction (up or down) during aging in both species ($P = 3.3*10^{-31}$; Fisher's exact test based on regulation of all genes). This finding matches the shared aging signatures (DEG function analysis, see above) in this tissue. Interestingly, in skin samples we found a contrasting overrepresentation of DEGs, which are regulated in opposite directions (P = 0.005). This finding points to the intriguing possibility that, in some tissues, expression changes that cause aging in the laboratory rat are counteracted by opposite changes during aging in the giant mole-rat.

In kidney tissues, most shared DEGs were regulated in opposite directions between species during aging (Fig. 1a). As an example, collagen metabolic process (GO:0032963) is one of the seven processes that are enriched in the kidneys of both laboratory rats and giant mole-rats. Although the enrichment in the laboratory rat was based on 20 collagen genes that were significantly up-regulated and one that was down-regulated during aging, in the giant mole-rat this enrichment resulted from four collagens and two genes that code for potent collagenases (*CTSK* and *CTSS*), all of which were down-regulated during aging. Of these six collagenases and collagens, five overlapped with those, which were significantly up-regulated in laboratory rats. Collagen regulation reflects well the molecular aging process because decreasing collagen levels attenuate kidney diseases in rats [14], whereas increased collagen levels in the kidney have been shown to induce the development of cysts in rats with polycystic kidney disease [15]. At the same time, kidney diseases are an important cause of death in rats [16] and perhaps also in (naked) mole-rats [17]. The opposite collagen regulation pattern in the giant mole-rat can be interpreted as an anti-aging program rather than as a signature of the aging process.

DISCUSSION

The gene expression stability of giant mole-rats during aging that we show here concurs with a general pattern of stability. This has emerged from numerous molecular and physiological comparisons of the extremely longlived naked mole-rat (Heterocephalus glaber, a close relative of giant mole-rats) with shorter-lived species of mice or rats: For example, during aging, naked molerats maintain an unchanged membrane lipid composition [3], a fairly stable production of reactive oxygen species [18], and relatively stable levels of oxidative damage to lipids [2], as well as high protein stability and integrity [19]. At the same time, all of these variables, which are known to be among the key factors for lifespan and age-related diseases [20], change significantly in an unfavorable direction during aging in short-lived mice or rats. Naked mole-rats also exhibit minimal decline of physiological functions and maintain activity, fertility, and body composition into old age; they are also remarkably resistant to cancer, and their cancer-associated mortality rates do not increase substantially with age [1]. Given that nakedand giant mole-rats are closely related [21], our own husbandry experience with giant mole-rats leads us to assume that several of the aforementioned properties are shared by both species.

In line with our results, an earlier study showed that gene expression in three types of tissue from naked mole-rats remains nearly unchanged during the first half of their lifespan [22]. However, the statistical power of this analysis was very limited because the study used only one replicate per age. Regarding laboratory rats, our results are in good agreement with the findings of the rat body map initiative [23]. This database shows many DEGs (491 to 14,062) across eleven types of tissue during rat aging; the time points used in this study are similar to ours (21 weeks vs. 2 years). The results of Kim et al. [22] and of the rat body map project cannot be directly compared with each other because those studies used different methods for sequencing and DEG detection. Therefore, in this study both species were examined with the same sequencing procedure and the same bioinformatic analyses. Thus, we confirmed that, the gene expression of a long-lived African mole-rat species - in contrast to those of a short-lived rodent indeed remains stable during aging from young to a elderly adulthood. Since gene expression is a basic regulatory process of the cell that determines many of the above-mentioned molecular phenotypes and physiological observations, we suggest that gene expression stability during aging is one of the key causal factors for the extraordinary long and healthy lifespan of this African mole-rat species, and potentially of the whole family.

In conclusion, we hypothesize that the higher gene expression stability observed in long-lived giant molerats compared to short-lived rats evolved under different evolutionary constraints and contributes to the considerably distinct life history traits of the short- and long-lived species: early onset and fast aging in one species, and delayed or slowed aging from youth to elderly adulthood in the other.

MATERIALS AND METHODS

Experimental design

This study compared the transcriptomes of young and elderly animals from two species: Wistar rats (Rattus norvegicus) and giant mole-rats (Fukomys mechowii). Samples from five tissues (blood, heart, kidney, liver, and skin) were taken from animals in both species and both age cohorts. All examined animals were nonbreeding males. Young and elderly laboratory rats were 6 and 24 months of age, respectively, and sampled in April, October and November 2016 (see table S1 for details). Library preparation and sequencing was performed for all but three rat samples in one batch in December 2016 - the remaining three were sequenced in January 2017 (table S3). Young mole-rats were 1.3 to 2.0 years old (grand mean across tissues: 1.5 years). whereas elderly mole-rats were 5.5 to 7.7 years old (grand mean across tissues: 6.8 years). Mole-rats were sampled in 5 distinct sampling sessions between February 2014 and December 2016 (table S1). Sequencing of mole-rat samples was performed in 7 runs across the same time frame (table S3).

We examined samples from 4 to 8 animals per tissue for each age cohort and species (Tables S1-S3). All animals were healthy at the time when they were sacrificed.

For tissue collection, rats were euthanized with CO₂. Mole-rats were anaesthetized with 6 mg/kg ketamine combined with 2.5 mg/kg xylazine and then euthanized by surgical decapitation. Immediately after dissection, tissue samples were transferred to tubes containing RNA-protective buffers and stored in -80°C until analysis.

For both species, the first age group consists of young, sexually mature adults. Their age was approximately one-fourth of the second group's age, which corresponds to a survival fraction of approximately 39% and 24% in rats and giant mole-rats, respectively (Tables S1-S3) [8, 9]. In relation to maximum lifespan the median age at the second time point represents 53% in rats (maximum lifespan in male Rattus norvegicus: 3.8 years) and 68% (maximum lifespan in Fukomys mechowii non-breeders: 10 years) [7, 8]. Thus, the chosen time points represent similar biological ages in the examined species with a wider age-range between the compared time points in giant mole-rats. The latter means that the observed smaller age-related changes of the transcriptomes in giant mole-rats compared to rats are conservative findings.

Animal housing and tissue collection was compliant with national and state legislation (breeding allowances 32-2-1180-71/328 (mole-rats) and 32-2-11-80-71/345 (rats), both Ordnungsamt Essen, Northrhine-Westfalia, Germany).

Transcript catalogue sequences

The giant mole-rat transcript catalog was assembled and annotated with human gene symbols on the basis of recently published read data [24] (European Nucleotide Archive [ENA] study PRJEB20584) and the assembly framework FRAMA [25] with default parameters. For laboratory rats, mRNA sequences were obtained from NCBI RefSeq. Ortholog relations between rat and human genes were downloaded from Ensembl Biomart. For both species, only the longest transcript isoform per gene was used, which is the method of choice for selecting a representative variant in large-scale experiments [26]. This resulted in 15,864 reference transcripts (genes) for the giant mole-rats and 23,479 reference transcripts (genes) for the laboratory rats of which 14,062 reference transcripts (genes) were annotated with the same human gene symbol.

RNA-seq, read mapping and quantification

Tissue samples were collected and stored in RNAlater (Qiagen, Venlo, Netherlands) after isolation. For all tissues except blood, RNA was purified with the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. Blood samples (100 μ l) were collected in RNAprotect Animal Blood reagent (Qiagen). The resulting RNA was purified with the RNeasy Protect Animal Blood Kit (Qiagen). Kidney

and heart samples were treated with proteinase K before extraction, as recommended by the manufacturer. Poly(A) selection and preparation of the RNA-seq libraries was performed with the TruSeq RNA v2 kit (Illumina, San Diego, USA). RNA-seq was performed by single-end sequencing with 51 base pairs on a HiSeq 2500 sequencing system (Illumina) and with at least 17 million reads per sample, as described in Table S3. The reads were aligned to the respective reference – rat or giant mole-rat (see above) – with the BWA aln algorithm of the Burrows-Wheeler Aligner (BWA) [27], allowing no gaps and a maximum of two mismatches in the alignment. Only those reads that could be uniquely mapped to the respective gene were used for quantification.

Read data for rats and giant mole-rats were deposited as ENA study PRJEB23955 (Table S3). Read counts per gene and sample can be found in Tables S30 and S31.

Method validation

To ensure the reliability of our RNA-seq results we determined pairwise Pearson correlation coefficients between all rat and all giant mole-rat samples, respectively, based on log-transformed read counts that were normalized for sample size (Tables S32, S33). For each species and tissue, we calculated the means and standard deviations (Table S34). The grand mean of the determined correlation coefficients across tissues was 0.96 and 0.97 for rat and giant mole-rat, respectively, and the mean standard deviation across tissues 0.02 for both species.

Furthermore, we estimated the statistical power of DESeq2 [28] based on the respective dispersion in our complete rat and giant mole-rat data sets, respectively, using the method of Ching et al. [10] and 10 simulation runs per species and tissue (Table S14).

Differential expression analysis

Differential expression analysis was performed with DeSeq2 [28]. In both species, the elderly animals were compared with their young conspecifics. Genes that showed a comparison p-value less than 0.05 after Benjamini-Hochberg correction for multiple testing were considered as DEGs. Initial numbers of DEGs per tissue and species were as follows: 4033 and 2002 (blood), 1506 and 227 (heart), 5015 and 57 (kidney), 635 and 94 (liver), 3231 and 18 (skin) DEGs were identified in rat and giant mole-rat, respectively (Tables S4-S13).

To acquire comparable numbers of DEGs and to determine the amount of DEGs that were found in both

species (overlap), only those genes were taken into account that were present in the transcript catalogs of both species based on human gene symbol annotation (Fig. 1a, n=14,062). The giant mole-rat transcript catalog was annotated against human (see above). Ortholog relations between rat and human were downloaded from Ensembl Biomart.

Biological processes that were enriched for DEGs were determined in both examined species by using the human gene symbol annotation of the DEGs (see above), their human gene ontology annotation (GO; annotation package: org.Hs.eg.db) and Fisher's exact test. The Benjamini-Hochberg method was used to correct the resulting *p*-values for multiple testing. Additionally, GO categories with a *p*-value of less than 0.05 after corrections for multiple testing were summarized with REVIGO (cutoff, 0.70; measure, SimRel; database, whole Uniprot) [13] (Fig. 2, Fig. S1-S9).

Abbreviations

DEG: differentially expressed gene; GO: gene ontology.

AUTHOR CONTRIBUTIONS

AS performed the sampling, the gene expression analysis and wrote the first draft of the paper. MB performed transcriptome assemblies. YH and CV performed giant mole-rat caretaking and sampling. MG oversaw the sequencing. MS oversaw rat caretaking and sampling. SH oversaw the statistics. MP acquired funding, wrote the first draft of the paper and supervised the project. KS performed the sampling, wrote the first draft of the paper and supervised the project. PD acquired funding, wrote the first draft of the paper, oversaw giant mole-rat caretaking, performed sampling and supervised the project. All authors have read and improved the first draft of the paper.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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SUPPLEMENTARY MATERIAL

Please browse the links in Full Text version of this manuscript to see Supplementary Tables.

Table S1. Overview of examined animals.

Table S2. Mean ages in years (with standard deviations) of examined animals by species, age cohort and tissue.

 Table S3. Samples that were sequenced in this study.

Tables S4-S13. Result of DESeq2-analysis for differentially expressed genes during aging in laboratory rats and giant mole-rats (one table per species and tissue).

Table S14. Mean estimated power per tissue and species using the method of Ching et al., 2014 and 10 simulations runs.

Tables S15-S24. Biological process gene ontologies that are enriched for differentially expressed genes (DEGs) (false discovery rate [FDR] < 0.05) in laboratory rats and giant mole-rats (one table per species and tissue).

Tables S25-S29. Overlap of genes that are differentially expressed in laboratory rats and naked mole-rat blood (one table per tissue).

 Table S30/S31. Read counts per sample and gene in rats/giant mole-rats.

Table S32/S33. Pairwise Pearson correlation coefficients of rat/giant mole-rat samples based on normalized and logarithmized gene counts.

Table S34. Means and standard deviations of pairwise Pearson correlation coefficients between samples based on normalized and logarithmized gene counts.

Supplementary figures

Figures S1-S9. REVIGO treemaps of gene ontology processes that are significantly enriched (false discovery rate [FDR] < 0.05) one figure for tissue and species; figure for giant mole-rat skin is missing because the number of enriched terms was too small for summarizing).

Figure S10. Expression levels of examined genes.

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amide	metabolic process tr	anslațional i	initiation	process ic regulation of	cellular	process	organonitrogen	cell activation	electron glycosyl	macromolocula complex assembly	ilar comple	k subunit o assem	rganization bly assembly drial	regula cytokine	tion of production
process	cellular macromolecule	processing	catabolic process n positive	biological quality cytokine	modification process organic	process	metabolic process	metabolic process	chain metabolic process	cellular	comple biogene	1 respirat 2X chair SIS biogene	x I assembly	regulatio	n of
organonitrogen compound	posttranscriptional	of protein metabo process mRNA	lic regulation o biological process	metabolic process celular	substance biosyntheti process	negative	cell activation	single-organism metabolic " process	nucleobase-containin compound metabolic process mitochondrial AT	component biogenesis	mitochon translatic elongati	drial of cell onal compo on organiz	ion ular cellular compone disassem	nt coagulat	tion
biosynthetic process	regulation of gene expression	metabolic process	gene expression	nitrogen compound biosynthetic process	IRES-dependen translational initiation	t regulation of transferase activity			proton transport	primary	cellular			cellular	nitrogen
	response to	stress r	esponse to	respons	se to	c receptor signaling	intracellular transpor	cellular localization	vesicle-mediated transport	process	process abolism	immune proc	system ess	organization or biogenesis	compound metabolism
immune response			or organism	Cytoki		pathway			organic	phosphorus metabolic	organic substance metabolic			multi-organi	sm process
	response biotic stim	immune reş ulus t	egulation	cellular response to stress	response to endoplasmi reticulum stress	o IC leukocyte degranulation	establishment ^{intra} protein localization	cellular transp	substance transport	process	process	macroautophagy	metabolic metabolism process	catabolic catabolism process	cell-cell adhesion
defense response		response to organic		I-kappaB kinase/NF-kappaE signaling	production of molecular mediator involve in inflammator response	mast cell activation		single-organi localization	sm endocytosis	interspecies i between or	nteraction janisms			macromolecule metabolism	biosynthetic biosynthesis process
derense response	regulation defense resp	regulation of substance defense response signal transductio		regulation of I-kappaB kinase NF-kappaE signaling	on response to response to agpaB external to wounding		localization	phagocytos	is single-organism transport			autophagy	localization	biological adhesion	generation of precursor metabolites and energy

Figure S1. REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in rat blood. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.

						J												
regulation of immune respor	egulation of anatomical structure morphogenesis biological quality to stres		response to stress	single-organisn metabolic proces	n 55	orga metabo	nic acid lic process	neutral lipid metabolic process		oxidatio pr	n-reduction poess	fatty acid metabolic process	phosphorus metabolic process		proteolysis	protein phosphorylation		
protein	respor	nse to	blood d	coaculation	coagulation			phosphati metaboli	dylcholine c process	monoc acid m	arboxylic ietabolic	respirate	ory burst ^P	hosphatidylethanolamir biosynthetic process	• phosobate-cor	protein p	hosphorylation rotein metabolic process	n protein metabolic process
cascade	organic si	ubstance		positive		single-organisn catabolic proces	n is	sing triglyd	e-organi	sm cata	nolecule	cellula	r lipid c	arboxylic acio biosynthetic	compoun metabolic pro	d xcess	regulation of cellular protein metabolic process	regulation of mitochondrial translation
response to wounding	acute inflammator ^{re} regulati	y of regu on of îmî	ilation sponse nune re	regulation Rho prote sponse transduction	of regulation in of body fluid levels	small molecule	,	metaboli	c process	pro	acid	nucleoba	ise bile ac	id lipid	mitochondrial respiratory chain complex	NADH dehydrogen complex	regulation of cellular component	
cholesterol	ER-associated ubiquitin-dependent protein catabolic process	positi regula of celli compo organiz	ve tion ular nent ation	regulation of molecula function	r inflammatory response	metabolic process		catabolic proce		met: pro	metabolic process		iC blosynth s proces	^{ss} process	respiratory chain complex		spiratory ation ssembly	localization
membrane	antigen	intrace sign	llular al	negative regulation mesenchymal to epithelial transitio	of response to	transat	vesicle	-mediated	single-or localiza	ganism ation	macromol localiza	lecule tion lo	cellular calization	intracellular transport	I biogenesis	org	anization	
depolarization during cardiac muscle cell	processing and presentation of peptide antigen via MHC class I	regulati	on of	high-densit lipoprotein	y midbrain dopaminergic	transport	tra	nsport vesi	cle-media transp	ated tra	nsportot	ein	organic	regulation	organonitrogen ccammoniu meimetabol process	m ion ism olic process	protein foldin in endoplasm reticulum	ic protein folding
defense response	positive regulation of homeostatic process	respon exter stimu	se to nal lus	assembly circulatory system proce	differentiation regulation of anatomical structure size	endocytosis	regul endo	lation of ocytosis	fatty a transp	acid port	monocal acid tra	rboxylic nsport	transpor regu cell r	lation of	cataboli	sm	immune system process	beta-amyloid metabolism

Figure S2. REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in rat heart. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.

immune response		defense response s		e cell sig	cell surface receptor signaling pathway		cell activation		cell migration		extracell matri organiza	lular ex x s ttion or	tracellular structure ganization	cytokine production		regulation of cytokine productior		cell adhe		esion	Test
regulation of infla response to stimulus re		nmator ponse	po ry regi of re	sitive ulation sponse	celli respor chen	ular nse to nical	vesicle-mediated transport	transport e		endocyto activatio	sis of p loca	blishment protein alization fatty acid oxidation		regulation multicellul organism process	ytokine p ar coag al	ulation	egulation of levelopmental process	immun	e syste	em proc	ess
		to stimu		imulus	stimulus		movement of cell or	movement of cell or		e-organism single-organis calization transport		regulated of c exocytosis com orga		blood ves developme	sel metal	gen muticellular bolic organism etabolic ess process					
response to stress	ir response biotic stime	immune response le to nulus other organism		positiv regulation of respon	e on reg nse o	gulation of cell	subcellular component	phago	cytosis	organic substanc	cellul localiza	ar monocarbo acid metab process	secretion			entre county	phosphorus metabolic	esponse	to		
				to extern stimulu	to external communicat stimulus mall GTPase		localization of cell	cell death mac		macromolec	t li modi ule a	pid fication ctin	platelet degranulation	biological	adhesion	phosphorylation protein		stimulu	5	signali	ng
intracellular signal	cellular resp	oonse	response to wounding	mediate signal	ed oxyge co	ponse to n-containin mpound	g		positive	localizatio	n filamer	positive	regulation of protein iomooligomerization			pnospn	orylation				
transduction		to stimulus		regulation ERK1 and E cascade	regulation of RK1 and ERK2 Ras protein signal		regulation of biological	r of	regulation molecula	n ofa ar p	poptotic	of peptidas activity	process	single-organism	single-organisn	loca	lization	locomo	ition	single-orç proce	janism ss
response to	regulation	chemical		antigen processing presentation of pe or polysaccharti antigen via MHC of	Cascade transductio		process p	ositive	regulation	on of biol	ogical pro ological	fluid leve	regulation of biological process	celular process cell proli	feration				develop	pmental	
external stimulus	signalin	signaling wo	vound healing	MAPK	B B	istagrin medicis signaling pathod	molecular functi	ion Io	ocalizatio	n inor hom	ganic ion eostasis	negative regulatio of sequence-speci DNA binding transcription facto activity	n regulation of actin Nament-based process	cell proli	feration	cell com	munication	leukocyte proliferation	biolo regul	gical lation	ulli engarian edistar proma

Figure S3. REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in rat kidney. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.

response to external stimulus	respons purine-cor compo	nse to ontaining chemical		res cj	ponse to /tokine	platelet degranulation	transport	vesicle-mediated transport	cell dea	th ce	II activation	coagulation	negative regulation of multicellular organismal process	regulation endoderm cell differentia	of muscle adaptation
response to organic substance	response t	se to sphorus external stimulu		defense	e response	cell migration	secretion	endocytosis	regulatic phagocy	on of tosis	egulation of icle-mediated transport	energy homeostasi	blood circulation	negative regulation developmen process	of ntal regulation of multicellular organismal process
		exte	external stimulus				localization of cell	lamellipodium assembly	single-orç transp	ganism ort	fatty acid transport	cinglo, organism	lipid moto	holia	protein
protein activation cascade	response to stress	response t	o response other orga	e to i nism s	sponse to norganic ubstance	secretion by cell	actin filament-based	actin cytoskeleto organization	nsingle-org	ganism la Ition c	mellipodium rganization	metabolic process	proces	is	protein maturation protein
	response	o externars					process					lipid me monocarboxylic aci	d single-org	anism	maturation
immune response	response to	regulatio	n hyperosi	notic re	cellular sponse to	positive regulation of heterotypic	n regulation of	regulation of	regulatio	on of c	ell volume	metabolic process	- catabolic p		mmune system
	stimulus	to stimulu	is respon	ise n	iechanical stimulus	cell-cell adhesio	n					or subcellular	biosynth proces	etic ss	process
wound healing	response to oxygen-containing compound	response t mechanica stimulus	o response al carbohydi	to re rate ERM	positive gulation of (1 and ERK2 cascade	regulation o ^{pc} hemostasis	regulation of sitive regulation of apoptotic proces	heterotypic cell s homeostasis	negativ -cell adhe ATPase ad	re sion tivity	lipid omeostasis	localization	response to stimulus	react oxyg spec	ive en leukocyte ies proliferation
		response t	0 inflammatory	response	positive regulation		positive regulation	steroid	regulation of reactive oxygen	regulation	cellular			metabo	lism
response to metal ion	biotic stimulus	low-densit lipoproteir particle	y response	response to of response to external stimulus		regulation of biological quality	of steroid biosynthetic process	metabolic process	species metabolic process	of localizatio	n metabolic process	locomotion	biological adhesion	FasL	FasL biosynthesis process

Figure S4. REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in rat liver. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.

immune response						regulation of single-organism			small molecule metabolic process			extracellular matrix organization		atrix n	localization of cell	endocy	tosis cor mo	gulation cellular nponent vement	regulation of hydrolas activity	n reg se bio	gulation of ological quality
immune respons	e	defe	ense respo	onse	regui defense	lation of e response	single-organ metabolic pro	cess				extr	acellula	r	single-org loca transport	lization	n of cell	gulation smooth uscle cell	eg bihydrola	ation o se act	of ivity
									c	ell activatio	n	structure	organia	zation	phagocytosis	single-organise localization	regulation	muscle cell	regulation	che home regu	emical eostasis ulation
			1							small mole	cule		ster	rol	progeoficere		localization	migration	roliferation	of ma fun	olecular
response to external stimulus	cellular res chemical s	ponse to stimulus	o respo biotic	onse to stimulus	cellular cytokir	response to ne stimulus	oxidation-reduction process	n lipid m pro	etacell a cess	proces	s p	metabolic rocess	metal proc	bolic ess							
	imi	mune re	sponse					cellula	ar lipid	organic			uns	aturated	immune s	system	process	cell cell	all adhesion		onse to
regulation of	cell surface mast cell activation		ell po regi	sitive ulation	response to	organic acid metabolic process	meta proc	metabolic process		catabolic process	oxida	oxidation metabolic process						T cell		muius	
response to stimulus	signaling p	tor athway		of si	gnaling					secondary	carboxylic biosynthe	acid etic cell	death					cos	timulation		
	positive reg	gulation	glial cell-derived neurotophic factor receptor signaling pathway	receptor signaling pathway	accessi masargar mala ayusha	" acute inflammatory response	single-organism biosynthetic proces	single-c s cellular	process	alcohol metabolic process	organophos metaboli process	hate biosj	ATP (nthetic ocess	of cell or subcellular component	biological ad	hesion	single-	organisr	n signalii		eactive oxygen pecies tabolism
response to stress	external st	timulus	cellular	protein activation	intracellu sional	lar positive regulation				re	negative gulation of	f multice	ilular col	lagen							eniratoru
	respons	se to	response to	cascade	transduct	tion 3-kinase signaling	o delline -	cytokin	e secreti	on n	ulticellular rganismal	organ metab	ism polic pro	tabolic			cofacto	actor	locomot	ion	burst
	chemi	cal	stimulus	MyD88-independ toll-like recepto	metal	bolic	production	C	cytokine	productio	process	proci	ess .		bic cofactor	biquinone or olic	me process	bolism			
other organism	respons oxygen-cor compor	se to ntaining und	wound healing	signal transduction by protein phosphorylati	on stimu	2055 Openance and an and a second sec		regulation organis	of multic mal proce	ellular ess mor	phogenes	el sis ^{coagula}	ation sys pro	enal stem icess	biosynthe sulfur comp biosynthetic p	esis ound xocess	cell com	municatio	hydroxy compound metabolism	organon/trope compound metabolism	en sulfur compound metabolism

Figure S5. REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in rat skin. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.

			-												
			posit resp	positive regulation of response to stimulus		cytokine production		regulation of catalytic activit	y hemosta	isis n I	gulation f protein etabolic process				
immune res	ponse	defense response						positive regulation	negative		regulation				
			cellular r	esponse to	o stimulus			of NF-kappaB transcription factor activity	of biological process	homeostatic process	of body fluid levels	immune	e system pro	cess	stimulus
					cytokine production		coagulation	ne production regulation of	on maintenance	negative regulation					
	regulation of response to biotic stimulus cell surface signaling pathway regulation of regulation of molecular of regulation of m								localization	of location	of cellular process				
regulation of response to stimulus			regulation of autophagy	regulation of autophagy protein phosphorylatio		ion single-organism	cell communicatio		sin	naling					
	im	regulation				function	biological	regulation of	regulation of phosphorus	s va	cuolar			si	
			regulatio	on of me	diated signal			biological quan	process	acio	ification				
	response to stre	ss signaling	Central pro	tr	ansduction						ocalization			cell proicell tion	biological
intracellular signal transduction		Fc receptor signaling	response to	cellular response t	transmembrane receptor proteir		all salls a	a all death	phagocy	tosis	of cell	leuko celi-celi :	ocyte adhesion	proliferation cellular process	adhesion
	reconnec to	pathway	compound	chemical stimulus	signaling pathway	ceir a	ictivation	ceil death		cytosoli					multi-organism
	external stimulu	IS response to		response	B		cell activa	tion	ph endocytosis	agocytos	is iron ion			locomotion	process
		cytokine	response to chemical	to	macrophage activation					transpor	t			cytokine	
response to other organism	I-kappaB			compoun	d			novement		secret	on transition	leukocyte proliferation	biological regulation	metabolism	autophagy
	kinase/NF-kapp signaling	aB wound healing	response to wounding	response t peptidoglyc	to transduction to by protein phosphorylation	apoptotic p	process	subcellular salvage	vesicle-mediated transport	single-org	ion anismtranspor ort			interspecies interaction between organism	localization

Figure S6 REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in giant mole-rat blood. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.

single-organism catabolic process	single-organism metabolic process	collagen catabolic process	or cata	rganic acid bolic process	renal system process involved in regulation of blood volume	response to amino ac	id regulation of smooth muscle contraction	blood coagulation, fibrin clot formation	organic organic hydroxy
fatty acid metabolic process	single-organism monocardoxylic acia	catabolismjanic acid metabolic process	oxid	dation-reduction	negative regulation of cellular resp.c.goal_system vascular endothelial growth factor stimulus	m proçe <u>şş inxolv</u> ed in body fluid levels	detoxification	umegative regulation of lamellipodium assembly	compound metabolism process
		-			multicellular organism metabolic process	aorta smooth muscl tissue morphogenes	negative regulation of very-low-density s lipoprotein particle remodeling	protein activation cascade	
		leukotriene B4 catabolic	process	steroid					cholesterol import
lipid metabolic process	small molecule metabolic process	small molecule biosynthetic process		metabolic process	extracellular matrix or	gaextracellular matri	x organizationular structu	ire organization	

Figure S7. REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in giant mole-rat heart. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.



Figure S8. REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in giant mole-rat kidney. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.



Figure S9. REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in giant mole-rat liver. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.



Figure S10. Expression levels of examined genes. Gene counts were first normalized for overall read number per sample. Then, separately for each tissue, across samples of, both, young and old animals, mean gene counts per kilobase transcript length were determined. Whiskers extend to the most extreme datum within 1.5 times inter quartile range. DEGs - differentially expressed genes. Overlapping DEGs - DEGs found, both, in rat and giant mole-rat.