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Lifespan and reproduction in brain-specific miR-29-knockdown mouse

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ABSTRACT

The microRNA miR-29 is widely distributed and highly expressed in adult mouse brain during the mouse's lifetime. We recently created conditional mutant mice whose miR-29 was brain-specifically knocked down through overexpression of an antisense RNA transgene against miR-29. To explore a role for brain miR-29 in maximizing organismal fitness, we assessed somatic growth, reproduction, and lifespan in the miR-29-knockdown (KD) mice and their wild-type (WT) littermates. The KD mice were developmentally indistinguishable from WT mice with respect to gross morphology and physical activity. Fertility testing revealed that KD males were subfertile, whereas KD females were hyperfertile, only in terms of reproductive success, when compared to their gender-matched WT correspondents. Another phenotypic difference between KD and WT animals appeared in their lifespan data; KD males displayed an overall increasing tendency in post-reproductive survival relative to WT males. In contrast, KD females were prone to shorter lifespans than WT females. These results clarify that brain-targeted miR-29 knockdown affects both lifespan and reproduction in a gender-dependent manner, and moreover that the reciprocal responsiveness to the miR-29 knockdown between these two phenotypes in both genders closely follow life-course models based on the classical trade-off prediction wherein elaborate early-life energetic investment in reproduction entails accelerated late-life declines in survival, and vice versa. Thus, this study identified miR-29 as the first mammalian miRNA that is directly implicated in the lifetime trade-off between the two major fitness components, lifespan and reproduction.

1. Introduction

Evolutionary theories of aging state that species optimize Darwinian fitness through life-history trade-offs between lifespan and reproduction in response to environmental cues [1]. A cost of reproduction, where longevity and fecundity counteract each other, is of widespread occurrence. Species with robust reproductive potential generally have a short lifespan, while intrinsically poor fecundity of a species is concurrent with its long survival [2]. More informatively, transplanting the ovaries of young mice into ovariectomized aged females prolongs their residual lifespan [3,4], implying that signals from reproductive tissues can influence organismal longevity. Given the leadership role of the animal brain in controlling whole-body physiology, it makes sense to postulate that for lifespan regulation, brain functions coordinate multilayered signaling interactions among neurons, somatic tissues, and gonads via the neuroendocrine system, but the molecular entities that preside over such neuroendocrine communications remain to be identified.

MicroRNAs (miRNAs) are endogenous, small non-coding RNAs that regulate gene expression via binding to specific sites at the 3'-untranslated region of their target mRNAs, resulting in translational arrest or mRNA degradation of the targets [[5] and accompanying papers in the same issue]. Each miRNA has the potential to silence hundreds of gene transcripts, and therefore miRNAs have emerged as global fine tuners of a very wide spectrum of biological events. Additionally, it is also becoming clear that most miRNAs concurrently target multiple genes with mutually related functions, thereby exerting significant effects on particular regulatory pathways. In the mammalian brain, numerous miRNAs have hitherto been shown to be differentially expressed in a spatiotemporally controlled manner during aging [6–9]. Although accumulating evidence indicates that mammalian brain miRNAs contribute to neuronal development and differentiation, synaptic plasticity, neuropathology, and other functions, nothing is known of their

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mechanistic involvement in lifespan regulation.

We previously performed a genome-wide screening of small non-coding RNAs expressed in developing mouse brain, and found that miR-29 is remarkably up-regulated in the adult brain [10]. miR-29 is a miRNA family comprising three paralogous members, 29a, 29b, and 29c [11–13]. Since the mature miR-29 family members share the same seed sequence that determines the targeting specificity, target genes for each miR-29 member almost completely overlap each other. miR-29 is distributed across the central nervous system and enriched in astrocytes [14,15]. We hypothesized that intensely and extensively expressed miRNAs, such as miR-29, play a critical role in controlling vital brain functions, and to test this idea, established a transgenic mouse line wherein the animal brains specifically overexpress anti-miR-29 antisense RNAs under transcriptional control of regulatory elements of *Ntrk2*, the gene for neurotrophic tyrosine kinase receptor type 2 [16]. miR-29 levels in the mutant adult brains were approximately half those in non-transgenic littermates.

Thus far, several mouse models with altered expression of a single protein-coding gene that displays a reciprocal relationship between reproduction and post-reproductive survival have been reported for a spontaneous mutation in the *Prop-1* [17], *Pit-1* (*Pou1f1*) [18], and GHRH receptor [19] genes, and a targeted disruption of the GH receptor [20,21] and brain IRS2 [22] genes, as well as a transgenic overexpression of the Klotho gene [23]. The functions of these genes converge on the insulin/IGF-1 signaling pathway. In the nematode worm *C. elegans*, the miRNA miR-71 [24] is localized to neurons and mediates the longevity-promoting effect of germline removal [25]. The function of miR-71 is normally suppressed by the germline, and its overexpression further strengthens the pro-longevity attribute of the germ-less animals. However, molecular details are unclear about the gene target for miR-71 and what signals from the gonad interfere with the expression of this miRNA. Here, we present initial data on the phenotypic characteristics of the brain miR-29-knockdown mouse strain. Our work is the first to investigate mechanistic roles of miRNA in the concept of fundamental lifespan-reproduction trade-offs in mammals.

2. Materials and methods

2.1. Mouse strains and husbandry

The strain of brain miR-29-knockdown mouse [16] was maintained in the heterozygous state on the C57BL/6N background, which has been deposited at RIKEN BioResource Center and is available via http://mus.brc.riken.jp/en/(strain name: Ntrk2_miR-29AS Rec BAC, RBRC No. 09372). For this study, the miR-29-knockdown (KD) mice and their normal non-transgenic (wild-type [WT]) siblings were produced through in vitro fertilization by crossing the heterozygous mutant males and wild-type C57BL/6N females. Pups were weaned on day 30 and genotyped by Southern hybridization. They were grouped into three cohorts of mating pairs: KD♂ × WT♀, 15 pairs; WT♂ × KD♀, 15 pairs; WT♂ × WT♀, 20 pairs. Each pair was individually caged in a barrier facility (23 ± 2 °C, 12-hr light/dark cycle) under specific pathogen-free conditions. Standard rodent chow (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan; 55.3% carbohydrate, 21.9% protein, 5.4% fat, 8.2% humidity, 9.2% mineral and fiber) and water were supplied *ad libitum*. Mice were monitored daily and weighed weekly, but otherwise left undisturbed until natural death. When a sole surviving male or female remained within a cage, this mouse was maintained alone for its remainder of lifespan. All animal experiments were conducted at Utsunomiya Office, Institute of Immunology, Co., Ltd. according to its institutional guidelines for the care of laboratory animals.

2.2. Statistical analyses

For group comparison other than survival curves, we used the Student's *t*-test, nonparametric Mann–Whitney *U*-test, and one-way ANOVA where indicated. Means were expressed with standard error of the mean (SEM). Survival profiles were constructed by Kaplan-Meyer survival analysis. The mutant and control survivorship curves were compared in pairs, and the significance of differences was evaluated using log-rank test. In all analyses, P-values < 0.05 were considered statistically significant.

3. Results and discussion

The KD and WT mice as study subjects for fertility and lifespan assessments were produced from heterozygous mutant sires and wild-type dams. Examination of gender and genotype ratios in the resulting pups revealed little deviation from the expected Mendelian ratio (KD♂:KD♀:WT♂:WT♀ = 1:1:1:1). The KD mice were healthy and behaved normally. We did not observe any substantial difference in body weight gain profiles throughout the course of this study between KD and WT mice for either gender (data not shown). These results indicate that partial loss of brain miR-29 activity has no impact on the development and postnatal function of mouse brain. Numerous recent articles have documented that the classical neural function of miR-29 appears to be a neuroprotective factor, acting against neurodegeneration and apoptosis [26–34]. However, the role of miR-29 in apoptosis seems context-dependent [35], and the onset and progression of apoptosis is supposed to be inextricably coordinated by synergism, antagonism, and compensatory mechanisms. Actually, increasing miR-29 had the effect of inducing neuronal cell death in focal ischemia by silencing an anti-apoptotic Bcl-2 family member, Bcl2L2 [36].

To evaluate the neural influences of miR-29 knockdown on fertility, we set up three cohorts of breeding pairs (KD♂ × WT♀, WT♂ × KD♀, and WT♂ × WT♀), and compared the reproductive performance between KD and WT mice in regard to maternal age at first delivery, litter size, pre-weaning pup mortality, total number of litters, and time interval between litters. These three mating groups showed comparable mean litter sizes at each reproductive cycle when childbirth was successful; the overall values across the reproductive period for KD♂ × WT♀, WT♂ × KD♀, and WT♂ × WT♀ groups were 5.8 ± 1.2, 5.8 ± 0.8, and 5.9 ± 1.5 (pups/litter), respectively. However, when monitored over repeated reproductive cycles of each breeding pair, the rate of reproductive success decreased more rapidly in KD♂ × WT♀ crosses, where severe reduction initiated from the fourth cycle. In contrast, fairly higher numbers of litters per pair were maintained in WT♂ × KD♀ crosses, when compared with WT♂ × WT♀ crosses (Fig. 1). There were little differences in other reproductive parameters among the three groups (data not shown). Thus, brain-targeted miR-29 knockdown was found to act against successful reproduction in males, but facilitated it in females without affecting sexual maturation and reproductive cycle duration. The compromised fertility in male mutants could be explained by impaired spermatogenesis as seen in mouse strains with somatotrophic defects [37–40], because there was no evidence for increased fetal mortality in the KD♂ × WT♀ crosses.

Next, we measured lifespan in KD males and females in comparison to their respective WT counterparts. The age of an individual mouse at its natural death was tallied and statistically analyzed for each gender and genotype (Table 1). Although we failed to obtain statistically significant differences, KD males outlived WT males in every respect of median, mean, and maximum lifespan, which is reflected in their survival profiles (Fig. 2). Conversely, lifespans of KD females were slightly shorter when

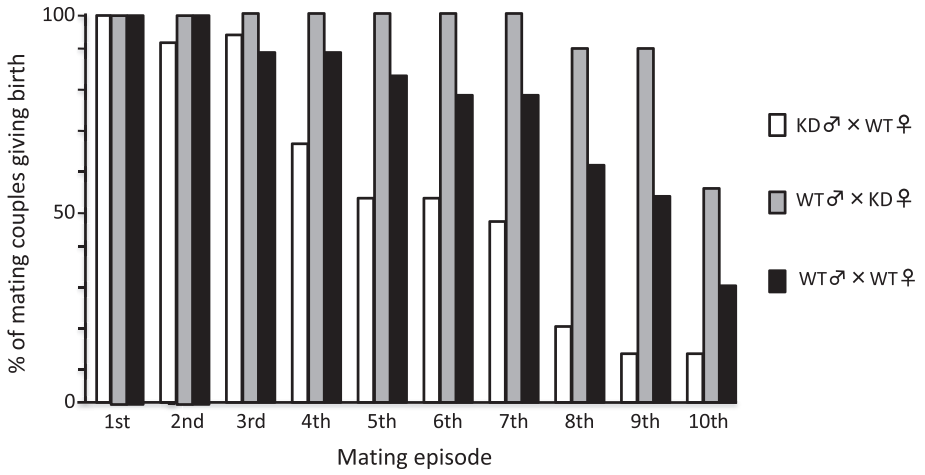

Fig. 1. Age-dependent kinetics of reproductive success. The rate of the number of pairs that produced offspring relative to the total number of pairs in each breeding group was expressed as a function of breeding cycle. One KD female failed to conceive and therefore its data was excluded from the analysis of WT♂ × KD♀ breeding. Statistical significance from the fourth cycle onward: P = 0.00047, one-way ANOVA; post-hoc test: P = 0.00032 for KD♂ × WT♀ vs. WT♂ × KD♀; P = 0.0032 for KD♂ × WT♀ vs. WT♂ × WT♀; P = 0.097 for WT♂ × KD♀ vs. WT♂ × WT♀.

Table 1
Survival characteristics of brain miR-29 knockdown mice.

	Median (days)	Mean ± SEM (days)	Maximum±SEM (days)[a]	*n*
Males				
KD	770[b]	780 ± 212[d]	1092 ± 208[f]	15
WT	679	683 ± 192	1020 ± 10	35
% Difference	13.4	14.2	7.1	
Females				
KD	623[c]	558 ± 163[e]	823 ± 94[g]	15
WT	637	628 ± 134	872 ± 25	35
% Difference	−2.2	−11.1	−5.6	

[a] Average of the lifespan of two longest-lived mice was used to estimate maximum lifespan from small populations.
[b] P = 0.078 vs. male WT, Mann–Whitney U-test.
[c] P = 0.46 vs. female WT, Mann–Whitney U-test.
[d] P = 0.060 vs. male WT, Student's t-test.
[e] P = 0.18 vs. female WT, Student's t-test.
[f] P = 0.34 vs. male WT, Student's t-test.
[g] P = 0.27 vs. female WT, Student's t-test.

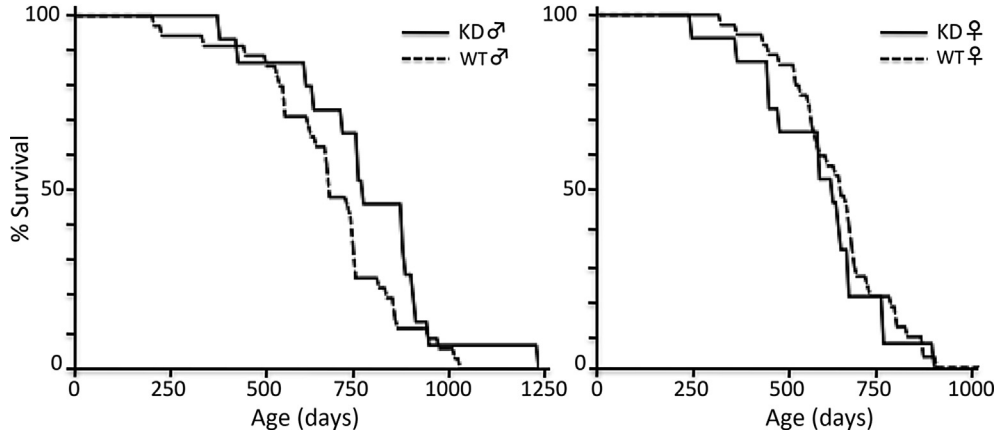

Fig. 2. Survival curves for brain-miR-29-knockdown mice. The age at death of individual mouse was plotted against the percent of mice still alive for each gender and genotype. Left panel, males; right panel, females; solid lines, KD mice; broken lines, WT mice. Statistical significance: P = 0.10 for KD♂ vs. WT♂, P = 0.62 for KD♀ vs. WT♀, log-rank test.

compared with WT females as evidenced in these survival parameters (Table 1 and Fig. 2).

Overall, it is conceivable that the lifespan in KD males was extended at the expense of reproductive output, whereas KD females underwent a relatively high reproductive cost, which slightly increased their mortality. Our observations underscored two notable aspects relating to the consequence of brain-specific miR-29 knockdown. One is that this intervention invokes sexually

different alterations in both of lifespan and reproduction, which are two competing, biologically pivotal traits. Sexual dimorphism during a lifespan is a ubiquitous phenomenon throughout the animal kingdom [41]. Males and females adopt different reproductive strategies and therefore acquire fitness differently, often in a mutually exclusive manner, through gender-specific genetic modifications, which could result in gender difference in their lifespans [42]. Such gender-related effects on survival have appeared in numerous genetically modified mouse models [43], but molecular explanation for the sexual differences in lifespan has not yet been addressed. A finding related to our study has recently been reported for a mouse model of amyotrophic lateral sclerosis, a chronic neurodegenerative disorder [44]; the male mutant mice displayed a trend toward improved survival as a result of intracerebroventicular injection of an antagonistic oligonucleotide against miR-29a, whereas the same manipulation did not affect the female lifespan. This observation is fundamentally consistent with our data representing a male-biased pro-longevity effect of miR-29 down-regulation. Secondly, the opposing male and female reproductive responses to lowered brain miR-29 levels are faithfully manifested as a trade-off with life potential, such that the severity of reproductive cost in each gender is reciprocally proportionate to its survival duration. Thus, brain miR-29 may hold the key to understanding the molecular basis of these sexually dimorphic life-history traits and their functional connections. In addition, it is possible that gender-specific, dose-dependent effects of brain miR-29 may underlie a dynamic balance of the lifespan-reproduction trade-off. Brain miR-29 may work on optimization of organismal fitness, so that males and females subjected to neural alterations in miR-29 levels in the face of environmental perturbations induce gender-specifically a physiological shift in this trade-off toward maintenance of sexually dimorphic fitness optima. Presently, molecular signatures involved in the miR-29-guided phenotypic coordination observed here are unknown. It is well acknowledged that the insulin/IGF-1 signaling cascade is an evolutionarily conserved pathway that intimately participates in the central control of organismal lifespan [45]. Intriguingly, IGF-1 was identified as a post-transcriptional target for miR-29, and its expression was inversely related to miR-29 levels in adult mouse brain [46]. As deduced from our study, the underlying process of insulin/IGF-1 signaling in lifespan modulation may partially overlap with the action mode of miR-29. Given the multi-functionality of miRNA that potentially regulates a plethora of target genes, miR-29 could be the master integrator to resolve life-history trade-offs, thereby optimizing fitness through yet unknown neuroendocrine mechanisms. Our understanding of how miRNAs act in the neuroendocrine control of organismal lifespan is in its early stages.

Transparency document

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