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Influence of Increased Amounts of the CHD1 Protein on Salivary Gland Secretion Genes Expression in Drosophila Salivary Glands

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ABSTRACT

BACKGROUND: The genetic material of eukaryotes exists in the nucleus in the form of a nucleoprotein complex named chromatin. Realization genetic information requires chromatin remodeling mediated by ATP-dependent chromatin remodeling proteins of the SNF2 family. Evolutionarily conserved chromatin assembly and remodeling factor CHD1 is associated with the development of prostate cancer. Development of prostate cancer is promoted both by deletions and by increase in the amount of CHD1 protein in the cell.

AIM: To analyze the effect of increased expression of the CHD1 protein in a model organism — Drosophila — on the transcription of hormone – dependent tissue-specific genes in the salivary glands.

METHODS: We used a genetic model based on the overexpression of either wild-type Drosophila CHD1 protein or its catalytically inactive form in the salivary glands under the control of the GAL4-driver *P{GawB}AB1*. The level of gene transcription in the salivary glands was investigated by reverse transcription followed by real-time PCR.

RESULTS: We have shown that increased production of the CHD1 protein in the salivary glands leads to a disruption in the attachment of pupae to the surface. It is shown that this phenotype is caused by specific suppression of transcription of *Salivary gland secretion* (*Sgs*) genes.

CONCLUSION: A model system has been created for studying genetic effects caused by an increase in the amount of CHD1 protein in *Drosophila* cells. This model can be used to investigate the mechanisms of transcriptional regulation by CHD1 and its disturbance as a result of increased production of CHD1 protein.

Keywords: *Drosophila melanogaster*; prostate cancer; CHD1; SNF2; *Sgs*.

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Оригинальное исследование
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Изменение экспрессии генов, контролирующих белки секрета слюнных желез дрозофилы, при повышенной продукции белка CHD1

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АННОТАЦИЯ

Обоснование. Генетический материал эукариот в ядре находится в виде нуклеопротеинового комплекса — хроматина. Реализация генетической информации требует ремоделирования хроматина, которое осуществляется АТФ-зависимыми белками ремоделирования хроматина семейства SNF2. Эволюционно консервативный фактор сборки и ремоделирования хроматина CHD1 связан с развитием рака предстательной железы. Развитию этого заболевания способствуют как делеция CHD1, так и увеличение количества белка CHD1 в клетке.

Цель — анализ влияния повышенной экспрессии белка CHD1 в модельном организме — дрозофиле — на транскрипцию гормон-зависимых тканеспецифичных генов в слюнных железах.

Материалы и методы. Использовали генетическую модель, основанную на сверхэкспрессии белка CHD1 дрозофилы дикого типа либо его каталитически неактивной формы в слюнных железах под действием GAL4-драйвера *P{GawB}AB1*. Уровень транскрипции генов в слюнных железах исследовали методом обратной транскрипции с последующей полимеразной цепной реакцией в реальном времени.

Результаты. Нами показано, что повышенная продукция белка CHD1 в клетках слюнных желез приводит к нарушению прикрепления куколок к поверхности. Этот фенотип обусловлен специфическим подавлением транскрипции генов, кодирующих основные белки секрета слюнных желез SGS (Salivary gland secretion, *Sgs*).

Заключение. Создана модельная система для изучения генетических эффектов, вызванных увеличением количества белка CHD1 в клетках дрозофилы. Эта модель может быть использована для исследования механизмов регуляции транскрипции белком CHD1 и ее нарушений в результате повышенной продукции белка CHD1.

Ключевые слова: *Drosophila melanogaster*; секрет слюнных желез; CHD1; SNF2; *Sgs*.

Как цитировать

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BACKGROUND

In eukaryotic cells, genetic material is arranged into a chromatin complex made up of DNA and histones. All DNA functions, including transcription, replication, repair, and recombination, rely on chromatin modifications; moreover, they serve as the foundation for epigenetic regulation. Chromatin remodeling by motor proteins (ATPases from the SNF2 family) is one of the primary mechanisms of chromatin modification [1]. Among these, the chromo-ATPase/helicase-DNA-binding protein (CHD) subfamily is distinguished by double chromodomains [2, 3]. Drosophila, a classic model organism, has all three groups of this subfamily: CHD1, CHD3-4, and kismet (CHD6-9), which are also found in the mammalian genome [4]. CHD1 is the most conserved protein in this subfamily, and its uniqueness lies in its involvement not only in chromatin remodeling, but also in its assembly from DNA and histones *in vitro* [5] and *in vivo* [6–8]. Nucleosomes are removed and assembled by ATP-dependent chromatin remodeling factors and histone chaperones acting together [7, 9]. *In vivo*, CHD1 is necessary for the integration of variant histone H3.3 in replication-independent chromatin assembly during post-fertilization transformation of the Drosophila male pronucleus [6]. A similar function of CHD1 has been described for mammals [10].

CHD1 plays an essential role in transcription regulation. It was initially characterized as a chromatin-remodeling protein that binds to active transcription sites (puffs and interbands) in the polytene chromosomes of Drosophila third-instar larvae [11]. Studies in yeasts and human cell culture found that CHD1 is secreted in complex with various transcription elongation factors and splicing system components. CHD1 regulates transcription initiation, elongation, and termination [3, 12–15]. During transcription elongation in Saccharomyces yeast, CHD1 interacts with the elongation factors Spt4-Spt5 and Spt16-Pob3, as well as the Rtf1 subunit of the PAF1 complex, which regulates elongation [13]. According to studies in mice, CHD1 is a component of the preinitiation complex during transcription initiation [16]. During its formation, CHD1 is recruited to active gene promoters through interaction with the Med1 component of the Mediator complex [16]. In fission yeast, Chd1 is also part of this complex [17, 18]. In yeast, Drosophila, and human cell gene promoters, CHD1 likely overcomes the transcription barrier, which is represented by the first nucleosome after the transcription start site [19–22]. In Drosophila, CHD1 acts as both an activator and a repressor of gene transcription [23, 24].

The human genome has two homologues of Drosophila *Chd1* (CHD1 and CHD2), which appeared as a result of whole-genome duplication during vertebrate evolution and are both involved in carcinogenesis. Mutations in the *CHD1* gene are associated with prostate cancer [25–27]. In this case, CHD1 acts as a cancer suppressor, essential for the development of prostate cancer and metastasis. Deletions, mutations, and rearrangements in the *CHD1* gene are among the most prevalent abnormalities seen in prostate cancer. *CHD1* recruits the androgen receptor to the promoters of cancer suppressor genes such as *NKX3-1*, *FOXO1*, and *PPARγ* [28]. However, deletions have a minor role in carcinogenesis compared to an excess of the CHD1 protein. The most common mutations in prostate cancer are in the *PTEN* gene; these mutations disrupt the proteolysis of the CHD1 protein [29]. Excessive CHD1 levels activate genes involved in the TNF-NF-κB pathway, which promotes cancer. *PTEN* mutations in cancer cells exhibit fatal synthetic interactions with *CHD1* mutations or inactivation of this gene by RNA interference. Therefore, in tumors with *PTEN* deletions that cause CHD1 protein accumulation, it may be a promising target for the development of targeted therapy. However, given the multiple roles of CHD1 in genetic regulation, its use as a target for targeted therapy necessitates comprehensive research into all of its functions and the mechanism of action. Therefore, we developed a genetic model to assess the effects of increased CHD1 production using the classic model organism *Drosophila melanogaster*. For this purpose, we induced the expression of this protein in salivary glands to assess the effect of increased CHD1 production on both the structure of chromosomes and chromatin, and on gene expression. We used the GAL4 driver *P{GaWB}AB1*, which expresses the GAL4 protein in salivary glands from the embryonic stage to the end of the larval stages, to induce transcription of transgenes encoding either the wild-type CHD1 protein (*P{UAST-Chd1$^{(wt)}$}*) or its catalytically inactive form (*P{UAST-Chd1$^{(KR)559}$}*). The substitution of lysine for arginine at position 559 of the conserved ATPase domain completely eliminates the protein's ATPase activity and its ability to remodel or assemble chromatin [6]. During these studies, we found that pupae of species with increased CHD1 expression in both forms are very loosely attached to the walls of Drosophila culture tubes and easily fall off when touched with a dissecting needle. Pupae attach to the substrate using salivary gland secretions. That is why this study assessed the effect of increased CHD1 production on the expression of genes encoding the key proteins of this secretion (*salivary gland secretion, SGS*).

The work aimed to assess the effect of increased CHD1 protein expression in a model organism, Drosophila, on the transcription of hormone-dependent tissue-specific genes in salivary glands.

METHODS

The study used the following Drosophila strains: wild-type Oregon-R strain; GAL4 driver *w; P{GaWB}AB1* strain; strains carrying the transgenes *w; P{UAST-Chd1$^{(wt)}$}*, encoding the wild-type protein, and *w; P{UAST-Chd1$^{(KR)559}$}*,

encoding a protein with an inactive ATPase [6]; as well as two strains carrying deletions in *Chd1—w; Df(2L) Df(2L) Chd1^{1}/T(2;3) SM6b-TM6B Tb* and *w; Df(2L) Exel7014/T(2;3) SM6b-TM6B Tb*. The *Df(2L)Chd1^{1}* deletion deletes three genes, including *Chd1*, whereas the large *Df(2L)Exel7014* deletion overlaps with *Df(2L)Chd1[1]* in only one gene, *Chd1* [6]. When crossing these two lines, null mutants for the *Chd1* gene were selected [6]. All cultures were maintained on a standard medium at 25 °C.

To quantitatively assess pupae attachment to test tube walls, cultures were grown in collapsible test tubes with removable bottoms. After pupation, the medium-filled bottoms were replaced with ones filled with room-temperature water and turned over a specified number of times. Then, the number of pupae that remained attached to the walls and the number of those washed away were counted. Statistical analysis was performed using the χ^2 test with Yates's correction [30].

Total RNA was isolated using acid guanidinium thiocyanate-phenol-chloroform extraction with Trizol reagent (Invitrogen, USA) in accordance with the instructions. RNA was extracted from isolated salivary glands or whole larvae at various stages of development. The isolated RNA was treated with DNase, and reverse transcription was performed using the LunaScript kit (New England Biolabs, USA). Real-time PCR was performed using the master mix for RT-PCR SyberGreen + Rox (2.5X) (BioRad) and the BioRad CFX96 amplifier. RNA was isolated from larvae at the puff stage PS1-2, 0-hour prepupae (stage PS10), and 0-hour prepupae + 2 h (stage PS10+2). To determine the physiological age, larvae were grown on a medium containing bromophenol blue dye, which allows distinguishing between development stages based on the amount of colored medium in the larval intestines [31]. Genes of U6 spliceosomal RNA (*SnRNA:U6*), actin *Act42A (Actin42A)*, and ribosomal protein *RpL32* (*Ribosomal protein L32*) were used as reference genes. Statistical analysis was performed using REST 2009 [32]. Primers used for RT-PCR are available upon request.

RESULTS

This study was based on the observed loose attachment to test tube walls in pupae with the genotypes *w*; *P{GaWB}AB1/P{UAST-Chd1$^{(wt)}$* and *w*; *P{GaWB}AB1/P{UAST-Chd1$^{(KR)559}$}*. Given that pupating PS10 larvae attach to the substrate using their salivary gland secretions, which serve as "glue", we hypothesized that this phenotype may be associated with a disruption in the synthesis of the main component of this "glue": salivary gland secretion (SGS) proteins. The disruption of synthesis was evidenced by the almost complete absence of secretion in the lumen of salivary glands at all studied stages of development. The glands of such species are somewhat reduced and do not exceed the fat body thickness, but otherwise retain normal morphology. To quantify the degree of pupal attachment to the surface, we washed off the pupae by inverting the culture cups with water and counting the number of pupae washed off vs those that remained attached. The findings are presented in Table 1.

According to Table 1, after 50 inversions, 88.7% of pupae with the genotype *w*; *P{GaWB}AB1/P{UAST-Chd1(wt)*, 92% of pupae with the genotype *P{GaWB}AB1/P{UAST-Chd1$^{(wt)}$}*, and only 5% of pupae with the genotype *w*; *P{GaWB}AB1/+ were washed off.* Therefore, we obtained qualitative differences in the attachment phenotype, indicating a nearly complete disruption of salivary gland secretion with increased expression of both the CHD1 protein and its inactive form in salivary gland cells.

In the next stage, we assessed the expression of salivary gland secretion genes *Sgs3* (*Salivary gland secretion 3*), *Sgs4* (*Salivary gland secretion 4*), *Sgs5* (*Salivary gland secretion 5*), *ng2* (*new glue 2*), and *Pig1* (*Pre-intermoult gene 1*), which are only expressed in salivary glands. The expression of all genes was assessed in salivary glands of larvae at the PS1-2 stage, when extremely active transcription of all three *Sgs* genes begins, but active transcription of *ng2* and *Pig1* ceases [33]. The U6 spliceosomal RNA (*SnRNA: U6*) gene, which is transcribed by RNA polymerase III, was used as a reference gene. Unlike genes transcribed by RNA polymerases I and II, the CHD1 factor had no effect on the expression of genes transcribed by polymerase III. The results of the analysis are shown in Fig. 1; the data are presented using a logarithmic scale.

In this stage, expression of both *Chd1* transgenes results in a nearly complete absence of transcription of all studied genes encoding salivary gland secretion proteins (SGS3, SGS4, and SGS5). At the same time, the expression of a catalytically inactive form of the CHD1 protein (*ng2*) causes transcription of two other tissue-specific salivary gland genes to remain unchanged (*Pig1*) or even increase. Therefore, the suppression of transcription of the *sgs* genes is highly specific and does not result from a general suppression of transcription in salivary gland cells. This is further supported by the preservation of the virtually normal structure of salivary glands, except for the absence of secretion in their lumen, which is primarily composed of SGS proteins.

In the next stage, we analyzed changes in the amount of RNA transcribed from the *Sgs4* and *Sgs5* genes during development. In this experiment, RNA was isolated from whole larvae, because expression of both the studied genes and the GAL4 driver *P{GaWB}AB1* occurs only in salivary glands, and separation from the fat body is difficult in larvae expressing *Chd1* transgenes. The experiment used two controls: the wild-type *Oregon-R* line and *P{GaWB}AB1/+* heterozygotes, and null mutants for the *Chd1* gene (Fig. 2). The *Act42A* and *RpL32* genes were used as reference genes. To assess changes in RNA

Table 1. Relationship between pupal attachment to the surface and expression of CHD1 protein in salivary gland cells

Baseline number of pupae	Number of pupae washed off depending on the number of tube inversions			Number of pupae that remained attached	Total number of pupae washed off (%), *p**
	10 inversions	30 inversions	50 inversions		
			P{GaWB}AB1		
39	0	0	2	37	2
10	1	0	0	9	1
12	0	1	0	11	1
40	1	0	1	38	2
52	1	0	1	50	2
12	0	0	0	12	0
16	1	0	0	15	1
42	1	0	0	41	1
24	2	0	0	22	2
19	0	1	1	17	2
266**	7	2	5	252 (94.7%)	14 (5.2%)
			P{GawB}AB1/P{UASTCHD1(WT)}		
30	12	8	8	2	28
36	15	11	7	3	33
48	15	16	11	6	42
31	6	16	2	7	24
33	15	11	7	0	33
36	9	17	7	3	33
58	24	28	3	3	55
272#	96	107	45	36 (11.3%)	282 (88.7%), $p = 3.3 \times 10^{-89}$
			P{GaWB}AB1/P{UAST-Ch1(KR)559}		
49	17	18	10	4	45
21	9	7	3	2	19
20	4	8	8	0	20
18	5	9	4	0	18
37	9	13	11	4	33
16	3	8	4	1	15
25	12	7	3	3	22
21	11	6	3	1	20
38	19	11	5	3	35
19	6	9	1	3	16
264##	95	96	52	21 (8.0%)	243 (92.0%), $p = 4.1 \times 10^{-88}$

Note. **p*, probability that the ratio of pupae washed off to pupae that remained attached does not differ from that in the control line *P{GaWB}AB1;* **, the ratio of pupae washed off to pupae that remained attached does not differ between tubes ($p = 1.00$), therefore the data were pooled; #, the ratio of pupae washed off to pupae that remained attached does not differ between tubes ($p = 0.18$), therefore the data were pooled; ##, the ratio of pupae washed off to pupae that remained attached does not differ between tubes ($p = 0.98$), therefore the data were pooled.

levels during development, relative expression is shown in relation to the reference genes and the minimum relative expression during development in control species with the *P{GaWB}AB1/+* genotype [34].

At the PS1-2 stage, *Chd1* null mutants also had reduced transcription of *Sgs4* but not *Sgs5.* Therefore, the effect of the catalytically inactive form of the CHD1 protein on the expression of *Sgs* genes cannot be explained

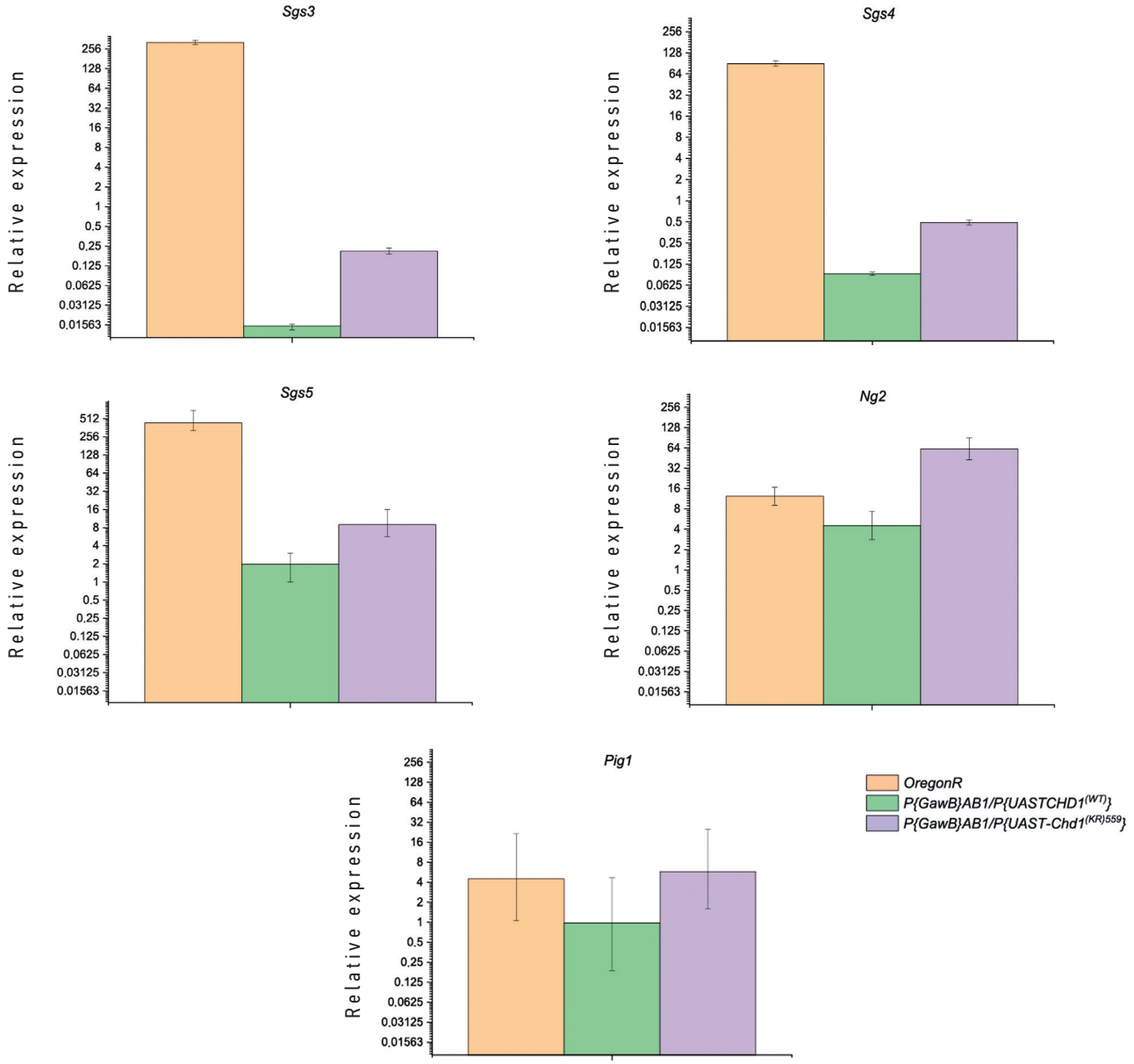

Fig. 1. Effect of increased production of constructs encoding normal (*P{UAST-Chd1(wt)}*) and catalytically inactive (*P{UAST-Chd1$^{(KR)559}$}*) forms of the CHD1 protein on the expression of tissue-specific genes of salivary glands in Drosophila. Relative expression and a 95% confidence interval are shown.

by its lack of activity and is comparable to the effect of increased wild-type CHD1 protein production on the expression of salivary gland secretion genes. Over time, the relative expression of *Sgs* genes increases slightly with increased production of CHD1 (especially its inactive form), which can be attributed to a slow increase in their transcription as CHD1 protein levels in the cell rise. Therefore, CHD1 does not completely inhibit *Sgs* gene transcription as a result of increased recruitment to chromatin, interfering with RNA polymerase recruitment.

DISCUSSION

The key finding of this work is that elevated CHD1 protein levels in salivary gland cells cause highly specific suppression of salivary gland secretion gene transcription, resulting in disruption of pupae attachment to the surface. This easily analyzed trait allows creating an experimental model for assessing the factors influencing the phenotype associated with an increase in CHD1 protein levels in Drosophila cells. In humans, mutations in the PTEN phosphatase cause the CHD1 protein to stabilize, resulting in an increase in CHD1 levels in the cell and malignant transformation of prostate cells. The identification of a highly specific phenotype associated with increased CHD1 production in salivary gland cells in Drosophila lays the groundwork for studying transcription regulation in a model organism, Drosophila, as a result of an increase in this protein levels. Such studies may shed light on both the mechanisms of action of the CHD1 protein and the mechanisms of

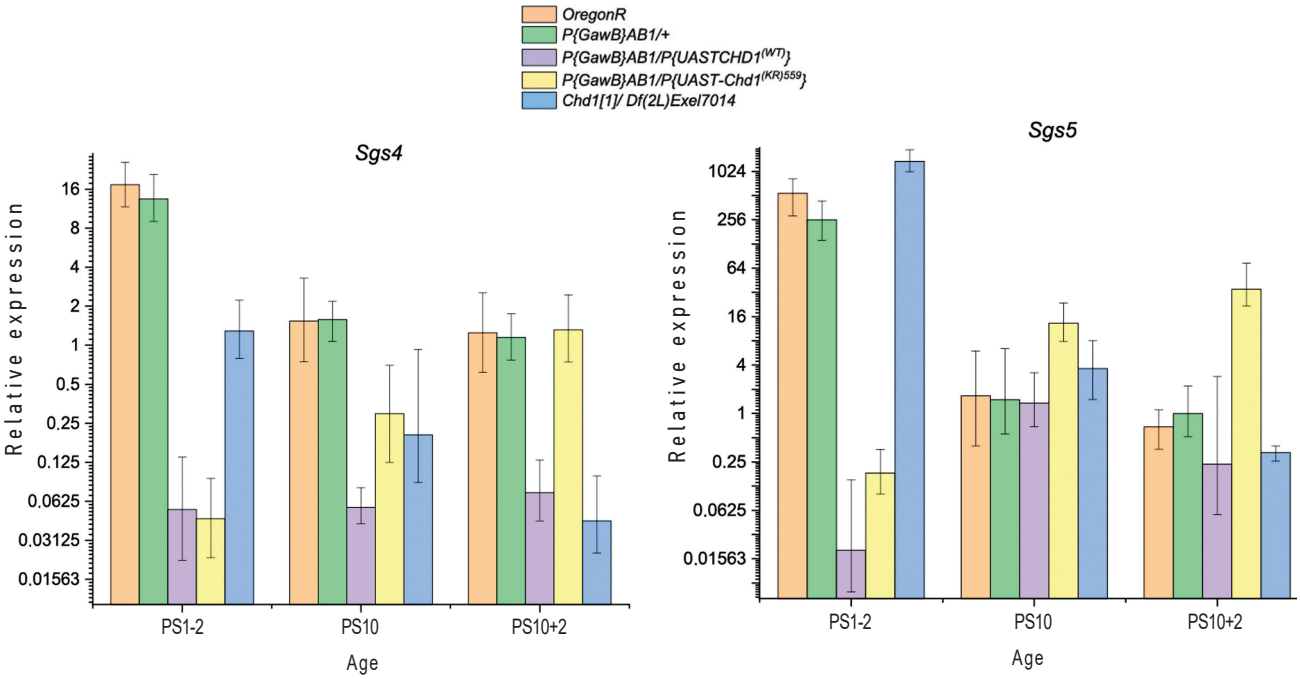

Fig. 2. Age-related changes in the expression of the salivary gland secretion genes *Sgs4* and *Sgs5* in Drosophila. Relative expression and a 95% confidence interval are shown.

hormone-dependent regulation of gene activity. Prostate cancer in humans is hormone-dependent [35]. Multiple transcriptional cofactors, including the pioneer factors FOXA1 and GATA2, strictly regulate hormone-induced gene expression via the androgen receptor (AR) [35]. The expression of salivary gland secretion genes depends on the steroid hormone ecdysone and is mediated by the ecdysone receptor EcR, the closest homologue of the androgen receptor [36]. The expression of the *Sgs4* and *Sgs3* genes is dependent on both the ecdysone receptor EcR and the transcription factor *forkhead*, a direct homologue of FOXA1 [36, 37]. Therefore, hormone-dependent regulation of gene activity in salivary glands of Drosophila is comparable to androgen-dependent transcription regulation in human prostate cells.

CONCLUSION

The proposed model system for assessing the genetic effects of increased CHD1 protein levels in Drosophila cells can be used to study the mechanisms of transcription regulation by the CHD1 protein and its disruption as a result of increased CHD1 protein production.

ADDITIONAL INFO

Author contribution: A.V. Toroshchina: sample collection and preparation, real-time PCR, data analysis, writing the text; A.Yu. Konev: research concept and design, data analysis, writing the text, literature review, final editing of the text. All authors have approved the publication version and also agreed to be responsible for all aspects of the each part of the work and ensured reliable consideration of the issues related to the accuracy and integrity.

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Statement of originality: The authors did not use previously published information (text, illustrations, data) to create this paper.

Data availability statement: data generated in this study are available in the article.

Generative AI: Generative AI technologies were not used for this article creation.

Provenance and peer-review: This work was submitted to the journal on its own initiative and reviewed according to the standard procedure. Two external reviewers, and a member of the editorial board participated in the review.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. А.В. Торощина — сбор и подготовка образцов, проведение ПЦР в реальном времени, анализ полученных данных, написание текста; А.Ю. Конев — концепция и дизайн исследования, анализ полученных данных, написание текста, обзор литературы, окончательная редакция текста. Авторы одобрили версию для публикации, а также согласились нести ответственность за все аспекты работы, гарантируя надлежащее рассмотрение и решение вопросов, связанных с точностью и добросовестностью любой ее части.

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Этическая экспертиза. Исследования проведены с использованием плодовой мушки *Drosophila melanogaster*. Заключения этического комитета не требуется.

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Раскрытие интересов. Авторы заявляют об отсутствии отношений, деятельности и интересов за последние три года, связанных с третьими лицами (коммерческими и некоммерческими), интересы которых могут быть затронуты содержанием статьи.

Оригинальность. При создании настоящей работы авторы не использовали ранее опубликованные сведения (текст, иллюстрации, данные).

Доступ к данным. Все данные, полученные в настоящем исследовании, доступны в статье.

Генеративный искусственный интеллект. При создании настоящей статьи технологии генеративного искусственного интеллекта не использовали.

Рассмотрение и рецензирование. Настоящая работа подана в журнал в инициативном порядке и рассмотрена по обычной процедуре. В рецензировании участвовали два внешних рецензента и член редакционной коллегии.

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