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Worming Insights: A comprehensive review on application of Caenorhabditis elegans in Research

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The nematode worm Caenorhabditis elegans (C. elegans) was first employed as a laboratory organism fifty years ago, and since then, it has been utilized as one of the most popular and adaptable models for almost every area of biological and genetic study. This review highlights the application of C. elegans in research, covering its historical significance, basic features, life cycle, and diverse applications, including obesity, aging, toxicity, and CNS disorders. The historical overview details the key milestones in C. elegans research, from its discovery by Dr. Sydney Brenner to the development of essential techniques and breakthroughs such as the discovery of RNA interference and completion of the genome sequence. The advantages of using C. elegans include its ease of growth, rapid life history, facile genetics, and molecular genetics resources. It is noted that the self-fertilizing nature of C. elegans minimizes inbreeding depression and allows for straightforward assessment of quantitative traits. Additionally, the model's genetic tractability and ability to systematically downregulate gene function through RNA interference are emphasized. The limitations of C. elegans as a model organism, include its evolutionary distance from mammals, simplified tissue complexity, absence of certain organs and systems, short lifespan, limited behavioural complexity, environmental sensitivity, and limited drug metabolism. The versatility of C. elegans in various research areas, its unique characteristics, and its potential to complement other model systems are emphasized. This review concludes by highlighting the importance of C. elegans as a model for studying aging and disease, particularly due to its ease of manipulation and lower cost compared to vertebrate models.

Keywords: C. elegans, obesity, toxicity, ageing, Alzheimer's disease

INTRODUCTION

Over the past several decades, the nematode Caenorhabditis elegans (C.elegans) has emerged as a model organism, revolutionizing our understanding of fundamental biological processes. Initially discovered by Dr. Sydney Brenner in 1963, this microscopic roundworm has since played a great role in understanding the intricacies of developmental biology, genetics, neurobiology, and an array of other disciplines.

C. elegans possesses a compact genome, comprising approximately 20,000 protein-coding genes, providing a manageable system for genetic studies, while its fully sequenced genome has paved the way for extensive functional genomics research. The transparency of the worm's body allows access to visualize cellular processes, making it an ideal model for investigating embryogenesis, organ development, and cellular

differentiation in vivo. One of the hallmark features of C. elegans is its well-defined and invariant cell lineage. The genetic tractability of C. elegans has significantly propelled research in molecular genetics and functional genomics. The worm's short life cycle, spanning only a few days, facilitates rapid experimental turnaround and allows for the observation of multiple generations, rendering it an efficient and cost-effective model for genetic studies.

Beyond its genetic utility, C. elegans has become a focal point for neurobiological research. With a nervous system comprising precisely 302 neurons, researchers have been able to decipher the complete neural circuitry, enabling studies on synaptic function, behavior, and sensory perception. Furthermore, the worm's behavioral responses, such as chemotaxis and thermotaxis, provide an excellent platform for investigating neural circuits and their role in guiding complex behaviors.

As we delve into this review, we will explore the history, the life cycle, and diverse applications of C. elegans as a model organism. From its contributions to the study of aging and longevity to its role in drug discovery, disease modeling, and environmental toxicology, C. elegans continues to offer unparalleled insights into biological phenomena across various disciplines.

HISTORY

The use of Caenorhabditis elegans (C. elegans) as a research model has a rich history that dates back several decades. Here's an overview of the key milestones in the history of C. elegans as a model organism:

Discovery and Isolation (1960s): C. elegans was discovered by Dr. Sydney Brenner in 1963 during his time at the Laboratory of Molecular Biology in Cambridge, United Kingdom. He chose C. elegans as a model organism due to its simple anatomy, short life cycle, and ease of cultivation.[1]

Genetic Characterization (1960s-1970s): In the late 1960s and early 1970s, Brenner, along with John Sulston and Robert Horvitz, extensively characterized the genetics of C. elegans. They established the complete cell lineage of the organism, mapping every cell division from fertilization to adulthood.[2]

Development of Techniques (1970s-1980s): During this period, researchers developed essential techniques for working with C. elegans, including methods for culturing, synchronization, and genetic manipulation. Brenner, Sulston, and Horvitz were awarded the Nobel Prize in Physiology or Medicine in 2002 for their work on the genetics and development of C. elegans. [1-4]

Introduction of Forward and Reverse Genetics (1980s): The use of forward and reverse genetics approaches in C. elegans became more widespread. The introduction of mutagenesis screens and the ability to identify and isolate specific mutants contributed to the understanding of gene function and development. [5]

RNA Interference (RNAi) Discovery (1990s): In 1998, Andrew Fire and Craig Mello discovered RNA interference (RNAi) in C. elegans, a breakthrough that revolutionized the field of molecular biology. This finding allowed researchers to selectively silence genes, providing a powerful tool for studying gene function.

Completion of the Genome Sequence (1998): The C. elegans genome was fully sequenced in 1998, making it the first multicellular organism to have its entire genome sequenced. This milestone facilitated genomic and functional studies, enabling researchers to identify and study all the genes in the organism.

Research on Aging and Longevity (2000s): C. elegans became a prominent model for studying aging and longevity. The short lifespan of the worm, combined with its well-defined genetics, allowed researchers to investigate the molecular mechanisms underlying the aging process.[6]

Disease Modeling (2000s-Present): Researchers started using C. elegans as a model for various human

diseases, including neurodegenerative disorders like Alzheimer's and Parkinson's disease. This provided insights into disease mechanisms and potential therapeutic targets.

Functional Genomics and Systems Biology (2000s-Present): Advances in functional genomics and systems biology allowed researchers to study complex biological processes, such as development, behavior, and metabolism, in a holistic manner. C. elegans served as an excellent model for these studies.

Integration with Other Research Areas (2000s-Present): C. elegans research has become integrated with other fields, including cell biology, neurobiology, immunology, and environmental science. The versatility of C. elegans as a model organism has contributed to its widespread use in interdisciplinary research.

BASIC FEATURES AND LIFE CYCLE

C. elegans is a harmless, free-living nematode that consumes microorganisms for food. It is very affordable and simple to maintain in a laboratory environment. When cultured at 20°C, adult C. elegans, which are self-fertile hermaphrodites measuring 1 mm in length, have a typical lifespan of roughly 18–20 days. Their reproductive cycle lasts 2.5–4 days at room temperature.2 When C. elegans hatch, they can either develop into the four larval stages (L1–L4) right away or wait until the L2 larval stage to reach the L3 larval stage before moving on to the Dauer larval stage. The dauer larval stage is a dispersion stage with developmental arrest that helps organisms endure harsh environments. C. elegans can recover and molt into the L4 larval stage, continuing normal development, after the unfavorable conditions pass. [3]
The important features of each stage are as follows:

Embryonic Stage: The embryonic stage of C. elegans is crucial for studying early development, cell fate determination, and organogenesis. The transparent nature of the embryos allows for real-time observation of cell divisions and differentiation.

Larval Stages (L1 to L4): C. elegans goes through four larval stages, and each stage involves specific developmental events. These stages are essential for studying growth, organ development, and the effects of genetic mutations on the overall development of the organism.

Adult Stage: The adult stage is crucial for investigating various biological processes, including reproduction, behavior, aging, and longevity. Researchers can study the effects of genetic manipulations or environmental factors on the adult phenotype.

Reproductive Stage: C. elegans has a well-defined reproductive system, and studying this stage provides insights into fertilization, gamete development, and the genetic control of reproduction. It is also valuable for understanding the molecular mechanisms underlying fertility and sterility.

Dauer Stage: The dauer stage is a non-feeding, stress-resistant larval stage that C. elegans can enter under adverse conditions. Studying the dauer stage is important for understanding environmental responses, survival strategies, and the molecular mechanisms regulating entry into and exit from dauer. [7]

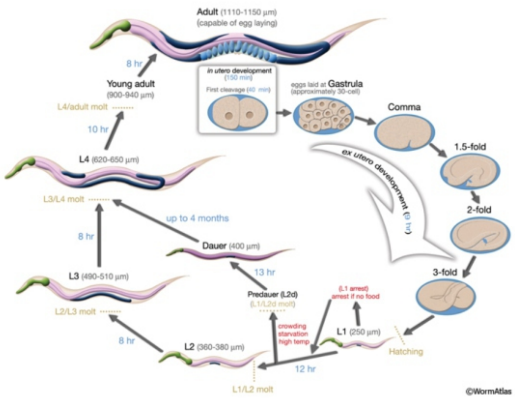

Figure1: Life cycle of C. elegans at 22°C.

0 min is fertilization. Numbers in blue along the arrows indicate the length of time the animal spends at a certain stage. First cleavage occurs at about 40 minutes post-fertilization. Eggs are laid outside at about 150

minutes post-fertilization and during the gastrula stage. The length of the animal at each stage is marked next to the stage name in micrometers (μm). 8-9

C. elegans in Obesity research

The anatomy of C. elegans is simple, with only a few tissues and organs. One of its primary organs, the intestine of C. elegans, performs the role of the fat metabolic organ, mimicking the functions of the mammalian liver and fat tissue.[9] It is simple to quantify accumulated fat in C. elegans using chemical/biochemical or dye-based techniques, as it is mostly found in hypodermal and intestine cells.[10] Chemicals are absorbed by C. elegans through two main pathways: cuticle diffusion and ingestion. Ingestion is controlled by the neurological system and is influenced by satiety, the food source, and/or the availability of food. For C. elegans to feed, it must first suck in liquid that contains suspended bacteria, trap those germs in its pharynx, and then release the liquid. At the conclusion of each embryonic stage, the underlying epithelial cells of C. elegans generate and secrete the cuticle, which is then re-established through moulting. A compound can diffuse through the cuticle which could be a barrier for absorption of compounds thus, mutants with increased cuticle permeability, such as bus-5, can be used for the increased chemical diffusion into the body. [11]

A successful method for performing a whole-animal chemical screen of pharmaceuticals is to utilize the nematode worm Caenorhabditis elegans as an alternative screening system. [12] C. elegans is, in fact, in a perfect position to find pharmacological molecules that could potentially be anti-obesity drugs. There is a significant degree of conservation in the distribution and storage mechanisms of fat between mammalian species and C. elegans [13]. The routes for amino acid metabolism, glycolysis, gluconeogenesis, mitochondrial and peroxisomal fatty acid β-oxidation, fatty acid biosynthesis, elongation, and desaturation, and gluconeogenesis are all similarly conserved.[14] In the skin-like epidermal cells that directly border the gut, C. elegans stores fat in droplets. Environmental conditions and other factors cause substantial changes in droplet size, quantity, and distribution. The vital dye Nile Red has been used to stain and quantify fat storage in worms without affecting growth, development, or survival[15] to visualize the fat content in C. elegans.

C. elegans in Ageing research

Anatomical and functional traits including immunity, learning and memory, motility, and tissue integrity all deteriorate with age in C. elegans. Age-related modifications to tissues primarily affect the neurological, muscular, and reproductive systems. With aging, the reproductive system's structure degrades, and the rate of reproduction sharply declines. In C. elegans, aging is accompanied with structural alterations and a decline in neuronal function.[16] Approximately two thirds of all human disease genes have homologs in the genome of C. elegans. [17] C. elegans is a useful model for studying aging mutations because of its easy maintenance in the lab, transparent body for anatomical observation, high genetic homology (60–80%) with humans, availability of the complete genome sequence, conserved biological molecular responses, high fertility rates (~250 eggs/worm within several days), and availability of molecular biology tools (i.e., transgenic, gene knockouts, and RNAi knockdowns)[18]. Many substances that considerably lengthen this nematode's life have been found, based on mutations in the age-1(PI3K) or daf-2(INSR) genes and a decrease in the daf-16 (foxo) mutant. In comparison to untreated controls, C. elegans treated with 25 and 50 μM silymarin had mean lifetime increases of 10.1 and 24.8%, respectively[19]. Another study showed that fullerenol increased lifespan by protecting C. worms under stress circumstances by up-regulating stress-related genes in a DAF-16-dependent way. These reduced endogenous levels of ROS [20].

C.elegans in Toxicology

Neural networks and somatic cell sites in C. elegans have been mapped21, enabling detailed neurological/behavioral correlations and

morphological evaluations of defects caused by toxins. This model has great potential for assessing human-relevant pathways of toxicity because: (a) C. elegans was the first multicellular organism whose genome was completely sequenced (b) genes and signalling pathways are well conserved [13]; (c) studies to understand C. elegans genetics have been ongoing for over 40 years [24] and (d) mutant and transgenic C. elegans strains are readily available.

Research aimed at rating toxicity in C. elegans has regularly demonstrated a strong association with the oral LD50 ranking in rodents. Rat and mouse oral LD50 ranking at one-tenth the cost of rodent testing coincided with the toxicity order for eight metal salts based on C. elegans adult mortality, according to an early ranking study that used C. elegans maintained on plates with test materials dissolved in agar[24]. Certain classes of poisons have also been successfully detected by focused C. elegans screening. An elegant test that was developed lately to identify aneugens—toxins that cause changes in the number of chromosomes per cell—is one example. Over 99.8% of the wild-type population of C. elegans is generally made up of self-fertilizing XX hermaphrodites, while XO males are rare and develop spontaneously by chromosomal non-disjunction. Transgenic C. elegans males will illuminate due to the expression of green fluorescent protein driven by a male-specific promoter. Seven out of eight known mammalian aneugens significantly enhanced the amount of XO male progeny when this transgenic strain was exposed to chemicals and the number of male offspring was examined; five known non-aneugens tested negative.[26]

C.elegans in CNS Disorders

C.elegance in Alzheimer's Disease

Caenorhabditis elegans (C. elegans) has been used as a model organism to study various aspects of Alzheimer's disease (AD). While C. elegans does not naturally develop Alzheimer's disease, researchers have utilized genetic and biochemical approaches to express human genes associated with AD in the worms. Researchers introduce genes associated with Alzheimer's disease, such as the amyloid precursor protein (APP) and presenilin, into C. elegans. The expression of these genes in C. elegans can lead to the formation of beta-amyloid aggregates, a characteristic feature of Alzheimer's disease. C. elegans provides a simple system for studying the aggregation of beta-amyloid peptides. Researchers can monitor the formation and accumulation of amyloid plaques in the transparent worms, facilitating visualization and analysis. Expression of Alzheimer 's-associated genes in C. elegans can lead to neurodegeneration and behavioural changes. C. elegans models of Alzheimer's disease are used in drug screening assays to identify potential therapeutic compounds. Compounds that modulate beta-amyloid aggregation or mitigate neurodegenerative effects in the worms may be further investigated for their relevance to Alzheimer's treatment.[28]

Through genetic studies in C. elegans, researchers can identify genes that modify the effects of Alzheimer's-associated genes. These genetic modifiers may represent potential targets for therapeutic intervention or provide insights into the underlying molecular pathways. Since aging is a significant risk factor for Alzheimer's disease, C. elegans, with its short lifespan, is used to study the interplay between aging and AD-related pathology.

While C. elegans may not fully recapitulate the complexity of human neurobiology, its amenability to genetic manipulation, short generation time, and simple nervous system makes it a valuable tool for investigating fundamental aspects of Alzheimer's disease and for screening potential drug candidates. Findings from C. elegans studies can complement research conducted in other model organisms and cell culture systems, contributing to our overall understanding of Alzheimer's disease pathogenesis.[27]

C.elegans in Parkinson's Disease

Caenorhabditis elegans (C. elegans) has also been

utilized as a model organism in Parkinson's disease (PD) research. Like Alzheimer's disease studies, researchers introduce genetic and molecular alterations into C. elegans to mimic aspects of Parkinson's disease. Here are some ways in which C. elegans is employed in Parkinson's disease research:

Researchers introduce genes associated with Parkinson's disease, such as alpha-synuclein, parkin, and PINK1, into C. elegans. The expression of these genes in C. elegans can lead to the formation of aggregates or other pathological changes associated with Parkinson's disease.

Parkinson's disease is characterized by the accumulation of alpha-synuclein aggregates in the brain. C. elegans allows scientists to study the aggregation of alpha-synuclein and its impact on neuronal function. Expression of Parkinson 's-associated genes in C. elegans can result in neurodegenerative changes, including damage to dopaminergic neurons. Behavioral assays can be conducted to assess movement abnormalities, as impaired locomotion is a hallmark of Parkinson's disease.[29]

Genes like PINK1 and parkin are associated with mitochondrial function, and mutations in these genes are linked to familial forms of Parkinson's disease. C. elegans is used to study mitochondrial dysfunction in the context of Parkinson's. C. elegans models of Parkinson's disease are employed in drug screening to identify compounds that may modulate alpha-synuclein aggregation, protect against neurodegeneration, or improve motor function. Through genetic studies, researchers can identify genes that modify the effects of Parkinson 's-associated genes, offering insights into potential therapeutic targets.[31]

While C. elegans may not fully capture the complexity of human neurodegenerative diseases, its advantages, such as a short lifespan, genetic tractability, and simple nervous system, make it a valuable model for investigating fundamental aspects of Parkinson's disease. Findings from C. elegans studies can contribute to our understanding of the molecular mechanisms underlying the disease and aid in the discovery of potential therapeutic targets.[30]

ADVANTAGES

1.1 Ease of growth and rapid life history

The utilization of C. elegans for aging research offers several inherent advantages, with its life history being particularly noteworthy. One primary advantage is its remarkably short life expectancy, lasting approximately 14 days, and a maximum lifespan of only 25–30 days when cultivated on agar at 25°C.

Lifespan assessment in C. elegans is not limited to solid substrates; it can also be conducted in liquid environments. Worms demonstrate adaptability to a temperature range spanning from 16 to 27°C. Moreover, it is worth noting that C. elegans can be cultivated axenically, thriving even in conditions where a fully defined medium is employed.[34]

The developmental process in C. elegans is swift, with eggs maturing into reproductive adults within 3 days. Reproduction reaches a significant stage by the 5th day of adult life and is essentially concluded by the 10th day.32. On average, a single worm can generate approximately 300 progenies, leading to an astonishing potential of about 100,000 offspring within just one week.

1.2 Facile genetics

Sydney Brenner, often recognized as the pioneer of nematode genetics, selected C. elegans due to its unique feature of being a self-fertilizing hermaphrodite (XX) that occasionally produces spontaneous males (XO) at a low frequency.[1]

Mating the spontaneous males with hermaphrodites allows the generation of cultures with a 50% male population. This makes the genetic system nearly ideal as it seamlessly combines the ease of homozygous expressing recessive mutations for straightforward assessment, along with the flexibility to readily cross strains for complementation tests, constructing double

mutants, and other intricate genetic analyses.

A less recognized challenge faced by research labs currently investigating the genetics of aging is the presence of hybrid vigor and inbreeding depression. These factors can significantly complicate the tracking of quantitative phenotypes, such as the length of life, making the task almost inherently difficult.

The self-fertilizing nature of C. elegans implies that the genome undergoes constant evaluation for fitness in the homozygous state.

These selective processes lead to the swift fixation of new mutants and work against deleterious recessive alleles responsible for hybrid Vigor. Consequently, C. elegans does not exhibit inbreeding depression or hybrid Vigor, allowing for a straightforward assessment of quantitative traits. [33],[35]

The ability to keep the worm in a cryogenically frozen state facilitates the convenient maintenance of numerous strains. As a result, there is minimal variation among lab strains since they do not undergo evolutionary changes through continuous propagation in the laboratory.

The entire anatomy of C. elegans is well-documented, and the development of individual worms is highly uniform and nearly identical36; indeed, at the neuroanatomical level, there are about the same number of neural connections between identical cells in different individuals. 21 A hermaphrodite of C. elegans possesses 959 somatic cells and an indeterminate number of sperm, typically around 300. Oocytes are generated as needed, and in the adult stage, the only actively dividing cells are those in the germ line.

1.3 Molecular genetics

The genetic resources accessible in the C. elegans nematode species are unparalleled among metazoans and, in certain aspects, even surpass those available in yeast. The genetic resources accessible in the C. elegans nematode species are unparalleled among metazoans and, in certain aspects, even surpass those available in yeast.

Similar to other rapidly advancing research areas, there exists a collection of early and pertinent studies that may be underappreciated due to their lack of online accessibility. Notably, transgenic worms can be effortlessly generated through the physical injection of DNA from any species. 40,41. This DNA is maintained extra chromosomally as tandem arrays until it is integrated into the genome. The transparency of the worm allows for remarkable microscopic studies and enables in vivo examinations of proteins tagged with GFP. [42],[43]

Undoubtedly, the most significant advantage of C. elegans is the ability to systematically downregulate gene function through RNA interference (RNAi). The injection of double-stranded RNA or the feeding of bacteria-carrying constructs that produce double-stranded RNA leads to the specific inhibition of the targeted gene in C. elegans 44. In addition to elucidating a model for genetic resistance to viral infection, the RNA interference (RNAi) methodology has facilitated screens of every gene in the genome for various phenotypes, with a particular focus on aging-related traits. [45]

DISADVANTAGES

Despite its numerous advantages, the use of C. elegans as a model system for the study of aging comes with several challenges. Foremost among these challenges is the diminutive size of nematodes, which poses a significant obstacle in acquiring biochemical quantities of these organisms as they age. While methods for cultivating substantial numbers of mature worms have been devised the implementation of these techniques remains challenging. [46] Consequently, there has been a limited number of biochemical studies focusing on worms in their later stages of life. Nevertheless, systematic investigations into genes exhibiting differential expression based on age, whether at the protein or mRNA level, have been documented. 43 Specific examinations of enzymes and physiological aspects, particularly in the realms of

metabolism and oxidative stress, have been conducted about chronological age. However, there remains a considerable amount of work to be undertaken in this regard. [48]

The diminutive size of nematodes also contributes to a lack of understanding regarding late-life pathology. Nematodes are deemed deceased when they exhibit a lack of spontaneous movement, do not respond to touch, and experience a loss of turgor pressure [33]. Many laboratories investigating nematode aging have moved away from utilizing the turgor pressure assay and now rely solely on the absence of movement to determine death. Observation of dead worms reveals them to be filled with bacteria emerging from various orifices such as the mouth, anus, and vulva. This observation suggests that the bacterial presence is a passive response to death rather than the primary cause of demise.[33]In-depth electron microscopic investigations have revealed that aging worms undergo degeneration in various tissues at distinct rates. Notably, the nervous system tends to be well-maintained in older worms, whereas the reproductive system and muscles appear to deteriorate more rapidly [50]

LIMITATIONS

While Caenorhabditis elegans (C. elegans) has proven to be a valuable model organism for various biological studies, it does have limitations. Some of the key limitations of C. elegans as a model organism include

Evolutionary Distance from Mammals:
C. elegans is evolutionarily distant from mammals, and certain physiological processes and systems in nematodes may not precisely mimic those in humans.

Simplified Tissue Complexity:
The nematode has a relatively simple anatomy with fewer cell types compared to mammals, which might limit its ability to model certain complex biological processes.

Absence of certain organs and systems
C. elegans lacks certain organs and systems found in mammals, such as lungs, a circulatory system, and complex immune responses. This can limit the study of diseases or processes involving these specific organs or systems.

Short lifespan
The short lifespan of C. elegans can be both an advantage and a limitation. While it allows for rapid observations of aging-related changes, it may not fully capture the complexity and longevity of human aging.

Limited behavioural complexity
The behavioural repertoire of C. elegans is relatively simple. Although it displays a range of behaviours, it may not adequately represent the complexity of mammalian behaviours and cognitive processes.

Environmental sensitivity
C. elegans is highly sensitive to environmental conditions, and small changes in the laboratory setting can significantly impact experimental outcomes. This sensitivity may limit the reproducibility and generalizability of results.

Limited Drug metabolism
The nematode lacks a complete drug metabolism system comparable to that of mammals. This can affect the study of drug interactions and metabolism. [51],[52].

Dauer stage and Diapause
The Dauer stage, a type of developmental arrest, is a notable feature in the life cycle of C. elegans. However, its relevance to mammalian aging remains uncertain, raising questions about the translatability of certain findings.[53]

CONCLUSION

The selection of models for studying human disease frequently involves balancing the model's ability to accurately represent the human situation with its ease of manipulation. For the study of development, signalling pathways, and many other areas of biology, invertebrate models like those of C. elegans and D. melanogaster have shown to be invaluable. Throughout this review, we have provided numerous examples that demonstrate how simple these C. elegans studies can be. Because of its distinct

characteristics, C. elegans is a great model to use in research in addition to mammalian models. While still allowing for the testing of theories in a complete metazoan organism, C. elegans investigations are less expensive than those involving in vivo vertebrate models. The life history of C. elegans lends itself to high-throughput analysis, and the genetic techniques available for this organism make it a suitable model for exploring the involvement of particular genes in varied pathologies. Therefore, C. elegans is a great addition to both in vivo vertebrate models and in vitro or cell culture-based systems.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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