A novel approach to cancer screening using the nematode *Caenorhabditis elegans*-based detection assays

Nový prístup ku skríningu zhubných nádorov pomocou detekčných testov použitím nematódy *Caenorhabditis elegans*

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Summary

Background: Early diagnosis of cancer is essential for its effective treatment. Currently, established screening tests are cancer-specific and require screening for each type of cancer separately. The primary objective of cancer research is to develop methods that can detect multiple types of tumors from a single body fluid sample. Multicancer early detection tests aim to detect fragments of circulating tumor DNA, cell-free DNA, circulating microRNAs, or proteins released by cancer cells in the patient's body fluids. However, these tests are not suitable for routine cancer prevention due to their high cost. Therefore, in recent years, cancer screening tests have been developed to detect volatile organic compounds in urine using living organisms, such as nematodes, *Caenorhabditis elegans.* Measuring only 1 mm in length, *C. elegans* has the potential to offer a new, efficient, cost-effective, quick, and painless method to detect the presence of tumor. ***Purpose:*** The purpose of this review is to present an overview of the literature on the development and validation of *C. elegans*-based cancer detection methods. The potential benefits of these assays are significant, as they could become a valuable tool for the early identification and diagnosis of cancer, even though this research is still in its initial stages of development.

Key words

cancer – detection methods – cancer diagnosis – *Caenorhabditis elegans*

Súhrn

Východiská: Včasná diagnostika zhubných nádorov je nevyhnutná pre ich účinnú liečbu. V súčasnosti sú zavedené skríningové testy špecifické pre jednotlivé typy zhubných nádorov, čo si vyžaduje testovanie pre každý typ zhubného nádoru zvlášť. Hlavným cieľom výskumu zhubných nádorov je vyvinúť metódy, ktoré dokážu odhaliť viacero typov malígnych nádorov z jednej vzorky telesnej tekutiny. Testy na včasnú detekciu viacerých typov zhubných nádorov sú zamerané na odhalenie fragmentov cirkulujúcej nádorovej DNA, voľnej DNA, cirkulujúcej mikroRNA alebo proteínov uvoľnených nádorovými bunkami v telesných tekutinách pacienta. Avšak kvôli vysokým nákladom nie sú tieto testy na prevenciu zhubných nádorov v bežnej zdravotnej starostlivosti vhodné. Preto sa v posledných rokoch venuje pozornosť skríningovým testom na zhubné nádory, ktoré detegujú prchavé organické zlúčeniny v moči onkologických pacientov. Na takéto testy sa často využívajú živé organizmy, napr. hlístovce *Caenorhabditis elegans*. *C. elegans*, ktorý meria iba 1 mm, má potenciál ponúknuť novú, účinnú, nákladovo efektívnu, rýchlu a bezbolestnú metódu na zisťovanie prítomnosti malígnych nádorov. ***Cieľ:*** Cieľom tohto článku je predložiť prehľad literatúry o vývoji a overovaní metód detekcie malígnych nádorov pomocou nematód *C. elegans.* Potenciálne benefity týchto testov sú významné, pretože by sa mohli stať cenným nástrojom pre skorú identifikáciu a diagnostiku zhubných nádorov, aj keď tento výskum je stále v počiatočných štádiách vývoja.

Kľúčové slová

zhubné nádory – metódy detekcie – diagnostika zhubných nádorov – *Caenorhabditis elegans*

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Introduction

According to global mortality data, cancer is one of the leading causes of death and a major obstacle to increasing life expectancy [1]. Cancer trends may change in the coming decades due to sociological, economic, and lifestyle changes associated with human development. However, population growth and aging are expected to contribute to an increase in cancer rates [2]. Early diagnosis and treatment are critical for the cure of many cancers. The detection and treatment of cancer will become increasingly important as the population ages and the incidence of cancer increases [3]. During the development of cancer, the body produces substances known as tumor markers. Tumor markers are not used to support a diagnosis, but rather to assess a patient's response to treatment or to monitor recurrence of the disease [4].

A continuing challenge for healthcare is the effectiveness of routine cancer screening tests. These tests are cancer-specific, meaning that separate evaluations must be performed for each type of cancer. However, the goal of cancer research is the development of screening tests capable of detecting multiple types of cancer from a single sample of body fluid, such as peripheral blood. This is achieved through multicancer early detection (MCED), which looks for fragments of circulating tumor DNA (ctDNA), cell-free DNA (cfDNA), circulating microRNAs (miRNAs) or proteins released by cancer cells in a patient's body fluids. Liquid biopsy refers to tests that obtain information from a patient's body fluids without taking a sample directly from the tumor. The usefulness of liquid biopsy in the monitoring of patients has been investigated in several studies [5–10]. However, the high cost of the MCED screening tests is a limiting factor.

In recent years, there has been a rapid accumulation of research in the scientific literature linking the presence of volatile organic compounds (VOCs) in urine with various cancers [11–14]. In fact, urine has several advantages over other biological fluids, including affordability, metabolite richness, ease of handling, and availability in large quantities without invasive collection methods. Urine transports waste products from the blood and contains intermediates or end products of several metabolic pathways. Therefore, it can provide information not only from the urinary tract but also from distant organs through plasma collected by glomerular filtration [15,16]. Several studies have suggested that tumor growth is related to scent traces in urine, which can be detected by some animals such as dogs or mice [17–21]. However, the use of these animals in therapeutic trials is not feasible. For screening purposes, it is necessary to use simple organisms that are easy to handle in the laboratory, have low nutritional and growth requirements, and can reproduce rapidly in large numbers. A nematode, *C. elegans*, meets these criteria, as it is easily cultured in the laboratory, has a short life cycle with many offspring [22]. The olfactory system of this nematode is highly sensitive and effective in the detection and discrimination of a wide variety of chemical compounds, including volatile and water-soluble substances associated with food, danger, or other animals, including VOCs in urine from cancer patients [23]. Based on these findings, this review summarizes the basic facts and recent data that have led to the development of new cancer screening methods using *C. elegans*, with the aim of stimulating future research.

C. elegans – an organism used in cancer research and detection

C. elegans is a multicellular organism that has been used as a model in biological research for more than 50 years. It is a small translucent worm that is easy to grow and maintain under laboratory conditions. As an adult, the hermaphrodite reaches a size of about 1 mm, whereas males are a little smaller. Hermaphrodites, which make up 99.9% of the population, can reproduce through self-fertilization using their own sperm or by mating with males. The population consists of only 0.01% males who solely produce sperm. Hermaphroditism facilitates the maintenance and reproduction of genetically defined strains of *C. elegans* [24]. Under normal conditions, individuals undergo four developmental stages designated L1, L2, L3, and L4. Each larval stage begins with a cuticle exchange, where a new cuticle is created and the old one is removed. The time required to reach adulthood depends on the temperature at which the nematodes grow. At a temperature of 20°C, development is completed in approximately 3.5 days. If the larvae develop on crowded plates or under food-free conditions, they enter an alternate larval stage known as the Dauer larva [25]. These larvae do not consume food, are thinner and have a thicker cuticle to prevent dehydration. Under optimal conditions, especially when the food source is restored, they can continue to develop. Cultivation of *C. elegans* typically involves the use of nematode growth medium supplemented with the *Escherichia coli* OP50 bacterial strain. Due to its well-understood genome and nervous system, *C. elegans* is a powerful tool for studying the molecular basis of disease. Its olfactory system is particularly sensitive and useful, as it can detect volatile chemicals at a distance [26].

Cancer is believed to change the metabolome of the organism, which can release its characteristic waste products into biofluids, including urine [27,28]. Changes in metabolism can lead to the production of VOCs, which are commonly found in malignant tumors and contribute to the odor of cancer [29]. *C. elegans* detects chemicals by exposing its sensory cilia to the environment through the cuticle. The adult nematode has 959 somatic cells, of which 302 are neurons. It is assumed that 16 pairs of bilaterally symmetric neurons, approximately 10% of the nervous system, participate in chemosensation [30]. Chemosensory neurons are found in the amphid, phasmid, and inner labial organs, which are directly or indirectly exposed to the environment [26]. *C. elegans* uses three pairs of olfactory neurons, AWA, AWB, and AWC, to detect attractive or repulsive odors. Typically, worms use AWA and AWC olfactory neurons to identify appealing substances, while AWB neurons are used to detect volatile aversive compounds [30–32]. Chemotaxis to volatile substances is

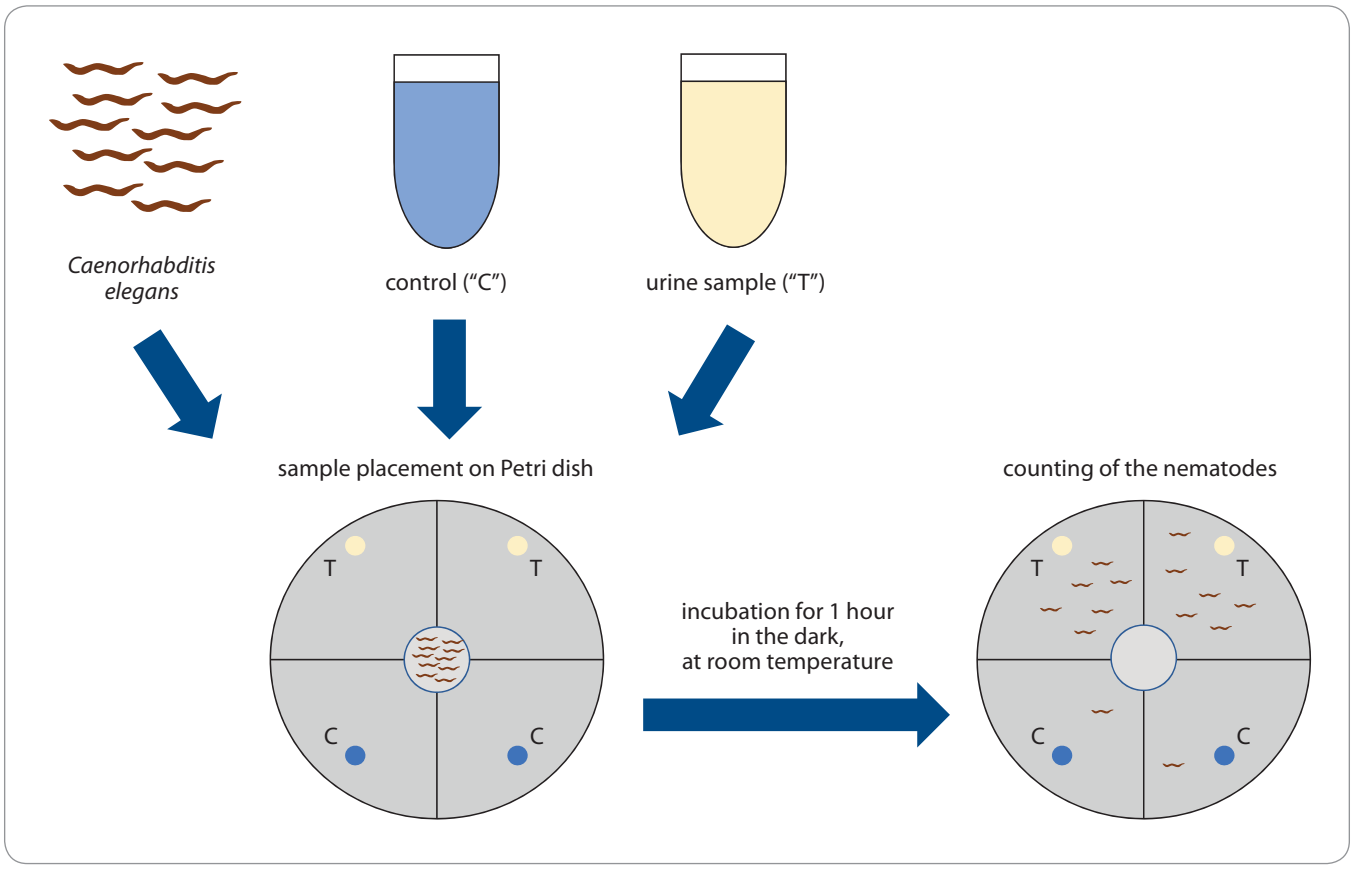

Fig. 1. An overview of a common scheme for chemotaxis assays using *Caenorhabditis elegans*. The assay involves placing test samples on one side of a Petri dish and a control on the opposite side. The Petri dish is divided into four quadrants by two perpendicular lines, creating four sections of equal size. A circle with a radius of 0.5 cm is marked at the point where the quadrant lines converge. Each quadrant has a designated point labeled either "T" or "C" that is 2 cm away from the dish's central point. These points are used as the application sites for the test substances: urine samples obtained from cancer patients or healthy volunteers (marked as "T") and a control solution consisting of buffer (marked as "C"). The worms are typically equidistant between the test and control areas. After exposure time, the worms are tracked and counted at both locations. Worms that are attracted move towards the tester region, while those that are repelled move away from it.

dependent on a trimeric G-α protein ODR-3, which is expressed in the AWA, AWB and AWC olfactory neurons [33].

Development of cancer detection assays using *C. elegans*

Following the information mentioned above, scientists have developed chemotaxis assays using *C. elegans* to evaluate the attractiveness or repulsiveness of volatile chemicals [33–39]. The complete genome sequence of this nematode is now known, encoding at least 1,500 G-protein coupled receptors, including olfactory receptors. The compact and well-characterized nervous system of this organism, together with access to transgenic strains, allows in-depth investigation of the neuronal mechanisms controlling behavioral responses [25,40].

In 1973, Ward et al. performed the first *C. elegans* chemotaxis experiments [34]. Following these experiments, numerous studies of chemotaxis in *C. elegans* have contributed to the understanding of the neuronal mechanisms responsible for behavioral responses to various stimuli. Since then, these experiments have been modified and used in various fields of research [35–39,41,42]. Chemotaxis assays in *C. elegans* usually involve placing test samples on one side of a Petri dish and a control on the opposite side, with the worms equidistant between the test and control areas (Fig. 1). After a specified exposure time, the worms are tracked and/or counted at both locations. Attracted worms migrate toward the tester region, while repelled worms move away from it [33,35,36]. Hirotsu et al. documented that *C. elegans* nematodes were attracted to the urine of cancer patients, but avoided urine samples from healthy individuals [37]. In contrast, nematodes with ablated olfactory neurons did not show attraction to the urine of cancer patients, indicating that *C. elegans* can detect specific odors in the urine samples of these individuals [37]. The results of this study formed the basis for new cancer screening tests called "nematode nose" (N-NOSE), which are based on the chemotaxis characteristic of *C. elegans* [43,44]. For example, Kusumoto et al. investigated the diagnostic performance of N-NOSE using 76 samples from healthy participants and 180 samples from cancer patients [43]. They found that *C. elegans* olfaction allowed highly sensitive detection of gas-

trointestinal malignancies from urine through N-NOSE tests [43]. In another study, researchers used this clinical approach to detect early pancreatic cancer in humans [45]. The urine of pancreatic cancer patients (stage 0 or IA) and healthy volunteers was examined. Based on chemotactic indices, it was found that *C. elegans* nematodes were attracted to cancer-associated scents. This method has a higher sensitivity to detect early pancreatic cancer compared to existing diagnostic markers, based on changes in the preoperative and postoperative chemotaxis index [45]. Inaba et al. developed the N-NOSE combination method using information from two dilutions (10× and 100×) of urine samples from cancer patients, resulting in a significantly higher sensitivity for cancer detection [44]. Using the *C. elegans* behavioral assay, Thompson et al. successfully discriminated the urine of patients with early-stage prostate cancer from that of controls [46]. Currently, prostate-specific antigen (PSA) has the highest sensitivity of any biomarker for non-invasive detection of prostate cancer. However, it is characterized by low specificity, which can lead to overdiagnosis, as it can be excreted by malignant and nonmalignant epithelial cells. Therefore, there is a need for biomarkers that can complement or replace PSA, as needle biopsies resulting from overdiagnosis of prostate cancer can be invasive and dangerous [46].

It has previously been shown that *C. elegans* can detect malignant VOC signatures in canine urine samples. Namgong et al. conducted a study comparing *C. elegans* chemotaxis between urine samples from dogs with cancer and urine samples from healthy dogs without cancer [47]. Their results demonstrated a sensitivity of 85%, suggesting that *C. elegans* can detect the presence of cancer in a variety of prevalent canine malignancies. Furthermore, with a urine specificity of 90%, the test showed a low percentage of overidentification of cancer risk [47]. Based on the results of chemotactic assays using *C. elegans*, Kaiglová et al. hypothesized that urine from cancer patients contains specific odor traces. The study found a significantly higher chemotactic index of the urine samples from the cancer group compared to the control group (urine samples from healthy volunteers, $P < 0.0001$) [48].

Together, these findings demonstrate that *C. elegans* may be a valuable tool for the early detection of cancer. The high sensitivity of the *C. elegans* chemotaxis assay indicate its potential to detect cancer at an early stage when treatment is most effective. Nevertheless, VOCs have also been associated with non-tumor pathologies, including tuberculosis [49] and diabetes [50], potentially compromising the specificity of the test. While the other studies mentioned above suggest the potential utility of *C. elegans* in cancer detection, further research is needed to validate the test in larger clinical trials.

Conclusions

The use of *C. elegans*-based screening tests has the potential to provide a sensitive, cost-effective, and non-invasive solution for cancer screening. However, it is important to note that this method is still in its early stages. Urine samples can be collected routinely and painlessly and the test can be performed quickly in a straightforward laboratory setting. The objective of a primary screening test for early cancer detection using the nematode olfactory system should be simplicity, non-invasiveness, and affordability. Although this review presents promising findings, additional research is necessary to validate *C. elegans* cancer-based detection methods. With the further development of this technique, *C. elegans* could become a valuable tool for the early detection and diagnosis of cancer.

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