Original Article

Odor detection of cancer cell metabolites by scent-detection dogs

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Abstract

Dogs have a superior olfactory system; thus, they have been trained and used to detect various nonbiological and biological scents. In addition, many studies have recently reported dogs' ability to detect the odor of certain cancers or cancer cells. A previous study documented that a dog trained to detect a certain malignant cancer cell could also detect another, unfamiliar malignant cancer cell well, implying that these two cancer cells share a certain specific odor. Thus, given the hypothesis that malignant cancer cells of different origins may contain a common cancer-specific odor, the purpose of the present study was to evaluate odor detection ability for various cancer cells (prostate, lung and breast cancer) by dogs trained on prostate cancer cells. Two dogs were trained and participated in the tests. Sensitivity, specificity and the value of area under the curve (AUC) by receiver operating characteristic curve analysis were evaluated. According to the AUC value, the two dogs showed excellent and perfect detection abilities in detecting the odor of a trained prostate cancer cell (PC3), respectively. Both dogs also showed good detection ability for another, unfamiliar prostate cancer cell (LNCaP-LN3). When evaluating the detection ability for lung (A549) and breast (MCF-7) cancer cells, the two dogs showed excellent and good detection abilities, respectively. In conclusion, it is presumed that a certain common cancer-specific odor exists in cancerous cells when compared with normal cells. Scent-detection dogs have promising potential in training for cancer detection. Further study is needed to determine whether the detection ability of dogs trained to cancer cells affects the detection ability for real cancer.

Keywords: cancer cell line, carcinoma, odor, prostate cancer, scent-detection dog

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Received April 26, 2022
Accepted July 10, 2022
https://doi.org/10.14456/tjvm.2022.61

Introduction

Dogs have been reliable in odor detection because of their superior olfactory system. With this advantage, dogs have been trained and utilized to detect various substances such as explosives, hazardous chemicals or drugs. They have also been used to detect biological scents, including human odors and scents of other animals (Beebe *et al*., 2016). With the ongoing COVID-19 pandemic, a previous study reported the olfactory ability of dogs to discriminate SARS-CoV-2 infected individuals from normal people (Jendrny *et al*., 2020). Recently, detection of cancer metabolites has been discussed as a relatively new approach in cancer diagnostics (Aboud and Weiss, 2013). In addition to the various uses of dogs' olfaction, an attempt to detect tumor by canine olfaction was first reported in 1989 (Williams and Pembroke, 1989). Although various studies reported detecting cancer using advanced diagnostic tools such as gas chromatography or real-time polymerase chain reaction (Chen *et al*., 2007; Dijkstra *et al*., 2014), utilizing canine olfaction in detecting cancer has the advantage of cost-effectiveness and is often more practical than electronic devices (Beebe *et al*., 2016). From this point of view, many studies have been reported on dogs' potential ability in detecting the odor of tumors, such as prostate, lung and breast cancer (McCulloch *et al*., 2006; Taverna *et al*., 2015). However, numerous samples are needed for training medical-detection dogs because patient-derived samples are not standardized, as they contain many individual odors reflecting the patients' habits (Murarka *et al*., 2019). Using a cancer cell line as an alternative is being discussed because of its advantage of circumventing individual patients' confounding factors, such as age, diet and medication use (Lima *et al*., 2018). Several proof-of-concept studies have reported training detection dogs by using metabolites of cancer cells instead of the real cancer (Murarka *et al*., 2019; Jeong *et al*., 2022). In addition, unlike previous studies that trained dogs to detect single cancer, a recent study evaluated how dogs trained on one cancer cell (breast or colorectal cancer) responded to another unfamiliar cancer cell. It was revealed that dogs trained to detect breast or colorectal cancer cells could detect another, unfamiliar cancer cell well (Seo *et al*., 2018). Similar results were obtained by another previous study that trained dogs to detect prostate cancer cells. Dogs trained to detect a certain prostate cancer cell can detect not only another prostate cancer cell but also bladder cancer cell well (Jeong *et al*., 2022). Therefore, given the hypothesis that malignant cancer might share a similar odor, the purpose of the present study was to identify whether dogs trained to detect a certain malignant cancer cell respond to other malignant cancer cells of different origins.

Materials and Methods

Cell culture: A prostate cancer cell line (PC3) was cultivated for training the scent-detection dogs. Other cancer cell lines included LNCaP-LN3 (prostate cancer cell line from another origin), A549 (lung cancer) and MCF-7 (breast cancer). Normal cell lines used as controls included CCD-18Co (human colon), CCD-986sk (human skin), 3T3 (mouse embryo) and bovine knee chondrocyte. Cancer and normal cell lines were purchased from the Korean Cell Line Bank. Only the bovine knee chondrocyte was isolated from our laboratory. The identity of each cell line is described in Table 1. Cancer and normal cells were cultivated in RPMI 1640 (HyClone, Utah, USA) and DMEM (HyClone, Utah, USA) media, respectively. Each medium contained 10% fetal bovine serum (FBS) (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) and 1% penicillin/streptomycin (HyClone, Utah, USA) and the cells were grown in 75-cm² tissue culture flasks under 5% CO_2 at 37°C. The medium was collected and stored at -20°C until the experiment after the cells reached over 80% confluence.

Experimental setup: Following a previous study (Jeong *et al*., 2022), a wheel with 10 ports was used for the experiment. The ports were composed of 1 target (cancer cell) and 9 controls or 10 controls without a target. An acrylic plastic cup with a diameter of 7 mm and a height of 80 mm was used to contain the target or control. Each sample was added with a volume of about 0.2 ml. To avoid contamination by the dogs, the same cup was superimposed and replaced each trial. A 4-mm-diameter hole was made in the superimposed cup for scent detection. In the training or test procedures, a trial was defined as the dog circling the wheel once. Each test was composed of 30 trials and performed over several days while considering the physical condition of the dogs (Fig. 1).

Table 1 Source of cell line

Cell line	Source
PC3	Prostate adenocarcinoma with bone metastasis from a 62-year-old patient
LNCaP-LN3	Metastatic prostate carcinoma from a 50-year-old patient
A549	Lung carcinoma from a 58-year-old patient
MCF-7	Breast adenocarcinoma from a 69-year-old patient
CCD-18Co	Normal colon epithelial cell from a 3-month-old human
CCD-986sk	Normal skin fibroblast cell from a 22-year-old human
3T3	Swiss albino mouse embryo cell

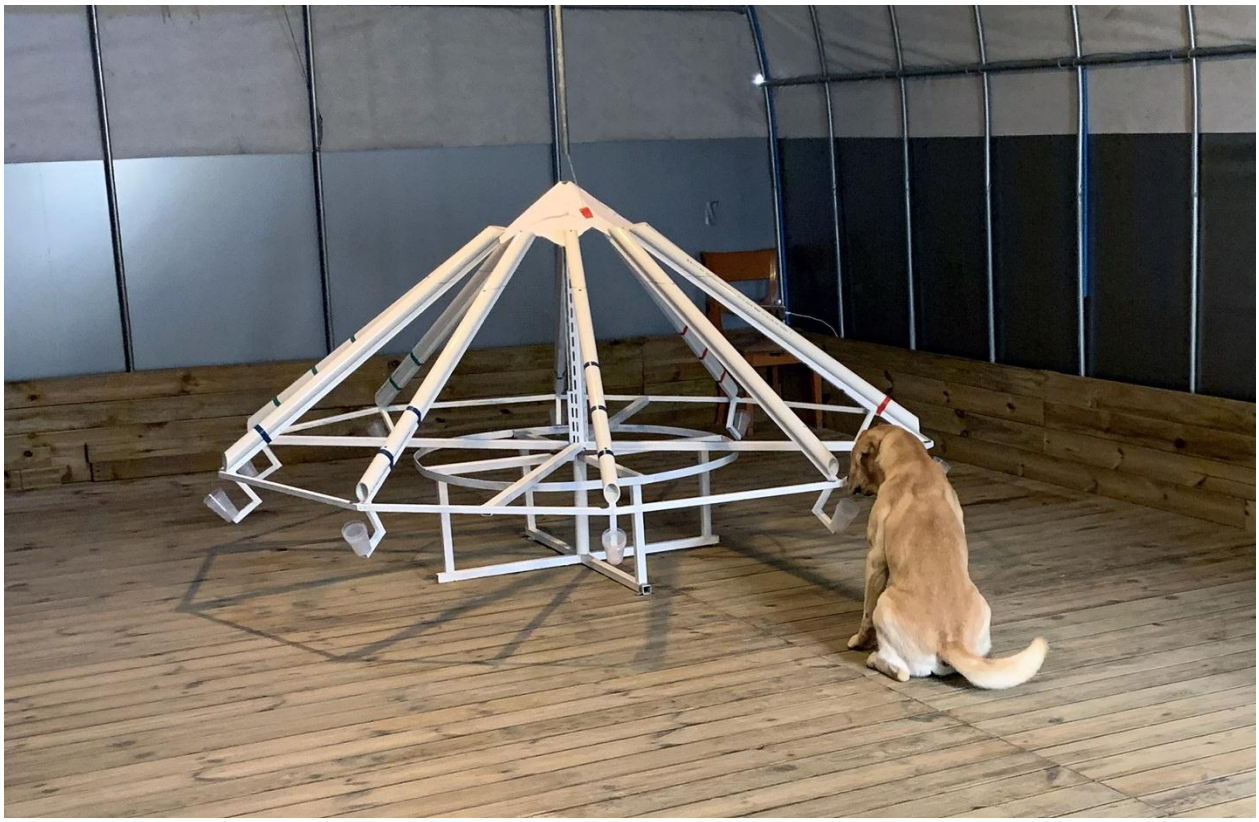

Figure 1 An experimental setup composed of a wheel with 10 ports and a scent-detection dog performing the training or test procedure.

Training: A 4-year-old male (referred to as Dog-1) and a 4-year-old female (referred to as Dog-2), both Labrador Retrievers, participated in training. The dogs were trained to detect metabolites of prostate cancer cell (PC3) from various controls. The training consisted of several stages and lasted for nine months. The dogs moved to the next stage after achieving a sensitivity of over 90% and the next stage was composed from the previous stage by adding a new control. The controls included empty cup, RPMI 1640 or DMEM medium, medium that contained 10% FBS and 1% penicillin/streptomycin and FBS with various concentrations (0.5-10%). The dogs expressed a positive response by sitting in front of the sample. When a dog showed hesitation or moved from the target, it was regarded as a negative response. Expressing a positive response to the control was also considered as a negative response. If there was no target on the wheel, dogs were trained to stare at the handler after sniffing all ports (Jeong *et al*., 2022).

Test: Both dogs passed the training regimen and participated in the tests. In the test procedures, metabolites of normal cells were included as the control (CCD-18Co, CCD-986sk, 3T3 or bovine knee chondrocyte). Tests were performed single-blinded; th us, the handler did not know the identity of each port. Another observer arranged the samples and evaluated the detection ability of the dogs. The initial test was performed to detect PC3 as the target. Additional tests were done by replacing the target from PC3 to LNCaP-LN3, A549 or MCF-7.

Data analysis: Sensitivity and specificity were analyzed to evaluate the ability to detect metabolites of cancer cells. To interpret detection ability, receiver operating characteristic curves and area-under-the-curve (AUC) values were calculated using Sklearn and Matplotlib packages by Python 3. Following the previous criteria, AUC values of 1.0 were considered perfect, AUC values of 0.9 to 0.99 were considered excellent, AUC values of 0.8 to 0.89 were considered good, AUC values of 0.7 to 0.79 were considered fair, AUC values of 0.51 to 0.69 were considered poor and AUC values of 0.5 were considered of no value (Carter *et al*., 2016).

Results

The scent-detection dogs were trained to detect various substances but only the odor of prostate cancer cells (PC3) was imprinted during the whole experiment to avoid confusing the dogs. During test procedures, the odor of trained cancer cells (PC3) was imprinted after every 10 trials to maintain detection accuracy. When evaluating 30 trials in each test, the overall sensitivity and specificity of Dog-1 were 93% and 100% (100% in Dog-2) in trained prostate cancer cells (PC3), 79% and 97% (75% and 99% in Dog-2) in unfamiliar prostate cancer cells (LNCaP-LN3), 100% and 99% (77% and 100% in Dog-2) in lung cancer cells (A549) and 88% and 94% (69% and 98% in Dog-2) in breast cancer cells (MCF-7), respectively. Controls including metabolites of normal cells did not affect the detection ability of the dogs. According to the AUC value, two dogs showed excellent and perfect detection abilities in distinguishing trained prostate cancer cells

(PC3) from controls, respectively. When detecting another unfamiliar prostate cancer cell (LNCaP-LN3), both dogs showed good detection ability. In the final tests, evaluating the detection ability for lung (A549) and breast (MCF-7) cancer cells, Dog-1 and Dog-2 showed excellent and good detection abilities, respectively. Sensitivity, specificity and AUC value of test procedures are described in Table 2.

Table 2 Sensitivity, specificity and value of area under the curve of test procedures

	PC3		LNCaP-LN3		A549		MCF-7	
	Dog-1	**Dog-2**	**Dog-1**	**Dog-2**	**Dog-1**	**Dog-2**	**Dog-1**	**Dog-2**
Sensitivity	0.933	1.000	0.786	0.750	1.000	0.767	0.875	0.690
Specificity	1.000	1.000	0.968	0.989	0.993	1.000	0.942	0.983
AUC	0.967	1.000	0.877	0.870	0.996	0.883	0.909	0.836

AUC, area under the curve

Discussion

Given dogs' superior olfactory system, various studies have reported their ability in cancer detection (McCulloch *et al*., 2006; Taverna *et al*., 2015; Murarka *et al*., 2019; Jeong *et al*., 2022). In addition, a previous study documented that a dog trained on breast cancer cell (breast carcinoma) could also detect colorectal cancer cell (colorectal carcinoma) well, implying the existence of a common specific odor (Seo *et al*., 2018). Similar results were obtained in our previous study. Dogs trained to detect prostate cancer cell (prostate adenocarcinoma) showed excellent ability in detecting unfamiliar bladder cancer cell (transitional cell carcinoma) (Jeong *et al*., 2022). The same dogs were used to evaluate the ability to detect other unfamiliar cancer cells (prostate carcinoma, lung carcinoma and breast adenocarcinoma) in this study. Both dogs showed excellent or good abilities in the detection of the tumor cells. Taken together, it is presumed that malignant cancer cells or carcinoma might share some specific odor when compared with normal cells. Further confirmation by evaluating other malignant cancer cells of different origins, such as sarcoma, is needed to resolve the current hypothesis. Several limitations exist. In the present study, normal prostate, lung and breast cell lines were not included. However, our previous study revealed that there was no interference in a dog's ability to detect metabolites of prostate cancer cell, despite adding normal prostate cell as a control (Jeong *et al*., 2022). When using a cancer cell line as an alternative to real cancer, a previous study documented that further confirmation is needed because the cultured cell could not reflect metabolic alterations occurring in real tumor progression (Lima *et al*., 2018). One study documented that a dog could distinguish between the odor of cancer tissue and blood of an ovarian cancer patient from that of healthy female individuals, implying that cancer-specific odor is produced in both tissue and blood (Horvath *et al*., 2010). Another study trained dogs to detect the odor of ovarian cancer cell instead of that of real cancer and evaluated the ability to detect the odor of blood from an ovarian cancer patient. The study revealed that a dog's ability to detect the odor of cancer cell did not spontaneously switch to an ability to detect that of real cancer (Murarka *et al*., 2019). However, the study had the limitations of a small-sized sample and that the detection ability of only one dog was evaluated. From the present study's point of view, each test for evaluating the detection ability against cancer cell lines was composed of 30 trials and two dogs participated in the tests, as both dogs passed the training regimen in the present study. And recently, the use of urine samples from cancer patients has been discussed as reliable biofluid along with blood samples for cancer metabolomic studies (Carrola *et al*., 2011; Mohamed *et al*., 2019). As the present study has revealed that dogs can be sufficiently trained to detect the odor of prostate cancer cells, further study is needed for testing dogs' ability to detect biofluid (blood and urine) of prostate cancer patients. In conclusion, it is suspected that a common specific odor exists in malignant cancer cells or in carcinoma that makes them different from normal cells. Although there exists a more specific method for odor detection such as gas chromatography (Chen *et al*., 2007), scent-detection dogs can also be trained to recognize these odors. Dogs trained to detect the odor of a certain cancer cell can also detect that of other cancer cells well.

Ethical statement: This study was approved by the Chungbuk National University Institutional Animal Care and Use Committees (CBNUA-1692-22-01).

Acknowledgements

This research was supported by Chungbuk National University Korea National University Development Project (2021). The authors give thanks to Byungbu Kim for the housing and training of the two dogs, Dica and Diel.

References

Aboud OA and Weiss RH 2013. New opportunities from the cancer metabolome. Clin Chem. 59: 138-146.

Beebe SC, Howell TJ and Bennett PC 2016. Using scent detection dogs in conservation settings: A review of scientific literature regarding their selection. Front Vet Sci. 3: 96.

Carrola J, Rocha CM, Barros AS, Gil AM, Goodfellow BJ, Carreira IM, Bernardo J, Gomes A, Sousa V, Carvalho L and Duarte IF 2011. Metabolic signatures of lung cancer in biofluids: NMR-based metabonomics of urine. J Proteome Res. 10: 221-230.

Carter JV, Pan J, Rai SN and Galandiuk S 2016. ROC-ing along: Evaluation and interpretation of receiver

operating characteristic curves. Surgery. 159: 1638-1645.

Chen X, Xu F, Wang Y, Pan Y, Lu D, Wang P, Ying K, Chen E and Zhang W 2007. A study of the volatile organic compounds exhaled by lung cancer cells in vitro for breath diagnosis. Cancer. 110: 835-844.

Dijkstra S, Birker IL, Smit FP, Leyten GH, de Reijke TM, van Oort IM, Mulders PF, Jannink SA and Schalken JA 2014. Prostate cancer biomarker profiles in urinary sediments and exosomes. J Urol. 191: 1132-1138.

Horvath G, Andersson H and Paulsson G 2010. Characteristic odour in the blood reveals ovarian carcinoma. BMC Cancer. 10: 643.

Jendrny P, Schulz C, Twele F, Meller S, von Köckritz-Blickwede M, Osterhaus ADME, Ebbers J, Pilchová V, Pink I, Welte T, Manns MP, Fathi A, Ernst C, Addo MM, Schalke E and Volk HA 2020. Scent dog identification of samples from COVID-19 patients - a pilot study. BMC Infect Dis. 20: 536.

Jeong S, Kim D, Kim W-J and Kim G 2022. Detection of volatile organic compounds from human prostate cancer cell using canine olfaction. J Vet Behav. 49: 80-84.

Lima AR, Araújo AM, Pinto J, Jerónimo C, Henrique R, Bastos ML, Carvalho M and Guedes de Pinho P 2018. Discrimination between the human prostate normal and cancer cell exometabolome by GC-MS. Sci Rep. 8: 5539.

McCulloch M, Jezierski T, Broffman M, Hubbard A, Turner K and Janecki T 2006. Diagnostic accuracy of canine scent detection in early- and late-stage lung and breast cancers. Integr Cancer Ther. 5: 30-39.

Mohamed EI, Mohamed MA, Abdel-Mageed SM, Abdel-Mohdy TS, Badawi MI and Darwish SH 2019. Volatile organic compounds of biofluids for detecting lung cancer by an electronic nose based on artificial neural network. J Appl Biomed. 17: 67.

Murarka M, Vesley-Gross ZI, Essler JL, Smith PG, Hooda J, Drapkin R and Otto CM 2019. Testing ovarian cancer cell lines to train dogs to detect ovarian cancer from blood plasma: A pilot study. J Vet Behav. 32: 42-48.

Seo I-S, Lee H-G, Koo B, Koh CS, Park H-Y, Im C and Shin H-C 2018. Cross detection for odor of metabolic waste between breast and colorectal cancer using canine olfaction. PLoS One. 13: e0192629.

Taverna G, Tidu L, Grizzi F, Torri V, Mandressi A, Sardella P, La Torre G, Cocciolone G, Seveso M, Giusti G, Hurle R, Santoro A and Graziotti P 2015. Olfactory system of highly trained dogs detects prostate cancer in urine samples. J Urol. 193: 1382-1387.

Williams H and Pembroke A 1989. Sniffer dogs in the melanoma clinic? Lancet. 1: 734.