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Preservation through Cloning of Superior Canine Scent Detection Ability for Cancer Screening

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Abstract : This study was conducted to ascertain whether the scent detection ability of a donor dog having extraordinary talent in cancer detection can be conserved through cloning. A specially trained dog for colorectal cancer detection was cloned, and she was trained and tested to detect breast cancers using breath samples collected from patients and healthy volunteers. Scent detection sensitivity of the clone was 93.3% and specificity was 99.5%, similar with those of donor (91% and 99%). Furthermore, the clone successfully detected early stage of breast cancers. Therefore, superior canine scent detection ability for cancer screening could be preserved through cloning.

Key words **:** canine scent detection, cloning, dogs, breast cancers, diagnosis.

Introduction

Dogs have an outstanding olfactory sensitivity, up to 100,000-fold higher in detection of certain compounds than humans (24,25), which likely is due to a relatively higher number of olfactory neuroepithelium (11). In 1989, William and Pembroke (26) reported about a dog that became interested in its owner's skin lesion that turned out to be a malignant melanoma, and this was the first suggestion that a dog's sense of smell could be used for detecting human cancers. Since then, canine scent detection ability has been proved to be useful in diagnosing various cancers including bladder (27), melanoma (20), lung (17), breast (8,17), prostate (3,8), ovarian (9,10) and colorectal (22) cancers. Cancer screening with dogs using biological samples such as exhaled breath (17,22), urine (3,8,27), stool (22), tissue (9,10,20) or blood (10) is simple to perform, inexpensive and reproducible. Recently, many organizations are training dogs to screen various cancers and a good cancer detection dog can diagnose cancers with accuracy (22). However, only a small proportion of trained dogs are selected to be an elite service dog due to the low average detection rate (27) or unsuitable temperament (5,7,19).

This study was conducted to ascertain whether the scent detection ability of a donor dog having extraordinary talent for colorectal cancer detection can be conserved to detect other kind of cancer, breast cancer, through cloning.

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Case

Ear skin tissue from a specially trained, 7-year-old female black labrador retriever from St. Sugar Cancer Sniffing Dog Training Center (Chiba, Japan) (22) was collected aseptically in Japan and transferred to South Korea in phosphate-buffered saline (PBS, Invitrogen, Carlsbad, CA, USA) supplemented with 1% (v/v) penicillin-streptomycin (Invitrogen). The tissue was minced and cultured with Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% (v/v) fetal bovine serum (Invitrogen) at 39°C in a humidified atmosphere of 5% CO_2 and 95% air. Donor cells from passages 2-4 were cultured to confluence for somatic cell nuclear transfer and retrieved as single cells by trypsinization just after recovery of *in vivo* matured oocytes.

Dog cloning procedures were done as previously reported (15). In brief, a total of 163 *in vivo* matured oocytes were retrieved by flushing oviducts about 72 h after ovulation and cumulus cells were removed by repeated pipetting in 0.1% (w/v) hyaluronidase in Hepes-buffered tissue culture medium-199 (Invitrogen). Nuclear materials were removed from an oocyte, and a donor cell was injected into the enucleated oocyte. The oocyte-cell couplet was fused with two pulses of direct current (72 V for 15 μs) using an Electro-Cell Fusion apparatus (NEPA GENE, Chiba, Japan), then activated chemically. A total of 130 cloned embryos were transferred into 8 recipients, and one was revealed to be pregnant by ultrasonography. The pregnancy was monitored until delivery (13), and four healthy cloned black labrador retriever puppies were delivered by Cesarean section. Genetic identity of

Fig 1. Canine scent detection test by a cloned dog. (A) A cloned labrador retriever derived from a dog having superior ability to detect colorectal cancer. (B) Breath samples collected from patients and healthy volunteers. (C) Placing sample bags at the five sample stations having a cover with holes to allow exchange of air and exhaled breath chemicals. (D) Dog response by sitting down in front of a sample station after sniffing each of the five stations in a single trial.

the cloned dogs with the cell donor dog was proved by microsatellite analysis (data not shown). The experiment was approved by the Institutional Animal Care and Use Committees of Seoul National University.

Among these four cloned puppies, one was chosen to be trained for breast cancer detection (Fig 1A). Patients at Korea University Anam Hospital and Samsung Medical Center were recruited for collection of breath samples. Questionnaires about medications, medical history, smoking and food / drink ingestion were completed prior to the breath sampling. Breath samples were collected from the beginning of exhalation for 10 sec into a breath sampling bag (Otsuka Pharmaceutical Co., Ltd. Osaka, Japan) after deep inhalation. The sampling bags were sealed in ordinary grocery store Ziplock-style bags (Fig 1B) and stored at 4°C until analysis.

The training and testing of the dog were performed in a 3 m × 7 m room with five sample stations on the floor of the room in a single straight line spaced 45 cm apart (Fig 1C). Each station consisted of a black painted wooden storage container (45 cm × 45 cm × 30 cm) including a wall 15 cm deep in which the breath sample bags were placed. The top of each box was covered by clear polypropylene with holes to prevent the dog from directly contacting the samples and to allow exchange of air and exhaled breath chemicals. Correct responses were (1) indicating by sitting down in front of a sample station containing a cancer sample (a true positive in sensitivity calculations) (Fig 1D) and (2) sniffing but not indicating on a control sample (true negative). Incorrect responses were (1) indicating on a control sample (false positive), (2) sniffing but not indicating on a cancer sample (false negative) and (3) hesitation, considered to be an incomplete reaction to either cancer or control samples (either false positive or false negative depending on whether hesitation was on a cancer or control sample).

The training method was a reward-based approach in which the correct behavior is rewarded by simultaneous play with a tennis ball. Training the cloned dog was done with four controls and one target or five controls, 2-5 days per week for 14 months starting from 12-months-old. After placing the sample bag at the sample station, the dog handler called the dog and encouraged it to sniff the stations by the command 'Search!' without a leash. The dog passed and sniffed each of the five stations in a single trial and gave responses to the handler. The scent detection test was performed with four controls and one target. For this test, breath samples were collected from a total of 99 women at least 20 years of age with breast cancers (15 at stage 0, 72 at more than stage 1, and 12 under chemotherapy) and 260 age-matched controls (205 women and 55 men). The test was performed for five weeks and five trials were done on each day of testing.

Diagnostic accuracy was calculated as sensitivity and specificity of the dog's indication of samples compared with the true diagnosis confirmed by presence of breast cancer. The sensitivity (or the true positive rate) of the test was 93.9%, which is the proportion of cancer samples correctly identified by the dog, and the specificity (or the true negative rate)

Table 1. Canine scent detection results of a cloned dog using breath samples collected from breast cancer patients and healthy volunteers

Breath sample	Control[a)]	Breast cancer[b)]	Total
Negative	394	6	400
Positive	2	93	95
Total	396	99	495

[a)]Control breath samples included those from people with a history of medication (for colds, gastrointestinal disease, hypertension, etc.), disease (diabetes, hyperthyroidism, hepatitis, dental disease, etc.), food intake (coffee, soup, vitamin, orange, rice cake, etc.) before the sampling, or smoking.
[b)]Breath samples of breast cancer included 15 of stage 0, 72 of more than stage 1, and 12 under chemotherapy.

was 99.5%, which is the proportion of control samples (Table 1). Medication, smoking, or food / drinking ingestion prior to the breath sampling did not affect the results.

Discussion

Breast cancer has the highest incidence (22.9% of the total cancer cases, 1.4 million) and mortality rate (13.7% of the cancer deaths, 0.5 million) in women worldwide (6,12). Mammography is the most commonly used screening method to diagnose breast cancer, but it is not perfect. Its sensitivity declined as low as 48 to 50% in women with dense breast (1,14), which could result in a worse prognosis. Dense breast tissue is commonly observed in more than half of women younger than 50 years and at least one-third of women older than 50 years (23). Although digital mammography (21) or combined screenings with ultrasound and mammography (1) have been reported to improve diagnostic accuracy, these could not eliminate the limitation of dense breast parenchyma. However, this limitation can be overcome using canine scent ability, which is simple to perform, inexpensive and reproducible.

In general, only a small proportion of all trained dogs have proved suitable to be a service dog: 40% of German Shepherd breed in the Swedish Armed Forces (7), 42% of Guide Dogs NSW/ACT in Australia, 43% of Guide Dogs for the Blind Inc. in the USA (19), and 56% of Golden retriever, German shepherd, and Labrador retriever in Seeing Eye guide dog school in the USA (5). Therefore, it is very costly in terms of time, labor and financial resources not only for training and producing a good service dog but also for finding alternative homes for adult dogs that failed to qualify. Cloning could be the solution to reduce costs and effort for producing a good service dog, and to decrease the number of dogs that failed to qualify. Therefore, we aimed to produce a cloned dog from a donor dog having extraordinary talent in cancer detection and evaluated its scent detection ability.

It has been postulated that dogs can smell volatile organic compounds, such as alkanes, methylated alkanes, aromatic compounds and benzene derivatives, derived from cancer tissues (4,16). Although both breath and urine samples collected from breast cancer patients could be used for detection of volatile organic compounds by sniffing dogs, breath samples seem more efficient, with small individual differences (8,17). Trained dogs could even detect stage I breast cancer with 94% sensitivity and 95% specificity from breath samples (17). In this study, we also used breath samples and our cloned dog also successfully detected early stage of breast cancers including 15 at stage 0.

In 2009, somatic cell nuclear transfer technology was first applied to clone an elite service dog working on drug sniffing at the Korea Customs Service (18). All of the six cloned pups (100%) finishing a 16-month-training period passed the detector dog selection test, while only one of eight (13%) pups in breeding stock passed the test (2). Furthermore, the six cloned dogs have successfully performed their duties as drug sniffing dogs in airports (http://news.bbc.co.uk/2/hi/asia-pacific/8158097.stm). In line with these results, our cloned dog showed high sensitivity (93.9%) and specificity (99.5%) in breast cancer detection ability, similar to the cell donor dog (22) which detected colorectal cancer with 91% sensitivity and 99% specificity. Unfortunately, we could not compare the scent detection ability of the other three cloned dogs with the one used in our study because two of them were taken over by other organizations and the last one was trained for other purposes.

Conclusions

In conclusion, canine cancer scent detection ability could be preserved in a cloned dog produced by somatic cell nuclear transfer. This method of producing cancer-sniffing dogs is much more efficient and saves a great deal of time and effort compared with simply breeding elite dogs. Using cloned scent detection dogs could be widely applied not only to screen early stages of breast cancers but also to development of chemical sensor technology, which would result in good prognoses of cancer patients.

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복제를 통한 우수한 암탐지 능력의 보존

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요 약 : 본 연구는 암탐지에 우수한 능력을 보유하고 있는 공여견의 냄새 탐지 능력이 복제를 통하여 보존될 수 있을지를 알아보기 위하여 설계되었다. 직장암 탐지에 특화되어 훈련된 개를 복제하였고, 복제된 개는 환자와 건강한 지원자들로부터 채취된 호흡 샘플을 사용하여 유방암을 탐지하도록 훈련 되었다. 복제개의 암탐지 민감도는 93.3%, 특이도는 99.5%로 공여견의 암탐지 민감도 및 특이도 (91% 및 99%)와 유사하였다. 게다가 복제개는 유방암의 초기 단계까지 성공적으로 탐지할 수 있었다. 따라서 우수한 암탐지 능력은 복제를 통해서 보존될 수 있을 것이다.

주요어 : 개 냄새 탐지, 복제, 개, 유방암, 진단