

Thermography use as a Predictive Tool in Early Diagnosis of Breast Cancer

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Abstract

Thermography is a noninvasive diagnostic technique that measures thermal field and temperature distribution of a targeted surface and generates thermal patterns in the form of a color map. Even though thermography has been already used in detection of breast cancer, its predictive value has not been fully solved yet. The aim of our study was to evaluate the predictive role of thermography in the process of chemically induced mammary carcinogenesis in female Sprague Dawley rats. Breast cancer was induced to 20 female Sprague Dawley rats by 2 doses of N-Methyl-N-Nitrosourea (50 mg.kg⁻¹ intraperitoneal) on the 43rd and the 50th postnatal day. Digital infrared camera with a resolution was used to evaluate the thermal patterns of ventral part of the body. Symmetrical body areas were monitored to detect temperature patterns of intact breast in compare to potential affected one. Only tumors developing non-parallel were further evaluated. Most developed breast tumors were characterized as ductal carcinomas in situ. 19/28 tumors were characterized by the increased temperature before their appearance. 9/28 breast tumors showed either no temperature difference or decrease of the temperature of the place with the potential cluster of neoplastic transform cells before tumor appearance diagnosed by the palpation. The temperature increase $\geq 0.5^{\circ}\text{C}$ was seen in 38% ductal carcinoma in situ forms and the temperature decrease $\geq 0.5^{\circ}\text{C}$ in 11% ductal in situ forms. Thermography could be used as an effective noninvasive predictive tool in breast cancer diagnosis. However, more studies are required to describe the potential of this predictive method.

Keywords: Thermography; Breast cancer; Early diagnosis; Cancer prediction; Rats

Abbreviations: DCIS: Ductal *In Situ* Carcinoma; DIC: Ductal Invasive Carcinoma; NMU: N-Methyl-N-Nitrosourea; SD: Standard Deviation

Introduction

Breast cancer represents the most common cancer in woman worldwide with more than 1.7 million new cases diagnosed in 2012 [1]. On the other hand, breast cancer is a highly treatable disease, with 97% chance of survival if diagnosed early [2]. Currently, the most used diagnostic methods in preventive and early breast cancer as mammography, ultrasound or magnetic resonance imaging, are often supplemented with the alternative imaging techniques, f. e. thermography [3].

Thermography is a low-cost, non-invasive and non-contact skin surface screening method based on the theory that the microenvironment of especially breast tumors (but also other types of tumors localized near the skin) have an increased blood supply and metabolic rate which is mirrored in the increased temperature gradients compared to surrounding normal tissue [4]. Nowadays, there are some studies dealing with thermovision as one of the potential alternatives or supplements in standard diagnostic [2-4]. Protocols existing in clinical practice are precise and include detailed procedure how to prepare the patient for the screening [2-3]. The effectiveness of infrared imaging to evaluate mammographically suspicious lesions is up to 97% in the sensitivity [5]. When performed with mammography, the screening could correct the false signals from mammography screening (density of the breast, etc.) and have excellent results [2,3].

Tumor tissue is formatting progressively over time by the accumulation of somatic mutations in the progeny of a normal cell, leading to a selective growth advantage in the mutated cells and ultimately to uncontrolled proliferation [6]. The period from

the original cell mutation to a clinically detectable tumor can vary depending on the growth characteristics of the transformed cells and the microenvironment in which the tumor is growing. However, it is without debate a long time; sometimes the decades of years [6]. It was reported that the results of thermography could have a predictive value. According to Ng et al. thermography can be correct 8-10 years before mammography can detect a mass and that the error in thermography is that it is just "too right too early" [7]. The aim of our study was to evaluate the predictive role of thermography in the process of chemically induced mammary carcinogenesis in female Sprague Dawley rats.

Materials and Methods

Animals

20 female Sprague Dawley rats (Laboratory of Research Bio-models, Košice, Slovakia) were used in the experiment. The animals aged 30 days and weighing 100-130 g were adapted to standard vivarium conditions with a temperature of 21-24°C, a relative humidity of 50-65% and an artificial 12:12 h light:dark regimen. The rats were fed standard pellets (Peter Miško, Snina, Slovakia) and drank the tap water *ad libitum*. The animals were handled in accordance with the guidelines established by Law No. 377 and 436/2012 of Slovak Republic for the Care and Use of Laboratory Animals (Ro-2616/14-221).

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Breast cancer initiation

Mammary carcinogenesis was initiated with two intraperitoneal doses (50 mg.kg⁻¹ body weight each) of N-Methyl-N-Nitrosourea (NMU; Sigma, Deisenhofen, Germany), freshly dissolved in a physiological sodium chloride solution. Breast cancer was induced on the 43rd and the 50th postnatal day.

Experimental schedule

The rats were palpated once a week to record the presence and localization of each tumor. Every two weeks the rats were imaged using digital infrared camera (Figure 1). At the end of the experiment the animals were killed by quick decapitation. Mammary tumors were excised and the final tumor size was recorded.

Tumor palpation

To determine the tumor localization and size, the animal was immobilized (not anesthetized) by holding it in a firm grip with one hand and with the other hand measuring the approximate length, width, and height of the tumor; separately for left and right body part. The end points for data analysis were latency to tumor appearance, the volume of the tumor upon detection (calculated per the formula: $V = \pi \times (S1)^2 \times S2 / 12$; where S1 and S2 are tumor diameters; $S1 < S2$) and the number of tumors (expressed as tumor frequency).

Thermography procedure

Infrared thermography was used to evaluate the thermal patterns and temperature of ventral part of the body. Rats were maintained in temperature-controlled room for 15 min and then imaged at room temperature 22°C using digital infrared camera with a resolution of 160 × 120 pixels (FLIR E40, FLIR Systems OÜ, Estonia). The high-rainbow Color Palette was chosen for taking and displaying images. The emissivity of one ($\epsilon=1$) was set for the imaging. Anesthetized rats (isoflurane, 2 ml/L of induction chamber volume) were positioned in front of uniform pad and thermographic images were obtained in 0.5 m from rats.

The evaluation of thermal images

Thermographic images were analyzed and reviewed by software program (FLIR Tools version 2.0, FLIR Systems, Inc., Wilsonville, USA). Symmetrical body areas were monitored to detect temperature patterns of intact breast in compare to potential affected one. Only tumors developing non-parallel were further evaluated (Figure 2).

Histopathological classification of tumors

All tumors were inspected by experienced pathologists. Histopathological classifications were performed using the standard criteria for breast cancer classification adapted from [8].

Statistical analysis

The data are expressed either as mean ± standard deviation (SD) or as the difference of the temperature between potential tumor and/or real tumor place in compare to intact healthy breast of each parallel breasts.

Results

NMU developed breast tumors in each rat in a different time. The first tumors appeared in the 6th experimental week; thus 4 weeks after the first NMU dose. The incidence of breast cancer reached 100% in the 12th experimental week (Figure 3).

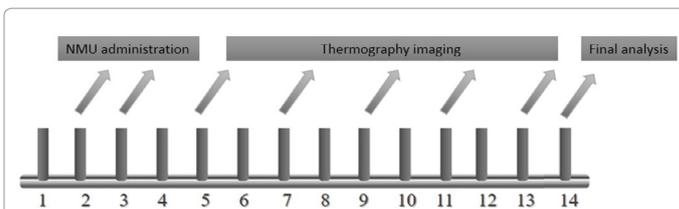


Figure 1: Experimental design. The experiment run 14 weeks. The animals were kept under standard vivarium conditions. Mammary carcinogenesis was induced by two doses of intraperitoneal N-methyl-N-nitrosourea (NMU) administration (50 mg/kg of body weight). The rats were palpated once a week and imaged using digital infrared camera every two weeks in anesthesia. At the end of the experiment the animals were killed by quick decapitation.

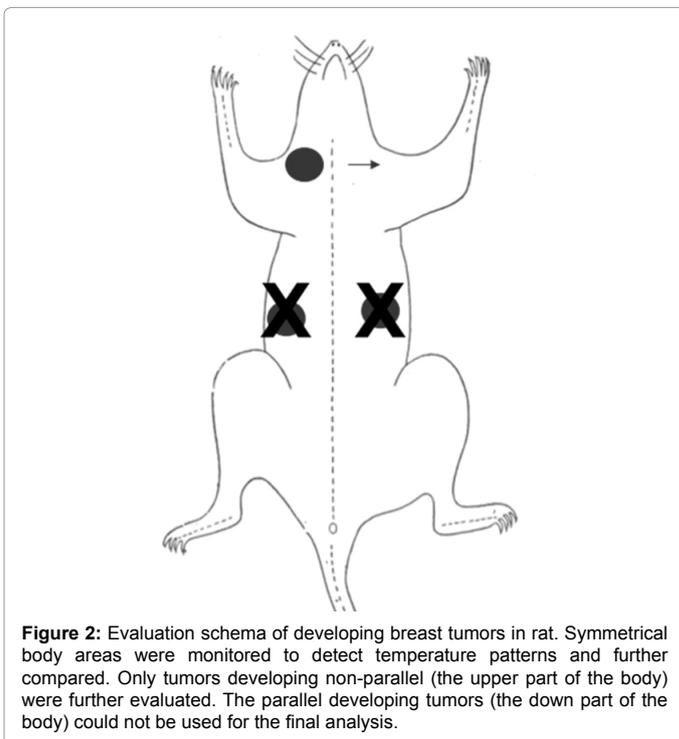


Figure 2: Evaluation schema of developing breast tumors in rat. Symmetrical body areas were monitored to detect temperature patterns and further compared. Only tumors developing non-parallel (the upper part of the body) were further evaluated. The parallel developing tumors (the down part of the body) could not be used for the final analysis.

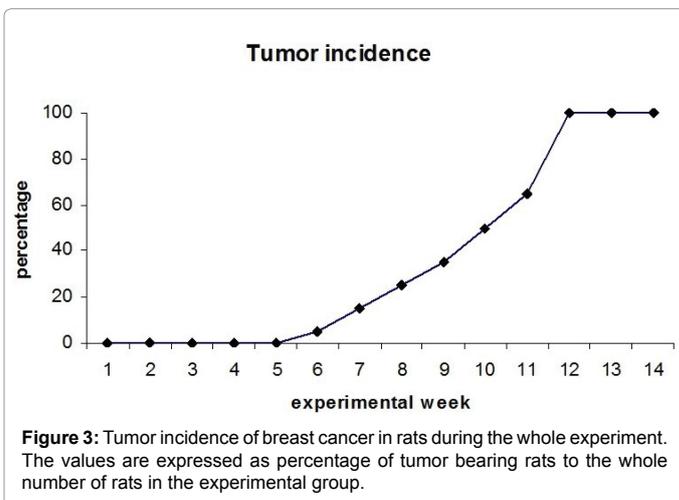


Figure 3: Tumor incidence of breast cancer in rats during the whole experiment. The values are expressed as percentage of tumor bearing rats to the whole number of rats in the experimental group.

As seen on the Figure 4, the rats developed usually more than two breast tumors already from 7th experimental week. At the final week,

the tumor frequency reached the value 3.84 ± 2.50 . Maximal number of tumors in one rat was nine, minimal one breast tumor.

The predictive role of thermography was evaluated maximal two weeks before the tumor appearance. After the NMU administration, more than 120 breast tumors developed. As expected, most breast tumors developed parallel. Thus, for the final analysis only 28 of them could be used. 19 tumors were characterized by the increased temperature before their appearance (Tables 1 and 2 and Figure 5).

Nine breast tumors showed either no temperature difference (2/9) or decrease of the temperature (7/9) of the place with the potential cluster of neoplastic transform cells before tumor appearance diagnosed by the palpation. Most breast tumors were characterized as ductal carcinomas in situ (DCIS, n=26; Table 1).

One breast tumor was medullar carcinoma (1/28) with the increased temperature of 0.2°C 14 days before the appearance. At the day of first palpation the tumor had the volume of 2.1 mm^3 . In addition, another one type of breast cancer (1/28) was characterized as ductal invasive carcinoma (DIC) with surprisingly lowered temperature of 0.3°C three days before the first tumor rise. The tumor volume at the time of first palpation was 16.75 mm^3 .

One DCIS form of breast cancer was characterized by 0.5°C temperature increase nine days before its first palpation. DCIS with calcification showed slight lowering of the temperature before its appearance. Tumors characterized as DCIS with intraductal papilloma either increased the temperature of the place with potential cancer site (2/3) or slightly decreased the temperature (1/3). DCIS cribriform showed slight temperature increase in 6 cases and more than 0.5°C in 7 cases. Two DCIS cribriform were characterized with no temperature difference of the skin near breast three days before tumor appearance; two with the lowering of the temperature more than 0.5°C . DCIS cribriform cystic showed 0.7°C increase of the temperature of the skin. On the other hand, DCIS cribriform with necrosis revealed 0.8°C diminution of temperature before tumor appearance.

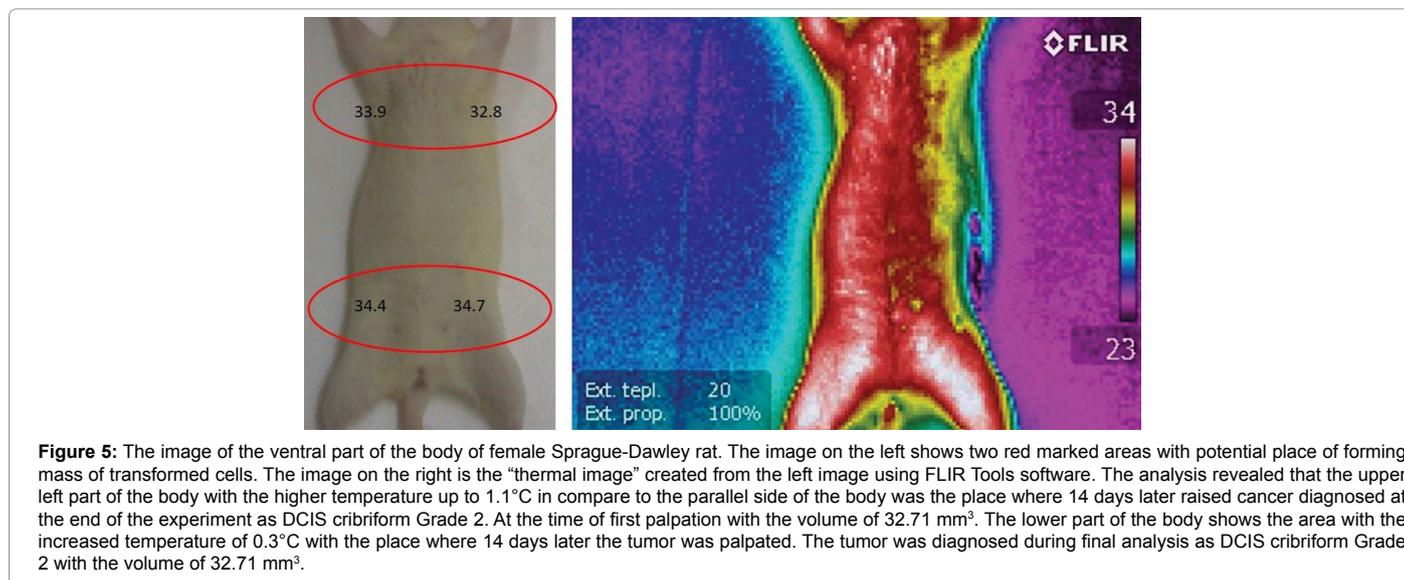
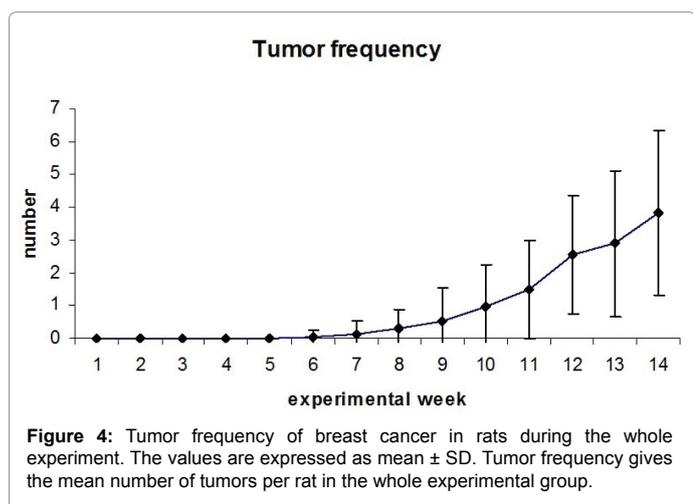
During the experiment, the temperature was constantly monitored every two weeks. At the time of the first tumor appearance, most tumors (27/35) were characterized by the increased skin temperature in compare to the parallel healthy breast (Figure 6).

4 tumors caused the decrease of the temperature and 2 caused no temperature difference (data not shown). At the final week, only 29 temperature differences could have been evaluated (Table 3).

Discussion

Mammography is currently considered to be the best available approach for the early detection of breast cancer; however, the positive predictive value is weak [9]. Thermography or digital infrared imaging is based on the principle that metabolic activity and vascular circulation in both pre-cancerous tissue and the area surrounding a developing breast cancer is almost always higher than in normal breast tissue [2,4]. Cancer cells forming a tumor mass before it is already palpable may need more energy to set the micro-conditions to survive. The skepticism about thermography use in human medicine partly remains. Of course, the relevance of thermal imaging is disputable, but when combined with another non-invasive technique it could bring new possibilities in very early breast cancer diagnostics.

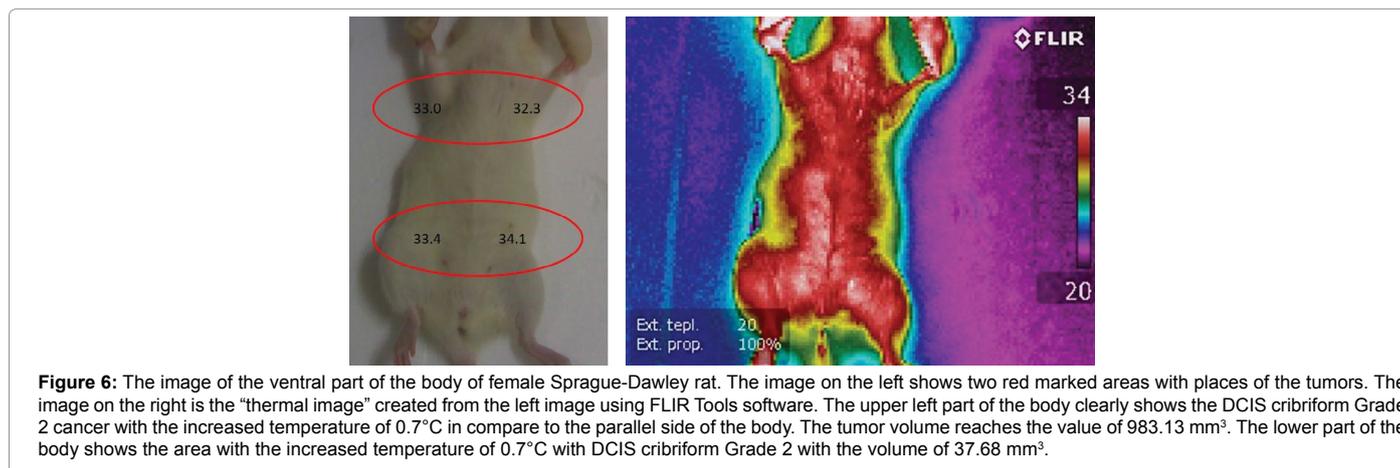
In our experiment, thermography was used to evaluate thermal patterns of skin surrounding breast with the aim to screen for breast cancer already before its appearance. The model of chemically induced breast cancer is a good model to screen continuously the developing



	Histological type	Grade	T difference (°C)		Days before tumor palpation	Tumor volume (mm ³) evaluated at the first appearance
			Increase	Decrease		
1	DCIS	G2	0.5		9	32.71
2	DCIS with calcification	G1-2		0.3	4	7.07
3	DCIS with intraductal papilloma	G2	0.2		14	16.75
4	DCIS with intraductal papilloma	G2	0.7		11	4.19
5	DCIS with intraductal papilloma	G2		0.2	14	89.75
6	DCIS cribriform	G2	0.1		11	32.71
7	DCIS cribriform	G2	0.3		3	32.71
8	DCIS cribriform	G2	0.3		11	32.71
9	DCIS cribriform	G2	0.3		14	16.75
10	DCIS cribriform	G2	0.3		14	32.71
11	DCIS cribriform	G2	0.3		14	452.16
12	DCIS cribriform	G2	0.5		11	07.7
13	DCIS cribriform	G2	0.5		14	261.67
14	DCIS cribriform	G2	0.8		3	38.47
15	DCIS cribriform	G2	0.9		11	32.71
16	DCIS cribriform	G2	1.0		3	301.44
17	DCIS cribriform	G2	1.1		14	32.71
18	DCIS cribriform	G2	1.1		4	32.71
19	DCIS cribriform	G2		0	3	32.71
20	DCIS cribriform	G2		0	3	32.71
21	DCIS cribriform	G2		0.5	11	261.67
22	DCIS cribriform	G2		0.8	3	16.75
23	DCIS cribriform papillary	G2	0.1		14	32.71
24	DCIS cribriform cystic	G2	0.7		11	261.67
25	DCIS cribriform with necrosis	G2		0.8	11	261.67
26	DCIS comedo	G2		0.3	14	89.75

Table 1: Prediction value of thermography in the preventive screening.

Temperature difference between the potential site of the future tumor and the parallel healthy breast few days before the first tumor appearance. Histological type of each tumor was evaluated at the end of the experiment. Tumor volume was calculated according to the size of each tumor during the first appearance during palpation



	T increase	T decrease
min-max (°C)	0.1-1.1	0-0.8
0°C (n=)	0	2
0.1-0.4°C (n=)	9	4
0.5-0.9°C (n=)	7	3
≥ 1°C (n=)	3	0
n total	19	9

Table 2: Temperature difference of the skin between the site of potential breast tumor and the parallel healthy breast in order to see the prediction value of thermography. T: Temperature

	T increase	T decrease
min-max (°C)	0.1-0.9	0-0.9
0°C (n=)	0	3
0.1-0.2°C (n=)	3	1
0.3-0.5°C (n=)	3	2
0.5 -0.9°C (n=)	12	1
≥ 1°C (n=)	4	0
n total	22	7

Table 3: Temperature difference of the skin between the site of breast tumor and the parallel healthy breast in the final experimental week. T: Temperature

cancer from the not only very early stages. Our results revealed 28 tumors characterized mostly by the increased temperature (67%) up to 1.1°C. The tumors developed non-parallel and were proper for evaluation of thermography prediction value. The temperature differences were seen 3-14 days before the first tumor palpation. So, it was exactly the time when cancer cells develop tumor mass and form a tumor which firstly could be seen during screening. This is the period when clinicians could pay special attention for the patient and may strongly influence the tumor development. When comparing the mean life expectancy of human and rats (people live approximately 60 years, rats live maximal 2-3 years), thermography could reveal thermal differences months or years earlier. After NMU induction, every rat developed one or more tumors. It was expected, because the rats have six pairs of mammary glands, three in the pectoral group and three in the inguinal group, extending from the salivary glands anteriorly to the perianal region posteriorly [10]. Human breast cancer usually develops parallel and this trend was seen also in rats. Therefore, only 28 tumors from totally 120 developed tumors could have been evaluated. After NMU induction, mainly DCIS and its forms developed. However, the histological evaluation of tumors was possible first at the end of the experiment and not during the experiment since biopsy may influence the processes of tumor forming and increase the temperature because of increased inflammatory responses induced by invasive biopsy. So, we assume that the final stage of the tumor may not correspond to its early or developing stages. Despite that, we applied the final classification of tumors to their developing stages. After the final analysis, some forms were presented only once and could not bring some general value (medullar carcinoma, DIC). Simple DCIS form was characterized by 0.5°C temperature increase nine days before its appearance (approximately 270 days in human). However, DCIS with calcification was characterized by the decreased temperature up to 0.3°C four days before appearance. Calcifications are associated with central necrosis of the tumor [11], but at the beginning of tumor forming, the tumor had no necrosis. So, each tumor probably develops various mechanisms to create ideal conditions to survive. These conditions depend on endogenous factors of individuals; they cannot be the same even in the same histological type of tumor.

The temperature differences were divided into two groups: <0.5°C and ≥ 0.5°C. According to the literature, in clinics, the temperature differences reliable are >0.4°C [2]. In our experiment, the temperature increase ≥ 0.5°C was seen in 10/26 tumors and the temperature decrease ≥ 0.5°C in 3/26 tumors. The temperature increase <0.5°C was typical for 8/26 cancer and the temperature decrease <0.5°C (with two cases with no temperature difference) was typical for 5/26 cases.

The temperature differences before tumor appearance may be influenced by many endogenous and exogenous factors. Firstly, the inflammation of the breast could increase the temperature [12]. The temperature increase could be detected also during the menstruation cycle [13]. In addition, as by humans the precise protocols before the

thermography screening avoid factors influencing the temperature patters [2], in rats the anesthesia influences only the process of falling asleep, not the position of the animal at this time. The rats usually fall asleep on one side and thus stymie the contact of the skin. Our results were probably influenced by the position of the animals at the time before screening.

On the other hand, every breast tumor develops in a different way. All the tumors evaluated were DCIS forms. DCIS consists of the clonal proliferation of cells that appear malignant and that accumulate within the lumens of the mammary duct [14]. Low grade DCIS is characterized by monotonous cell proliferation; nuclear size approximates that of a normal ductal cell with a variety of architectural patterns, including cribriform, micropapillary, solid and papillary growth. Cells are well-polarized, and mitotic figures are rare. Punctate necrosis may be found, but large foci of necrosis are uncommon and should not be more than focal in LG-DCIS. Small laminated microcalcifications are a common finding [11]. High grade DCIS has been associated with the breakdown of the myoepithelial cell layer and the proliferation of fibroblasts, lymphocyte infiltration and angiogenesis [14]. So, DCIS is characterized mostly by the intensive angiogenesis and elevated immune response. And indeed, the most DCIS forms were characterized by the increased temperature. DCIS lies along a spectrum of preinvasive lesions originating within normal breast tissue, with histologic progression from atypical hyperplasia to invasive breast cancer. Although the initiating steps and precise pathways of breast tumorigenesis remain poorly defined, it appears that nearly all invasive breast cancers arise from in situ carcinomas [14].

During the experiment, the monitoring of the temperature patterns revealed mostly elevated temperature of skin near breast cancer in compare to healthy breast when tumor appeared for the first time. This time could be named as the early breast cancer screening. The value of thermography screening in our experiment was 77%. Only four tumors caused the decrease of the temperature and two caused no temperature difference. However, when combining thermography with mammography screening, the sensitivity of 95% could be achieved by ductal carcinomas. The authors reported that the sensitivity for the detection of ductal carcinoma by clinical examination alone was 61%, by mammography alone was 66% and by thermography alone was 83%. A sensitivity of 95% was obtained when suspicious and equivocal mammograms were combined with abnormal thermal pattern images. However, when clinical examination, mammography and IR images were combined, a sensitivity of 98% was achieved [15].

During the experiment, each tumor rose in a unique way and the temperature difference in compare to parallel healthy breast varied in time. Some tumors showed only elevated temperatures but other ones showed mixed elevated/decreased thermal patterns during following weeks (data not shown). Therefore, we suggest that thermography could have better predictive or very early diagnostic value. Indeed, according to [7] thermography can be correct 8-10 years before mammography can detect a mass and that the error in thermography is that it is just "too right too early".

Conclusion

In conclusion, thermography could be used as an effective noninvasive predictive tool in breast cancer diagnosis. However, more studies are required to describe the potential of this predictive method.

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