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New Criterion on Cancer Detection Based on NLC Orientation

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The new optical criterion for objective diagnosis of malignant tumors in human beings and animals tissues is suggested. The molecules of NLC thin layers on sections of malignant tissues have a homeotropic orientation, while on the surface of benign and normal tissues the orientation is almost planar or tilted. Therefore malignant tumors in light microscope with crossed polarizer and analyzer always looks black. The different orientation of NLC molecules is explained by two orders different value of anchoring energy on the surface of malignant and benign tissues. The experiments on contact angles measurement of NLC and H_2O droplets on investigated surfaces proved the difference in their surface tension that also confirm authenticity of a new criterion for microscopic malignancies' identification.

Keywords: liquid crystals orientation; anchoring energy; malignant tissue

INTRODUCTION

The problem of malignant tumors is one of the most actual in modern medicine. It consists not only in development of effective patients treatment methods, but in objectification of microscopic diagnosis of neoplasms. The routine diagnostic methods are based on various optical microscopy modifications in view of spectral, luminescent and fluorescent features of the growth images.

The essence of luminescent and fluorescent methods is based on properties of some photosensitive materials to be collected in malignant tissues in the much greater degree, than in surrounded normal tissues, that under certain conditions allows to receive luminescent and fluorescent images of tumors [1, 2]. These methods are suitable for detecting malignant growths on outside surfaces of a body in dermatology and cosmetology, and also on internal surfaces observed with the application of endoscope's technique [3]. Advantage of these methods is, besides an opportunity to study efficiency of undertaken medical measures, in real time. However these methods are unacceptable for microscopic diagnosis.

For traditional histological diagnosis tissue sections of 4-5 μ thick are prepared of the block after its freezing or embedding in paraffin. Then sections are placed on a object glass and treated with organic dyes. Haematoxylin and eosin are usually used as dyes that impart different colors to the nucleus and cytoplasm in the cell. The malignant character of a tumor is verified by observing in light microscope cell atypism features and invasive particularities of malignant growths. The disadvantage of this method is, that the observable distinctions in a number of cases are so small, that the diagnosis is made in the greater degree on intuition of the expert (pathologist), rather than on objective criterion revealing. The absence of objective criteria for diagnosis can sometimes result in irreparable mistakes.

In the present paper the new optical criterion for objective diagnosis of malignant growths in tissues of the human beings and animals is suggested and its reliability is confirmed with experiments.

APPLICATION OF NLC THIN LAYERS TO OBJECTIVE DIAGNOSIS OF MALIGNANT TUMORS

The thin NLC layers (<1 μ), applied on the surface examined as a free film and observed in polarizing microscope are being used in a science and technology for decoration of defects and structural inhomogeneities detection on the surfaces of various materials [4]. Simplicity, high speed and data comprehension of the method, and also its high sensitivity to structural inhomogenieties, have given an opportunity of its application in medicine. Already first experiments, which have been carried out on detection of pathological changes in sections of human epithelial tissues with the help of NLC, have given positive results [5]. That was why the regular comparative researches of malignant and benign tumors were carried out. 20 samples of authentically malignant tumors of five various types from existing seven were studied.12 samples of benign tumors and non-tumorous tissues were the comparison group. Only frozen tissue sections were suitable for experiments. Routine histology was used for identification of malignant areas in micropreparates . In order to avoid the NLC molecules orientation induced by plotting operation the mixture of NLC and the solvent was used. The solvent transferred NLC to isotropic phase. When the mixture was plotted on the tissue surface the solvent evaporated and NLC orientation was obtained only by structural topography. Five histogenetic groups arised from epithelium (2), tissues of mesenchimal genesis, haemopoietic and melanin-producing tissues were studied.

Through a light microscope with crossed analyzer and polarizer in all investigated cases malignant growths decorated with NLC looked black (except for clearly distinct vessels and elements of linkage skeleton) (Fig. 1, a). At the same time NLC molecules on normal tissues and benign tumors always looked white or color [6, 7]. The similar pictures were observed in dog and human tissues.

The revealed phenomenon is suggested as objective diagnostic criterion for malignant growth. To confirm the reliability of the offered criterion it is necessary to realize careful analysis and many of experiments.

HOMEOTROPIC ORIENTATION OF NLC MOLECULES AS CRITERION ON MALIGNANT TUMORS DIAGNOSIS

It is known, that the orientation conditions of NLC molecules on a solid surface depends on relation between the surface tension on the boundary of materials [8]. When the surface tension of liquid crystal γ_{LC} is bigger than the surface tension of a substrate γ_s, the NLC molecules exhibit homeotropic alignment. If $\gamma_{LC} < \gamma_s$, the NLC molecules

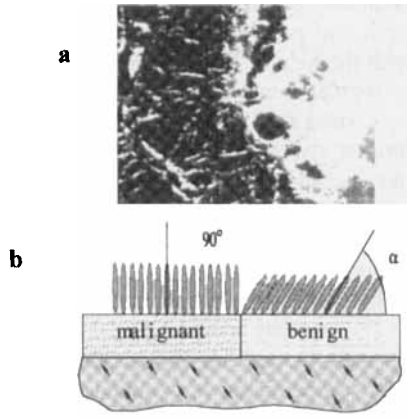

FIGURE 1. Photo of rectal cancer (adenocarcinoma): malignant (left) and benign (right) tissues decorated by the NLC layer (**a**). The NLC molecule orientation model on tissues (**b**).

alignment is planar. If γ_{LC} and γ_s have close values, the NLC molecules orientation is tilted.

When viewed through a microscope in crossed analyzer and polarizer the areas with homeotropically aligned molecules, look black (no light passes through the analyzer). If the molecules have orientation close to planar, such areas look white (intensity of light in connection with the arisen phase delay is distinct from zero) (Fig. 1, b).

All this implies that NLC layer is a unique recording medium for visualizing the distribution of a surface tension over the investigated surface.

The specified property allowed us to formulate a new biophysical criterion for objective diagnosis of malignant tumors. Thus the orientation of NLC molecules on a substrate is the micromolecular characteristic of a surface determined energy of NLC molecules coupling with a substrate. The different NLC molecules orientation on investigated tissues means a difference in anchoring energy of NLC molecules on a surface of malignant and not malignant tumors.

The interaction energy of NLC molecules with the tissue surface F^S is equal:

$$F^S = F^S_{IS} + F^S_{LC},$$

F^S_{IS} – isotropic part of surface energy, F^S_{LC} – anisotropic part.

The analytic approximation for NLC surface energy F^S_a is Rapini formula [9]:

$$F^S_a = F^S_{IS} + F^S_a(\theta) + F^S_a(\varphi) = F^S_{IS} + 1/2 W^\theta \mathrm{Sin}^2(\theta^S - \theta^{So}) + 1/2 W^\varphi \mathrm{Sin}^2(\theta^\varphi - \theta^{\varphi o})$$

Here $F^S_a(\theta)$ and $F^S_a(\varphi)$ are correspondently polar and azimuthal energy components. Usually $F^S_a(\theta) >> F^S_a(\varphi)$. From numerous experiments for homeotropic orientation $W_h^S(\theta) = 10^{-3} \ldots 10^{-2}$ erg/cm^2 and for planar orientation $W_p^S(\theta) = 10^{-2} \ldots 1$ erg/cm^2 [10]. It means that the anchoring energy of malignant growth at least in two orders less than of normal tissue. It explains the fundamental nature of the new criterion on cancer detection based on NLC molecules orientation.

For confirmation the reliability of entered criterion it is necessary to confirm the distinction of surface characteristics by independent experiments on macromolecular level, for example in measurement of a surface tension of malignant and not malignant tissues.

EXPERIMENTAL INVESTIGATIONS OF THE MALIGNANT AND BENIGN TUMORS SURFACE TENSION

The experiments purpose was the confirmation of distinctions in value of a surface tension of malignant and non-malignant tumors and normal tissues. The showing of this fact would serve as the additional proof of observable difference in orientation of NLC molecules on investigated tissues.

It is known, that on a number of the biophysical and biochemical characteristics the malignant tumors differ from normal tissues. However in the scientific literature there is no information on their surface tension value. It seams that these tissue properties simply were not studied before.

The research of tissues surface tension was made by wetting contact angles definition of investigated surfaces on the boundary with reference liquids. The photographic method allowing to a drop's profile at counter illumination was used for receiving its shadow image.

Tissue samples were fixed in 10 % formalin solution, of which on freezing microtome 5-7 μ thick sections were made. Some sections contained boundary areas of malignant and not malignant tissues, which could be observed simultaneously. Till two consecutive sections from each sample were used. One of them was exposed to traditional dyes processing for revealing malignant areas. Another section was located on subject glass, and after drying on it one (or several drops) 1-2 mm in

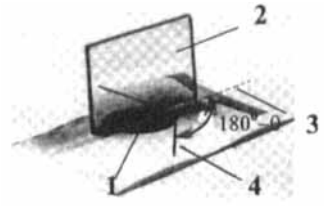

FIGURE 2. The mirror technique for tangent construction and contact angle θ measurement: 1- droplet's profile; 2- reflective mirror; 3- horizontal line; 4- tangent line.

diameter of a reference liquid was plotted. The sample was located on a special table, which could smoothly move in vertical and two perpendicular horizontal directions. The drop was shined with a collimated beam for recording with camera. For absorption of an infrared range of radiation the light source was equipped with special filter. As reference liquids water and MBBA were used.

The profile of a drop with current of time changed, as the researched tissues absorbed some part of a liquid. Therefore the recording of a drops profile was carried out repeatedly through the certain intervals of time after their drawing (through 1/2, 2, 5 and 10 minutes.).

The reflective mirror was used in the method for tangent construction to the drop profile up to complete sphere, that allowed to measure contact angles with the increased accuracy (Fig. 2).

The results of contact angles measurement of reference liquids on a surface of various types of malignant tumors, through the fixed intervals of time after drawing drops are given in Table 1.

In some cases the weak asymmetry of the drops form not depending on an error of measurements was observed.

DISCUSSION OF THE RESULTS

The comparative experiments which have been carried out on 11 samples of tissues have shown, that in all considered cases the wetting contact angles of a reference liquids on healthy tissues are more, than contact angles on malignant tumors. This difference makes essential size reaching on the average 30 % from absolute value of measuring parameter.

For the surface tension γ_{SV} calculations of malignant and normal tissues Young and Neuman equations were used [11]:

$\gamma_{SL} = 2(\gamma_{LV} \times \gamma_{SV})^{1/2} e^{-0,00015 (\gamma_{LV} - \gamma_{SV})^2}$; $_S$ - index of solid surface; $_L$ - liquid; $_V$ - vapour

$\gamma_{SL} = \gamma_{SV} - \gamma_{LV} \cos\theta$.

From our experiments γ_L for MBBA at room temperature is 30 dyn/cm^2

The results of calculations: γ_{SV} = 26 dyn/cm^2 for malignant tissues, γ_{SV} = 32 dyn/cm^2 for healthy tissues. The results of experiments and calculations are in good agreement with F. Kahn rule: γ of malignant

TABLE 1. CONTACT ANGLES OF REFERENCE LIQUIDS ON SURFACES OF VARIOUS TISSUES

	LIQUID / SAMPLE	H_2O				MBBA			
	minutes	0,5	2	5	10	0.5	2	5	10
1	Abdominal desmoid (fibrous tissue malignant tumor of the abdominal wall)	79	74	64	58	36	35	30	30
2	Breast fibroadenoma (fibrous tissue benign tumor)	84	74	-	74	30	25	20	18
3	Breast fibrosarcoma (fibrous tissue malignant tumor)	71	70	65	58	40	40	35	35
4	Scar of the esophagus (overgrowth on normal fibrous tissue)	83	76	74	65	15	12	10	10
5	Sclerotic ovary (overgrowth on normal fibrous tissue)	90	84	81	-	30	25	22	20
6	Scirrhous breast cancer	60	48	35	27	-	-	-	-
7	Uterine leyomyoma (smooth muscle tumor)	68	62	55	49	20	20	15	15
8	Uterine leyomyoma (smooth muscle tumor)	95	84	-	-	30	25	23	20
9	Skin melanoblastoma of the dog (malignant melanocytic tumor)	73	61	51	52	40	40	40	40
10	Human skin melanoblastoma (malignant melanocytic tumor)	48	48	37	-	40	40	35	35
11	Normal skin fibrous tissue of the dog	86	78	67	45	35	35	30	30

tissue (homeotropic orientation) < γ of MBBA < γ of normal tissue (planar orientation).

To conclude the direct determination of malignant and non-malignant surface tension values explain the different orientation of NLC molecules on their surfaces. The revealing of new criterion for objective diagnosis of malignancies can promote development of histological researches as applied and to other biological objects. The NLC application can be considered as an additional method allowing to raise reliability of the histological diagnosis in difficult cases.

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