ТЕОРЕТИЧЕСКАЯ МЕДИЦИНА

УДК 577.12:616-006

THE STRUCTURAL PECULIARITIES OF TUMOR DNA OF SARCOMA 45

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Key words: tumor DNA, 5-methylcytosine, melting temperature

The search of differences between DNA of normal and tumor cells was carried out on the level of primary structure and in physico-chemical aspect. Some reliable changes in the primary structure were established (the level of enzymatic methylation,the relative content of pyrimidine blocks) of some tumor DNAs in comparison with DNA of normal cells[1-4].

The investigation of the primary structure of DNA of leikozis lymphocytes shows that the content of 5-methylcytosine (5 MC) is 5 times more than in lymphocyte DNA of healthy man and the guanine and cytosine content is increased by 2-3% [1]. There are also data of the peculiarities of the secondary structure of DNA of tumor cells in comparison with DNA of normal cells [3, 4], and the differences are due to regeneration of tissues and are not a consequence of enzymatic degradation of DNA which is possible in the process of isolation[5].

For the investigation of peculiarities of the secondary structure of DNA of tumor cells [5] the kinetic formaldehide method was used which allows to registrate the existence of one defect in 10^4 nucleotide pairs [6]. It was discovered that in DNA of normal tissues, independent of their type and tissue specificity the concentration of defects does not exceed 0.3-0.6 in 10^4 nucleotide pairs. In DNA of injected tumors (hepatoma 22A, carcinosarcoma of Walker, ascite sarcoma 37) the level of defects is 2-4 in 10^4 nucleotide pairs [7]. Data were obtained which testify to the fact that the content of defects in the secondary strucrute in DNA of tumor cells is naturally connected with the development of tumor process [5]. However, as it was shown later [3], an increased content of firmly bound proteins is observed in tumor DNA. They may be centers of despiralization and misrepresent the results of defining the quantity of defective regions in tumor DNA by kinetic formaldehide method, which was not accounted in the paper [5]. Besides, the parallel measurement of the concentration of defects of the secondary structure and the content of 5MC in DNA

of tumor tissues revealed the linear dependence between these values, so that some of the defects in the secondary structure of tumor tissue DNA may be due to increased methylation of cytosine [3,8].

The aim of this paper is the elucidation of differences between DNAs isolated from normal and tumor (sarcoma 45) tissues on the basis of analysis of spectrophotometrical and microcalorimetrical melting curves, circular dichroism spectra and DNA nucleotide content.

Material and Methods

DNA samples from liver, testicle and spleen of healthy and tumor carrying rats and of the tumor sarcoma 45 were isolated by the method of Marmur in Vanyushin's modification (mol.weight~10^7 Dalton).

The content of proteins in DNA from tissues of healthy and tumoral rats was 1.5±0.2% and in DNA of sarcoma 45 – 1.3±0.2%.The content of RNA in DNA preparations was less than 1%.

Melting of DNA was carried out in neutral pH in 0.1 SSC and in 0.01 SSC (1 SSC=0.15 M NaCL+0.015 M Na citrate; pH 7.3) solution, in acidic pH – in 0.1 M Na acetate buffer and in alkaline pH-0.1 M borate buffer. Before measuring $5 \cdot 10^{-5}$M of neutralized with NaOH EDTA was added into the cell.

Attempts were made to fix the possible despiralized regions of tumor DNA by glyoxal. The preparation of glyoxal solution and fixing of melted regions of DNA was carried out according to the method given in the paper [9]. The degree of denaturation of DNA was controlled by spectrophotometer in the process of all stages of fixation.

The melting curves were performed with Cary 219 Spectrophotometer, in uninterrupted regime of heating of the DNA solution by rate $0.25^0/min$. The accuracy of temperature measurements ±0.05°C and that of the optical density – 10^{-4} opt.units [10].

Calorimetrical measurements were carried out on the differential adiabatic scanning microcalorimeter DASM-1M [11].

Circular dichroism (CD) measurements were carried out with Jasco J500A Dichrograph. pH was measured on pH-meter "pH-673". pH values were determined at 20°C. The DNA bases, including 5 MC, were determined by paper chromatography and then their quantity – spectrophotometrically [8].

Melting curves and those of heat absorption of each sample were obtained 10-12 times. As on the melting curves, obtained with the help of specrtophotometer, the structural peculiarities of DNA were displayed slightly, a transformation to differential melting curves (DMC) was realized according to the method presented in paper [12].

Results and Discussion

In Fig.1 DMC of liver DNA (hDNA) and of the tumor sarcoma 45 (tDNA) are presented. The microcalorimetric curves of heat absorption have

analogious appearances. The appearance of DMC and of heat absorption curves within the limits of measurement error are the same for all DNAs from tissues of healthy and tumoral rats. All further measurements of DNA from healthy tissues were carried out on DNA isolated from healthy rat liver.

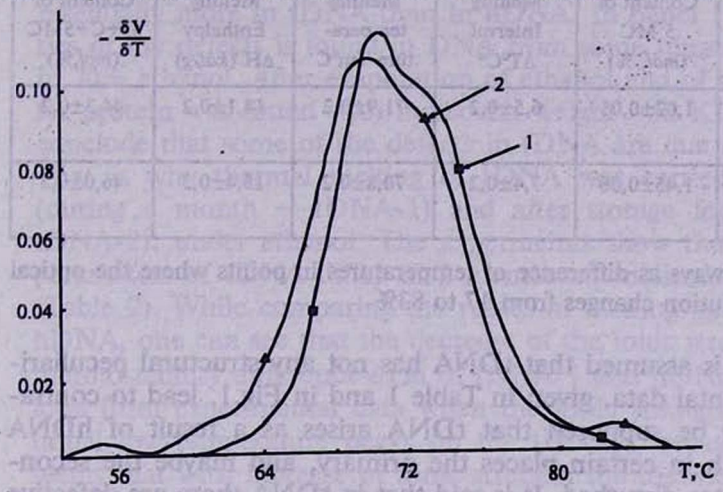

Fig.1. Differential melting curves of liver DNA (1), tumor DNA (2) in 0.1 SSC

As it follows from Fig.1, essential differences between DMC of tDNA and hDNA are observed.DMC of tDNA is shifted in respect of hDNA to the region of low-temperatures; on DMC of tDNA appear additional low-temperature peaks in the region 54-62^0 C. As the results summed in Table 1 show, besides the common shape of DMC of tDNA the thermodynamic parameters of the melting process change considerably: the melting temperature decreases (~for 1^{0}C), the melting interval (~for 0.7-1^{0}C) and enthalpy (~for 1.2 *kal/g*) increase. As it follows from Table 1, the primary structure of tDNA changes as well: both the content of 5 MC in tDNA approximately for 0.4 *mol%* and the content of G+C+5MC for 1.5-2 *mol%* exceed the corresponding values of hDNA. Increase in G+C+5MC content it tDNA must bring to additional stabilization of its molecule [13]. However, despite the expectation it follows that the melting temperature (Tm) of tDNA approximately for 1^{0}C is less than Tm of hDNA. Hence such a comparison of data by itself given in Table 1 and in Fig.1 shows that the primary and secondary structures of tDNA differ from corresponding structures of hDNA.

This means that either tDNA has almost "nothing in common" with hDNA, or that tDNAs arise at certain changes in primary and secondary structures of hDNA. Suppose that tDNA has a primary structure differing from that of hDNA and has not any differences in the secondary structure. Then the formation of additional peaks on DMC is due to specificity of tDNA primary structure. But as the GC-content of tDNA is more than the GC-content of hDNA it must melt at higher temperatures than the hDNA.

Table 1

The comparative characteristics of DNA isolated from healthy and tumor tissues in the solution 0.1 SSC pH 7.3

Living System	Source of DNA	Content of 5 MC (*mol*, %)	Melting Interval ΔT^0C*	Melting temperature Tm^0C	Melting Enthalpy ΔH (*kal/g*)	Content of G+C+5MC (*mol*,%)
Healthy animals	liver	1,02±0,03	6,5±0,2	71,9±0,2	14,1±0,2	44,3±0,2
Animals with sarcoma 45	tumor	- 1,45±0,06	7,4±0,2	70,8±0,2	15,4±0,3	46,0±0,3

* ΔT is determined always as difference of temperatures in points where the optical density of DNA solution changes from 17 to 83%

Consequently, if it is assumed that tDNA has not any structural peculiarities, then the experimental data, given in Table 1 and in Fig.1, lead to contradictory results. It may be supposed that tDNA arises as a result of hDNA transformation in which in certain places the primary, and maybe the secondary structures as well are disturbed. It is said that in tDNA there are defective regions (melting of which, probably, leads to formation of additional peaks and to shift of DMC), the nature of which is not clear yet. According to literature data the defective regions represent either regions with disturbed primary (mutations [14] and change of 5MC content [8,15]) or secondary (forming of open regions [1,3,5]) structure, either DNA regions with firmly bound nonhistone proteins[16] or regions with covalent crosslines between DNA strands. There is nothing strange in this consideration. It is known that in tumor cells the process of division is noncontrolled. That is why at isolation of DNA from such cells more probably DNAs with different stages of division of a partially despiralized shape are met. Probably, the mentioned defects in tDNA are due to existence of the despiralized regions and their consequent transformation.

In order to make choice between these considerations we investigated:

a) The thermal melting of tDNA and hDNA at low ionic strength of the solution ($2 \cdot 10^{-3}$M Na).

b) The repeated thermal melting of tDNA (heating of the solution was carried out up to the moment of entire melting of the low-temperature peak (~62^0C for the solution in 0.1 SSC characteristic for sacroma 45, then it was cooled to 45^0C with consequent increase of temperature).

c) The thermal melting of tDNA at acidic and alkaline pH.

d) The thermal melting of tDNA in the presence of urea (1-3 M).

e) The fixation of probable despiralized regions of tDNA by glyoxal.

f) The potential possibilities of tDNA to conformational reconstructions in the boundaries of keeping of double-helical structure at increasing temperature and NaCl concentration.

As it was said, one of the possible reasons of forming of tDNA defective regions may be the increased content of firmly bound nonhistone proteins [16]. These proteins stable to protease and alkaline treatment, are found both in tumor and normal cells. However, the content of firmly bound proteins is essentially more in tDNA than in hDNA. In paper [16] a decrease in quantities of the defects is found in DNA from some tumors after their long storage in 70% ethanol. After evaporation of ethanol and of solution of residue in water protein was found with molecular weight ~60 KD. This allows authors to conclude that some of the defects in tDNA are due to firmly bound proteins. That is why thermal melting of tDNA was carried out just after isolation (during a month – tDNA-1) and after storage for a long time (~1 year, tDNA-2), under ethanol. The experiments show that DMC and the melting parameters of tDNA differ in 2 mentioned methods of preparation solutions (Table 2). While comparing the values of melting parameters of tDNA-1 and hDNA, one can see that the decrease of the ionic strength leads to increase of thermostability difference δTm of tDNA-1 and hDNA. Besides that, as it follows from experimental data when the ionic strength is decreased, the additional low-temperature peaks on the tDNA-1 become more pronounced. It seems that with increase of ionic strength the thermostability of easily melted tDNA defective regions decreases, in consequence of which the melting of defective destabilized regions is not nearly recovered by melting of total DNA.

Table 2

Melting parameters of tumor DNA at two ionic strengths of the solution prepared by the first and the second methods

The method of preparation of solution	tDNA-1				tDNA-2			
Ionic strength of the solution	$2*10^{-2}$M Na(0.1SSC)		$2*10^{-3}$M Na(0.01SSC)		$2*10^{-2}$M Na(0.1SSC)		$2*10^{-3}$M Na(0.01SSC)	
Melting parameters	Tm	ΔT	Tm	ΔT	Tm	ΔT	Tm	ΔT
Source of DNA–liver	71.9±0.2	6.5±0.2	66.5±0.2	6.9±0.2	72.0±0.1	6.6±0.2	67.0±0.3	6.8±0.2
Source of DNA–sarcoma 45	70.8±0.2	7.4±0.2	63.5±0.3	7.7±0.2	71.2±0.2	6.8±0.2	67.2±0.2	7.4±0.2

As it follows from DMC of tDNA-2 the additional low-temperature peaks almost disappear. The melting parameters of tDNA change as well: Tm increases and ΔT decreases, approximating to corresponding values for hDNA. Probably these changes of melting parameters and of the shape of DMC of tDNA are due to separation from tDNA of firmly bound proteins because of tDNA longtime storage under ethanol. It must be mentioned that for hDNA

at 2 mentioned methods of preparation of solutions no difference of melting parameters and the shape of DMC is observed.

The repeated melting of tDNA at 0.1 SSC and 0.01 SSC was investigated as well. At repeated melting the additional low-temperature peaks almost disappear. Consequently the extension of easily melted regions, melting of which results in formation of additional peaks on DMC of tDNA-1 is of some hundreds of base pairs. If urea is added to the solution of tDNA urea being a universal denaturant for proteins, at low concentrations of urea (less than 1.5 M) additional low-temperature peaks remain and at higher concentrations (up to 3M) disappear only partially. Hence if denaturation of firmly bound proteins takes place under the influence of urea, they do not dissociate from tDNA. That is why, probably, the additional low-temperature peaks do not disappear either. The character of change of tDNA melting parameters from urea concentration differ next to nothing from analogous dependence for hDNA, obtained in the paper [17].

What else can explain the peculiarities of DMC of tDNA? As it follows from Table 1, the content of 5 MC in tDNA is more than in hDNA. Let us analyse where to hypermethylation of DNA can lead.Methylation of cytosine in the fifth position is of great biological significance and the change of methylation takes place in many pathologies of the cell [1,15]. In the paper [18] the reasons of the seldomly of GC dinucleotides in genoms of vertabrates in comparison with the value which must be at random consequnce of links are discussed. It is also shown in the work [18] that this is connected with high methylation of GC dinucleotides. Unamination of 5 MC leads to its transformation into uracil (or thymin) and the disappearance of GC dinucleotides. This process is difficult in regions enriched by GC-pairs because of great stability of such regions. Therefore because of enzymatic unamination of 5 MC the formation of destabilized regions in AT-rich regions tDNA is more probable. The noncomplementary G-T pairs are included into these destabilized regions. As it was already said, besides the formation of destabilized regions methylation leads to additional stabilization of DNA helical regions, which are capable more probably to transformation from B-form into Z-form DNA [19].

Generalizing the above mentioned one can confirm that due to hypermethylation in AT-rich regions destabilized regions arise, which contain G-T noncomplementary pairs. Melting of these regions may lead partially to the appearance of additional low-temperature peaks on DMC of tDNA. Indeed, in the paper [20] the defects of the secondary structure were found in DNA AT-rich regions. In order to clear up the existence of noncomplementary base pairs in tDNA, attempts were made to fix the possible despiralized regions by glyoxal. It is known that glyoxal interacts with adenine, cytosine and guanine, the adduct being stable only with guanine [9].

According to the methods presented in [9] fixation of easily melted regions of tDNA in 0.1 SSC (prepared by 2 methods). However the repeated melting of tDNA didn't show any considerable changes in the melting curve. Therefore

glyoxal did not interact visibly with denaturated or easily melting regions of tDNA. This indicates the high content of AT-pairs in easily melting regions of tDNA, or that guanines in these regions are in an inaccessible position for glyoxal molecules.

The thermal melting of tDNA at acidic and alkaline pH was investigated as well. Protonation of 5 MC somehow must differ from protonation of other DNA bases. Therefore investigating the melting of tDNA at acidic and alkaline pH one can judge of the character of 5 MC destribution along tDNA molecule according to changes of the total shape of DMC. pH of the solution was changed from 3.8-11.2 in the experiments. It was shown that the increase and the decrease of pH leads to decrease of Tm and ΔT [21].

Table 3

Melting parameters of DNA at different pH (ionic strength of the solution 0.1 M NaCl)

Animal type	DNA source	pH	Melting temperature (Tm°C)	Melting interval (ΔT°C)
Healthy animals	liver	3.8	46.5±0.2	3.2±0.2
		4.0	57.4±0.2	4.5±0.1
		4.5	69.2±0.2	5.2±0.2
		9.2	80.0±0.1	5.7±0.2
		9.7	76.0±0.2	4.8±0.2
Animals with sarcoma-45	tumor	3.8	46.3±0.1	3.7±0.2
		4.0	57.1±0.2	4.6±0.2
		4.5	68.8±0.3	5.3±0.2
		9.2	80.0±0.2	6.0±0.1
		9.7	75.9±0.2	5.1±0.2
		10.6	61.3±0.1	4.8±0.1
		11.2	47.9±0.2	6.1±0.2

In Table 3 values of melting parameters for tDNA and hDNA (the solutions made by both methods) are presented. As it follows from Table 3, at acidic and alkaline pH the general regularities for melting parameters of hDNA and tDNA remain unchanged: Tm of tDNA is always less than Tm of hDNA and ΔT of tDNA is more than ΔT of hDNA. The change of pH to the acidic or alkaline regions leads to a decrease in the difference between corresponding values of tDNA Let us compare DMC for tDNA-1 and hDNA in acidic (pH 4.0) and alkaline (pH 9.5) mediums (Fig. 2,3). In these mediums on DMC of tDNA again additional low-temperature peaks are observed. They are more pronounced and are seen even on the melting curve. However, in these conditions at repeated melting these peaks almost disappear. At repeated melting the melting rises more steeply in the initial stage and the melting is

late for~2°C, e.g. for the solution of tDNA at pH 4.0 melting begins at 48°C and at repeated melting – at 50°C. Therefore, at cooling of partially denaturated tDNA (heated up to 52°C for solutions pH 4.0) the despiralized regions do not renaturate entirely, leading to disappearance of low-temperature peaks at repeated melting. What is the reason for this phenomenon? It should be mentioned that in spite of DMC of tDNA-2 at neutral pH additional low-temperature peaks are almost not observed. At decreasing or increasing of pH these additional peaks become more pronounced. This proves once more that forming of defects is not due to firmly bound proteins only. It is known that in helical regions only guanine, and in melted regions - adenine, cytosine and guanine are protonated. That is why at change of pH to the acidic or alkaline regions additional weakening of bonds between nucleotide pairs and especially between pairs in easily melting regions of tDNA occurs. This leads to increase of melted regions and therefore the ability of restoration of low-temperature peaks at cooling decreases. Let us compare DMC of tDNA and hDNA presented in Fig.2 and 3. As it follows from Fig.2 and 3 the curves don't differ by their shape and localization on the temperature scale. The difference of tDNA and hDNA is observed again only in the initial stage of melting. Hence, probably, 5 MC are destributed in tDNA not in the form of claster but quasirandomly, along the all DNA molecules as, otherwise, on DMC new peaks would arise.

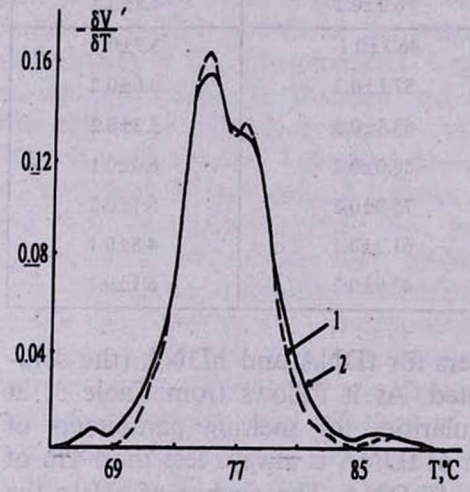

Fig.2. Differential melting curves of hDNA (1) and of tDNA (2) at pH 4.0

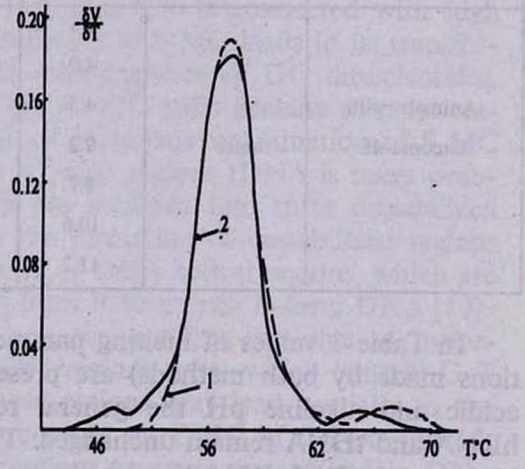

Fig.3. Differential melting curves of hDNA (1) and of tDNA (2) at pH 9.5

As it follows from Fig.2 and 3, additional peaks do not arise at hDNA melting in mentioned conditions either. So, even if the firmly bound proteins

make contribution in formation of low-temperature peaks on DMC of tDNA, they are not the only reason bringing to formation of these additional peaks.

Finally, the potential possibilities of tDNA to conformational reconstructions in the limits of keeping the double-stranded structure were investigated. As it is known [22], for normal DNAs, the hDNA, with increasing temperature in the interval, where it is still in a double-stranded position, some despiralization takes place (in the spectra of CD dichroic absorption at $l=276nm$ increases). As it follows from Fig.4, where the dependence of relative ellipticity at $276nm$ is presented ($\Delta\varepsilon_{276}$) on temperature for hDNA and tDNA, with increasing temperature the ellipticity almost is not changed for solutions tDNA-1. For tDNA-2 with increasing temperature $\Delta\varepsilon_{276}(T)$ is less than for hDNA. That means in tDNA there are regions preventing despiralization of DNA, this being stronger for tDNA-1. Thus, one can suppose that these may be regions with firmly bound proteins or regions with covalent crosslinks between the strands of DNA. In order to clear up what prevents the despiralization of tDNA, the dependence of $\Delta\varepsilon_{276}(T)$ was investigated for tDNA solutions in presence of 0.5-4 NaCl.

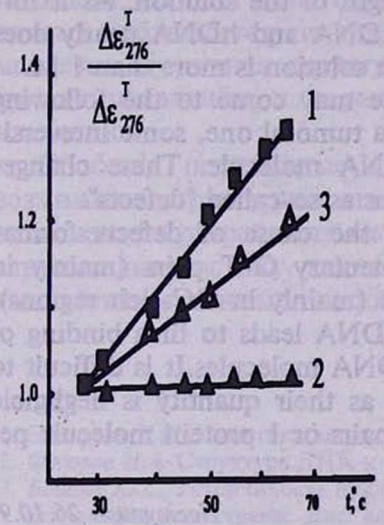

Fig.4. Dependence of relative change of $\Delta\varepsilon_{276}$ dichroic absorption at $276nm$ on temperature for hDNA (1), tDNA-1 (2) and tDNA-2 (3) in 0.1 SSC

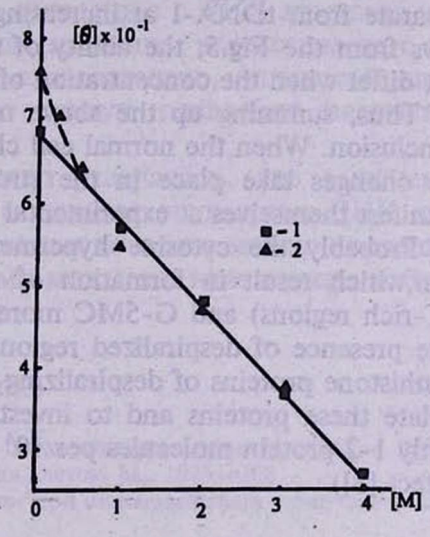

Fig. 5. Dependence of molecular ellipticity on NaCl concentration for hDNA (1) and tDNA-1 (2)

It is clear that the ionic strength will not change the peculiarities of tDNA very strongly if there are covalent crosslinks between the strands of tDNA. It follows from experimental data that with increasing concentration of NaCl the ability of despiralization of tDNA increases at increasing temperature. As it is known [23], with increasing concentration of NaCl twisting of DNA molecule takes place.Therefore for comparison in Fig.5 the dependence of molecular ellipticity$[\Theta]_{276}$ on the concentration of NaCl for tDNA-1 and for hDNA is shown. As it follows from Fig.5 the value of $[\Theta]_{276}$ decreases linearly for hDNA (as one could expect). The linearity of $[\Theta]_{276}$ curve is frunged for tDNA-1 in the region 0-1 M NaCl. In 0.1 SSC the value $[\Theta]_{276}$ for tDNA-1 is more than $[\Theta]_{276}$ for hDNA. It must be mentioned that for tDNA-2 the initial divergence of the curve $[\Theta]_{276}$ (M) nearly disappears. Let us try to explain the obtained value. It is known that proteins, not bound covalently, separate from DNA at high ionic strengths. That is why summing up the data on conformational transformations of tDNA in limits of keeping the double-stranded structure, one can conclude that tDNA-1 contains a hightened quantity of firmly bound proteins, possibly of despiralizing type, the binding of which with DNA leads to despiralization of DNA (this resulting in the increase of $[\Theta]_{276}$) and prevents the despiralization of DNA at increasing temperature. Possibly, these proteins separate from tDNA-1 at increasing ionic strength of the solution. As it follows from the Fig.5, the ability of twisting of tDNA and hDNA nearly does not differ when the concentration of NaCl in the solution is more than 1 M.

Thus, summing up the above mentioned we may come to the following conclusion. When the normal cell changes into a tumoral one, some inreversible changes take place in the structure of DNA molecule. These changes manifest themselves at experimental investigations as so-called "defects".

Probably the cytosine hypermethylation is the cause of defects formation,which result in formation of noncomplementary G-T pairs (mainly in AT-rich regions) and G-5MC more stable pairs (mainly in GC-rich regions). The presence of despiralized regions in tumor DNA leads to firm binding of nonhistone proteins of despiralizing type with DNA molecules.It is difficult to isolate these proteins and to investigate them, as their quantity is negligible (only 1-2 protein molecules per 10^4 nucleotide pairs or 1 protein molecule per defect [5]).

Поступила 26.10.96

ՍԱՐԿՈՄԱ 45 ՈՒՌՈՒՑՔԻՑ ԱՆՋԱՏՎԱԾ ԴՆԹ-Ի ԿԱՌՈՒՑՎԱԾՔԱՅԻՆ ԱՌԱՆՁՆԱՀԱՏԿՈՒԹՅՈՒՆՆԵՐԸ

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Ներկա աշխատանքում ուսումնասիրվել է սարկոմա 45 ուռուցքից անջատված ԴՆԹ-ի առաջնային եւ երկրորդային կառուցվածքի առանձնահատկությունները: Հայտնի է, որ չարորակ նորագոյացությունների առաջացման պատճառը ԴՆԹ-ի

առաջնային կառուցվածքի փոփոխություն է: Մուտացիայի ենթարկված ԴՆԹ իր կենսաբանական ֆունկցիաները իրականացնելիս ավելի է տրանսֆորմացվում:

Փորձնական առնետների վրա կատարված հետազոտությունները ցույց են տվել, որ առողջ կենդանիներից անջատված ԴՆԹ-ի համեմատ սարկոմա 45-ից անջատված ԴՆԹ-ի մոտ դիտվում է ցիտոզինի հիպերմեթիլացում, որի հետեւանքով փոխվում է ԴՆԹ-ի երկրորդային կառուցվածքը եւ, հետեւաբար՝ այն նկարագրող հալման կորի տեսքը եւ բնութագրերը: Ընդհանրացնելով ստացված փորձնական արդյունքները, կարելի է պնդել, որ ուռուցքային ԴՆԹ-ի հնարավոր կառուցվածքային փոփոխությունների պարզաբանումը թույլ կտա մշակելու չարորակ նորագոյացությունների նախնական ախտորոշման մեթոդ, երբ վիզուալ եղանակով հնարավոր չէ նկատել ուռուցքագոյացումը::

СТРУКТУРНЫЕ ОСОБЕННОСТИ ОПУХОЛЕВОЙ ДНК САРКОМЫ-45

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В работе исследованы особенности первичной и вторичной структуры ДНК саркомы-45. Известно, что причиной злокачественного перерождения ткани является изменение первичной структуры ДНК. В клетке вследствие репликации, транскрипции и т.д. ДНК еще больше трансформируется. Опыты, проведенные на эксперементальных животных, показали, что для ДНК саркомы-45 по сравнению с ДНК здоровых животных наблюдается гиперметилирование цитозина, вследствие чего изменяется вторичная структура ДНК и, следовательно, описывающая ее кривая плавления и характеризующие кривую плавления параметры плавления.

Обобщая полученные данные, можно утверждать, что выяснение возможных структурных изменений в молекулах опухолевых ДНК дает возможность разработать метод, при помощи которого можно проводить раннюю диагностику, когда визуальными методами невозможно наблюдать злокачественные новообразования.

REFERENCES

1. *Эмануэль Н.М.* Кинетика экспериментальных опухолевых процесов. М., 1977, с. 380.
2. *Федоров Н.А.* Структура ДНК и трансформация клеток. М., 1983, с128
3. *Бабаян Ю.С.* Устойчивость нуклеиновых кислот при взаимодействии с биологически активными веществами. Дис. докт. М., 1991.
4. *Waring M.J.* DNA modification and carcer. Ann. Rev. Biochem., 1981, 50, p. 159.
5. *Авакян К.А. Жижина Г.П., Пархоменко Н.И.* Состояние вторичной структуры и метилирование ДНК у перевиваемых опухолей и культур эмбриональных клеток, трансформированных онлогенными вирусами. Биохимия., 1979, 44, 8, с. 1436.
6. *Lazurkin Yu.S., Frank-Kamenetski M.D., Trifonov E.N.* Melting of DNA: its study and application as a research method. Biopolymers. 1970, 9, 10, p. 1253.
7. *Жижина Г.П., Бобович С.И., Авакян К.А., Петров О.Е.* Дестабилизация вторичной структуры ДНК эксперементальных опухолей. ДАН СССР, 1977, 235, 3, с. 703.
8. *Гарибян Д.В., Степанян Г.М., Даниелян И.С. Бабаян Ю.С., Арутюнян С.Г., Гарибджанян Б.Т., Асланян В.М.* Изменение специфического метилирования ДНК в тканях

здоровых и опухоленосящих крыс (с саркомой-45) под влиянием активного канцеролитика алкилирующего типа. Биохимия, 1980, 45, 11, с. 2028.
9. *Broude N.E., Budowsky E.I.* The reaction of glyoxal with nucleic acid components. Biochim. Biophys. Acta, 1973, 29 p. 378.
10. *Babayan Yu.S., Manzini G., Xodo L., Quadrifoglio F.* Base specifity in the interaction of ethidium with synthetic polyribonucleotides. Nucleic Acids Res., 1987, 15, 14, p. 5803.
11. *Бабаян Ю.С., Асланян Л.Л., Ананян Г.В., Гарибян Д.В.* Влияние фосфемида в сочетании с дексаметазоном на структуру ДНК саркомы-45. Биофизика, 1985, т. 30, 3, с. 414.
12. *Бабаян Ю.С., Вардеванян П.О., Гарибян Д.В., Арутюнян С.Г., Кулага Л.А., Асланян В.М.* Применение метода плавления в выявлении различий между ДНК из нормальных и опухолевых тканей. Биофизика, 1984, 28, 2, с. 313.
13. *Ehrlich M., Ehrlich K., Mayo J.A.* Unusual properties of the DNA from xanthomonas phage XP-12 which 5-methylcytosine completely replaces cytosine. Biochem. Biophys. Acta., 1975, 395, 2, p. 109.
14. *Reddy E.P., Reynolds R.K., Santos E., Barbacid M.* A point mutation is responsible for the asqaisition of transforming properties by the T24 human bladder carcinoma oncogene. Nature, 1982, 300, 11, p. 149.
15. *Doerfler W.* DNA methylation and gene activity. Ann. Rev. Biochem., 1983, 52, p. 93.
16. *Скалацкая С.И., Бунина Е.Ф., Круглекова К.Е., Эмануэль Н.М.* О приподе дефектов вторичной структуры ДНК опухолевых клеток. ДАН СССР, 1982, 265, 5, с 1268.
17. *Бабаян Ю.С.* Конформация и термостабильность двуспиральных нуклеиновых кислот в водных растворах мочевины. Мол. биол., 1988, 265, 5, с. 1204.
18. *Adams P.L.P., Eason R.* Increased G+C content of DNA stabilises methyl CpG dinucleotides. Nucleic Acids Res., 1984, 12, 4, p. 5869.
19. *Behe M., Felsenfeld G.* Effects of methylation on a synthetic polynucleotide:The B-Z transition in poly (dG-m5dc)*poly (dG-m5dc). Proc. Nat. Acad. Sci. USA, 1981, 78, 3, p. 1619.
20. *Лигина М.В., Шугалий А.В., Митрохин Ю.И. и др.* Модификации ДНК гепатомы Зайдела дибромэтилацетатом. Эксперим. онкол., 1982, 4, 2, с. 30.
21. *Сухоруков Б.Н., Мешковский Ю. Ш., Биршейн Т.М.* Спектроскопическое исследование перехода спираль-клубок в ДНК при разных температурах и pH. Биофизика, 1963, 8, 2, с. 294.
22. *Шелкина А.К., Минченкова Л.Е., Иванов В.И.* Влияние температуры на параметры двойной спирали ДНК в растворе. Мол. биол., 1977, 11, 2, с. 466.
23. *Зенгер В.* Принципы структурной организации нуклеиновых кислот (пер. с англ.). М., 1987, с. 584.