MITOGENETIC ANALYSIS OF THE PROTEIN FRAMEWORK OF PROTOPLASM COMMUNICATION III. SPECIAL FEATURES OF THE FRAMEWORK OF THE CANCER CELL

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In previous papers [4, 5] it has been shown that the protein framework of tissues in which cell division is proceeding is characterized by a reduction in the number of weak intermolecular bonds, i.e. an increase in the independence and, hence, of the mobility of the peptide chains over that found in tissues emerging from a meristematic state.

It seemed probable that the character of the protein framework of the cancer cell would be rather different. We know that the great difference between cancer tissue and normal meristems is shown, not by an increase in the mitotic coefficient, but the continuousness of division and, at the same time, by the topographical unrestraint of mitosis in the malignant foci, and the dissemination of mitoses throughout the tumor, in contrast to the confined nature of the foci of proliferation of normal meristems. It might have been expected that the protein framework of the cancer cell would also be distinguishable by some sign from the framework of the normal dividing cell. The present investigation is devoted to the partial explanation of this problem.

EXPERIMENTAL METHOD

We investigated implanted mouse adenocarcinoma (produced by subcutaneous inoculation of 0.2 cm^3 of a suspension of ascitic cells in the dorsal region), both in vivo and in isolation from the animal. The mitogenetic radiation of the tumor was studied on the 8-11th day after implantation.

For the experiment the mouse was fixed on a small operation table. A flap of skin covering the tumor was reflected, and the small exposed surface (approximately 3 × 3 mm) was centred in front of the collimator of the spectrograph (all the remaining surface of the tumor and adjacent areas of the trunk were covered). The tumor which had been isolated from the animal was treated in a similar way. Throughout the experiment the tumors were bathed in physiological saline, warmed to 38-40°.

As had previously been shown [1-3], tumors not separated from the body produce a constant and intensive (within the limits of mitogenetic intensity) spontaneous mitogenetic radiation. For this reason the method of spectral analysis of selective dispersion, as described in the previous communications [4, 5], could not be used in this part of the investigation.

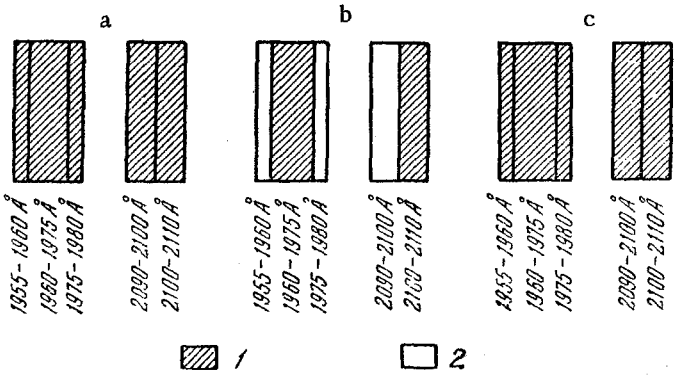
We studied the spectral bands corresponding to the emission from the carbonyl R – C – O and oxyphenyl R – C_6H_4 OH groups, and the bands of the peptide end groups R – OH and R – NH_2.

EXPERIMENTAL RESULTS

Even the first results showed a clear difference in the state of the protein frameworks of the malignant tumor and normal tissue.

The spectral bands for the carbonyl and oxyphenyl groups of the cancer tissue were wider than the standard values for these bands, and than the identical bands found during investigation of the radiation from normal tissues (see Figure).

In the normal tissues of the mucous membrane of the lip of a newborn guinea pig, the cells of which are capable of division, the R − C = O group was represented by bands between 1960-1975 A and between 2100 and 2110 A. In the tissues of the labial mucosa of the adult guinea pig, emerging from a meristematic state, these radicals also emit radiation in neighboring areas of the spectrum. The field of irradiation was thus widened. The field of irradiation of adult guinea pig tissue was 1955-1980 A and 2090-2110 A. The degree of widening of the bands which characterized the radiation emitted from cancer tissue was analogous to the widening of the same bands observed in the radiation emitted by tissues emerging from a meristematic state.



CO bands in the selective dispersion spectrum of adenocarcinoma and normal tissue.
a) Adenocarcinoma; b) mucous membrane of the lip of a newborn guinea pig; c) mucous membrane of the lip of an adult guinea pig; 1) active band; 2) inactive band.

From these findings the following direct conclusion could be drawn. A typical feature of the protein framework of the cancer cell was, evidently, the considerable preponderance of weak intermolecular bonds, joining the peptide chains in dimeride and trimeride systems, i.e. it was typified by a certain degree of aggregation of the framework, analogous to its condition in normal tissues in which no cell division was present.

This conclusion appeared to conflict with our views on the mobility of the protein and peptide systems of the framework of the dividing cells. The results of later spectral analysis of bands characterizing the peptide end groups $R-NH_2$ and R − OH went a long way towards removing this contradiction.

Investigation of the mitogenetic radiation of cancer tissue clearly revealed bands characteristic of amino- and hydroxyl groups at exposures of 21-24 seconds. This indicated that their amount was comparatively great, i.e. that the peptide chains undergoing aggregation were relatively short*. In normal tissue, containing dividing cells, from the labial mucosa of a young guinea pig, these bands were found at even lower exposures − 10-12 seconds. To judge by the intensity of the bands of malignant and normal tissue, the results can be arranged in the following order (see Table).

In the normal tissue of the adult animal we found no bands characteristic of amino- and hydroxyl groups at exposures of 6-40 seconds. The absence of bands at these exposures demonstrated that the intensity of radiation from the labial mucosa of the adult guinea pig was significantly less than that from normal tissue containing dividing cells and from cancer tissue.

* The criteria of the intensity (clarity) of the bands was the threshold of effective exposure. It was found experimentally that the intensity of the radiation and the threshold exposure were inversely proportional (It = constant).

In cancer tissue these groups were found at exposures of 21-24 seconds, and in tissue from the lip of a newborn guinea pig, at exposures of 10-12 seconds. From comparison of the results, the following concept emerges: aggregation of the protein framework is expressed in the cancer cell in a perfectly definite form – a predominance of short peptide complexes. Under this term we imply the combination of short segments of the peptide chains in dimeride, or even trimeride aggregates. Such a state may correspond to the considerable mobility of these peptide complexes, although it is possible that by comparison with normal dividing cells, characterized by free, short peptide chains [5], their mobility is slightly reduced.

Characteristic Radiation of Peptide End Groups

	Test object							
	mucous membrane of the lip of an adult guinea pig		mucous membrane of the lip of a newborn guinea pig		mouse adenocarcinoma			
					with no other agent		after administration of an extinguisher	
	effect (in %)	exposure (in seconds)	effect (in %)	threshold exposure (in seconds)	effect (in %)	threshold exposure (in seconds)	effect (in %)	threshold exposure (in seconds)
R – NH_2:								
2065-2070 A	2	6-40	41	12*	76	24	57	32
The same	–1	6-40	42	10	42	21	37	32
The same	1	6-40	35	12	50	21	38	32
The same	4	6-40	45	12	41	21	42	32
The same	3	6-40	43	10	36	21	40	32
2260-2270 A	1	6-40	32	12	47	24	75	32
The same	–2	6-40	46	10	30	21	36	32
The same	1	6-40	38	12	53	21	32	32
The same	–9	6-40	36	12	47	21	49	32
The same	–2	6-40	45	10	42	21	35	32
The same	5	6-40	51	10	44	24	48	35
R – OH:								
24-2450 A	–	–	49	10	46	24	52	32
The same	–	–	34	12	44	24	47	32
The same	–	–	43	10	55	21	49	32
The same	–	–	–	–	59	21	–	–

* At lower exposures – no effect.

In addition, however, the idea is very likely that directly after the onset of mitosis in the cancer cell, disintegration of the short aggregates takes place, i.e. free, short peptide chains are formed, analogous to those which predominate in normal dividing cells. The mobility of the framework of the various groups of cells would then be strictly comparable.

Some grounds for this hypothesis are provided by the following simple consideration: During the study of the mitogenetic spectra of normal and malignant tissues, we recorded the radiation of a large aggregation of cells, the majority of which, of course, were in a state of interkinesis, and a smaller number in the phases of mitosis. In other words, the results obtained related mainly to cells in different stages of preparation for division; we have no direct grounds for extending this criterion to the stage of mitosis also. Whether we adopt this point of view or not, however, it is obvious that the state of interkinesis of normal tissues and cancer tissue is typified by considerable difference in the spatial relationships of the structure of the protein framework.

In connection with the results obtained, the following question arose: Was the characteristic state of the protein substrate of the cancer cell associated to some extent with a distinctive mitogenetic pattern?

Whereas normal meristems are characterized by premitotic emission of radiation only, a continuous mitogenetic emission is specific for cancer tissue, probably due mainly to the presence in the cell of a continuously radiating carcinogen (unpublished work from this laboratory). We are not yet able to supply a sufficiently well-grounded or complete answer to this question, but in accordance with other findings, obtained some time ago in this laboratory [1, 2], it seems very likely to us that the small size of the peptide chains in the cancer cell is connected with the continuity of the mitogenetic radiation.

It was found that peptide synthesis was stimulated by mitogenetic radiation both in vivo and in experiments with aminoacid solutions, and that under these conditions, i.e. during excitation of the protein framework with high quanta of energy, the formation of peptide chains acquired the character of a chain reaction. At the same time it was found that intermittent radiation had the greatest stimulating effect; a continuous flow of photons, on the other hand, leads to frequent rupture of the developing chains.

In order to test the hypothesis of the importance of radiation for the state of the protein framework of the cancer cell, the cancerous mouse was injected subcutaneously with a so-called extinguisher of mitogenetic radiation – a solution of furfurol, which absorbs almost the entire ultraviolet region of the spectrum; this was injected in very small concentration – of the order of 10^{-6}, in a dose of 0.5 ml.

A fall in the genera intensity of radiation, especially after several repeated injections, and a fall in particular in the radiation from the malignant tumor, led to the expected result (see Table, last column). With the aid of spectral mitogenetic analysis it was found that after injection of the extinguisher a considerable weakening was observed of the characteristic bands of the peptide end groups (NH_2), whereas, for example, the bands of the carbonyl group did not alter in intensity. In these experiments we used the method of selective dispersion of mitogenetic radiation, which is known to allow the spectra of the functional groups to be detected even in an unirradiated or a feeble irradiated protein framework. When the mitogenetic emission is weakened, the length of the peptide chains in the cancer cells isthus increased.

In other words, the above-mentioned assumption that shortening of the chains was due to continuous radiation, specific for the cancer cell, was placed on a firm footing. We may add that a specific feature of the protein framework of the cancer cell is the predominance of dimeride and even trimeride systems of peptide chains, joined by intermolecular bonds, but of no great length and, hence, mobile. The mobility of the protein framework is one of the essential conditions of mitosis.

A fall in the continuous mitogenetic emission from the cancer cell, which may be brought about by injecting the animal with an extinguisher of mitogenetic radiation, leads to a significant lengthening of the chains, thereby to some extent bringing the state of its protein framework nearer to that of normal cells, not in process of division.

SUMMARY

By the method of mitogenetic spectral analysis it has been demonstrted that the protein substrate of cancer cells differs from the substrate of the normal tissue in which cellular division occurs. In 21-24 second exposures amino- and hydroxyl end groups have been revealed in the cancer tissue. Irradiations characterizing these radicals are found in 10 – 12-second exposures of the tissues of newborn guinea pigs containing dividing cells. At the same time the mitogenetic spectri of the cancer tissue characterizing the carboxyl (R – C – O) and the oxyphenyl (R – C_6 – H_4OH) groups are widened in comparison with the bands peculiar to the normal tissues with dividing cells. Hence it may be assumed that the cancer cell substrate consists of short (with relation to the main axis) two and "three-dimentional agreggates". Administration of the mitogenetic radiation extinguisher (fufurol solution) to mice has demonstrated the short chains to be related to the constancy of the radiation specific for cancer cells.

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* Original Russian pagination. See C. B. Translation.