THE STABILITY OF TRANSPLANTABLE CELL LINES

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Because of widespread occurrence of transplantable cell lines in the various divisions of biology and medicine, and especially in virology, particular importance is attached to the question of their stability and the immutability of their morphological and biological properties.

The problem of the evolution of transplantable cell lines is closely connected with the as yet unsolved problem of their malignant change. Many researchers [9] consider that transplantable cell lines have undergone malignant change. This point of view is based primarily on data relating to the morphology and physiology of these strains (the large number of irregular mitoses, the anisomorphism of their nuclei, the intensive multiplication of the cells). These signs are not, however, in themselves evidence of invariable malignant change—a certain number of irregular mitoses is seen, for example, in primary cultures [8, 11]. We have also confirmed this observation and postulated that the appearance of irregular mitoses characterizes the special metabolic conditions in these cultures, bringing about a disturbance of the mechanism of cell division [4].

The most convincing evidence in favor of malignant change is provided by the results of the injection of cell suspensions of transplantable strains into specially prepared animals (rats, monkeys) [9]. In these conditions tumor-like growths and true tumors develop. Some writers [8] even stipulate a direct relationship between the origin of a line (from normal or tumor cells) and its ability to produce tumors.

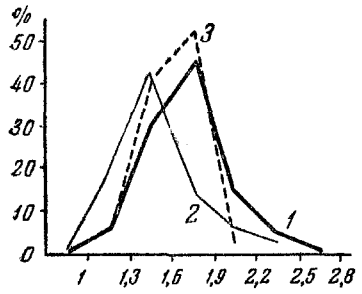

Fig. 1. Dimensions of nuclei in transplantable cell lines. 1) SCH; 2) Hep-2; 3) HeLa.

Without concerning ourselves with the whole of this complex and, in our opinion, far from solved problem of the malignant change in the cells when cultivated in vitro, we shall confine our attention to the question of the stability of transplantable cell lines, making use of morphological and biological criteria.

Our task is made easier by the fact that we have at our disposal a series of cell lines transplanted for more than 40 months (since August, 1957), kept in standard conditions, and subjected to systematic cytological and virological investigation. We have most information on HeLa, Hep-2, and cinomolgus heart (SCH) cultures, widely used in our institute. These cultures not only resemble each other morphologically, but also have many histochemical properties in common, suggesting that their metabolism is similar. It has been shown, for instance, that the pattern of activity of certain enzymes [5], like the pattern of the carbohydrate metabolism [6], in investigations of transplantable cell lines is very similar if not identical. At the same time, however, definite differences exist between the individual cell lines, suggesting the individuality of the various cultures and, hence, some degree of stability.

Our previous researches showed that the pattern of mitotic activity in each culture possesses its individual peculiarities, and may be regarded as one of its characteristic signs [2, 4].

In more recent work we have shown that, despite the considerable outward similarity between the cultures under study, they have definite morphological differences, not obvious during direct observation but revealed by more delicate methods. In particular, it has been found that the cultures from one another in the greatest diameter of their nuclei and in the change in this diameter in the course of cultivation. We investigated 6000 nuclei in HeLa,

Hep-2, and SCH cultures. The diameter of the nuclei was measured by means of an ocular micrometer on the third and sixth days of cultivation. The results, treated statistically and related to 100 nuclei, show that each of the cultures studied differs from the others in its characteristic distribution curve of nuclear diameters and in their largest diameter. A regular change in the diameter of the nuclei during cultivation, slightly different in each culture, was also observed (Table 1, Fig. 1).

TABLE 1. Distribution of Nuclei by Size in SCH, HeLa, and Hep-2 Cultures on the Third and Sixth Days of Cultivation (% of Total Number of Nuclei)

Culture	Time of cultivation (in days)	Diameter of nucleus (in μ)						
		<1	1-1.3	1.3-1.6	1.3-1.9	1.9-2.2	2.2-2.5	2.5-2.8
SCH	3rd	0.5	6	31	44	14	4.3	0.2
	6th	–	3	13	21	44	5	4
	3rd	1	24	42	19	11	3	–
Hep-2	6th	–	3.5	22	51	19	11	3
	3rd	–	5	41	52	2	–	–
HeLa	6th	–	26	33	37	4	–	–

These results point to the existence of fairly stable individual morphological and physiological differences between the three transplantable cell lines tested. The establishment of reliable morphological and physiological characteristics of a series of such cultures was a natural introduction to the study of the stability of the morphological and physiological properties of a single culture over a period of cultivation lasting several years. As test object we selected a SCH culture, about which it has often been suggested that gradual malignant change may take place.

Comparison of preparations of the SCH culture shows that over a period of several years (27th, 48th, 147th, and 256th passages) the morphology of the cultures remained quite unchanged. The preservation of certain biological properties of the SCH culture is suggested by the results showing the pattern of the mitotic activity of its cells in the course of a series of passages (from the 81st to the 256th), obtained on the 4th day of cultivation, i.e., in the period of intensive multiplication (Table 2).

TABLE 2. Mitotic Activity of the SCH Culture in the Course of 175 Passages

Passage	Date of investigation	Mitotic activity (in %)
81st	October, 1957	70
87th	October, 1957	74
105th	March, 1958	64
109th	April, 1958	59
135th	October, 1958	60
139th	December, 1958	58
147th	January, 1959	58
199th	January, 1960	47
256th	January, 1961	65

The figures in Table 2 show that the mitotic activity remained very constant throughout the period of investigation, the slight variations being associated, evidently, with slight differences in the conditions of cultivation (type of vessel, quality of serum, variations in temperature, and so on).

Besides the constancy of the cell morphology and the intensity of multiplication, a biological property of the SCH culture such as the sensitivity of poliomyelitis virus also remained substantially unchanged (Table 3).

TABLE 3. Sensitivity of the SCH Culture to Poliomyelities Virus During 111 Passages

Passage	88th	105th	110th	116th	126th	136th	144th	170th	178th	190th	196th	201th
Titer of virus (-log 10)	7	6	6.5	7	6.5	7	6	7	6.5	7	6.8	7

Our results showing the stability of the SCH culture may be compared with results obtained by other workers in our institute [1], indicating that passage of SCH cells through monkeys is not accompanied by irreversible changes in their properties. Cells (SCH-2) extracted 10 days after inoculation into monkeys and transferred to a nutrient medium reacquired the typical morphology of this culture, as morphological and histochemical investigations showed, after the 21st passage.

It is interesting to note that the sensitivity to poliomyelities virus was also associated with the recovery of the properties of the SCH-2 cells (Table 4).

It may be seen from Table 4 that the titer of virus, which fell at the 11th passage of the SCH-2 culture to $10^{5.5}$, rose considerably at the 21st and 26th passages at the same time as the morphological features of the cells were restored, and was practically identical with the titer of the virus on the SCH cells.

TABLE 4. Sensitivity of SCH and SCH-2 Cultures to Poliomyelitis Virus

Culture	Passage	Titer of virus in 1 ml
SCH	138th	$10^{7.5}$
	149th	$10^{6.5}$
	154th	$10^{6.8}$
SCH-2	11th	$10^{5.5}$
	21st	$10^{6.0}$
	26th	$10^{7.0}$

It is interesting to examine the data relating to the morphological and biological stability of the SCH culture in connection with some general ideas concerning the biological properties of transplantable cell lines. At present it may be accepted as firmly established that transplantable cell lines, despite certain individual peculiarities, possess and extraordinary resemblance as regards their morphology, their intensity of multiplication, and their metabolism. In all these features the cells of transplantable line differ sharply from the cells of primary cultures, and form a group with sharply defined properties, by means of which the most cursory examination can allocate a particular culture to the transplantable group. The principal cause of this distinction of the transplantable lines is their high adaptation to the conditions of cultivation in vitro. The high adaptation of the cells of transplantable lines is also mentioned by Cornell and co-workers [8]. On account of this adaptation, the cells of the transplantable lines lost many of their original features and acquired many new properties as a result of profound and, in all probability, genetic reorganization.

The problem of malignant change in transplantable cell lines cannot be considered finally solved. The individuality of the cultures of this group, and the stability of the SCH culture mentioned above, are in favor of the constancy of their properties and against the notion of the gradual development of malignant degeneration in the process of cultivation of the tissue.

We must point out that besides stability, all transplantable lines possess the ability to modify their morphology and biological properties in response to changes in the conditions of cultivation. These modifications take many forms, and affect both the size and shape of individual cells as well as their combination with each other (the time of formation of a continuous sheet of cells), which is determined by their intensity of multiplication. The biological properties of the culture may also vary within wide limits. For instance, the metabolic activity of the HeLa cells, determined by the concentration of hydrogen ions in the medium, changes sharply after a few days of cultivation, from one passage to another. The use of a new culture medium leads to a considerable change in the sensitivity of the cells to poliomyelities virus [6].

The conclusion that the properties of the cells of transplantable lines are stable when the conditions of cultivation are constant is not only of theoretical interest, but also of practical importance, because it means that a given cell strain can be used for a long time without the risk of a modification of its properties.

SUMMARY

An inquiry was made into the size of the nuclei and the mitotic activity of the transplantable cellular lines HeLa, Hep-2 and cinomolgus heart (SCH) during prolonged cultivation (for about 40 months). Each of the cultures studied was characterized by a definite size of the nuclei. For 40 months the SCH culture was characterized by constant morphology, degree of mitotic activity and sensitivity to the action of poliomyelities virus. These data are in favor of a marked stability of the transplantable cellular line. However, in individual cases considerable changes were seen in the morphology and mitotic activity of the same transplantable cellular line, depending on the conditions of cultivations.

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