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CELLS WITH FRAGMENTED NUCLEI IN ASCITES HEPATOMA 22A AND THEIR ROLE IN PROLIFERATION

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UDC 616.36-006-018.1-092.9

During aging of an ascites hepatoma 22A (AH22A) the number of cells with fragmented nuclei (especially multilobate) increases: in an AH22A aged 6 days they numbered $15 \pm 9.3^0/_{000}$, in a tumor aged 14 days $196 \pm 53^0/_{00}$, and in a delayed tumor aged 18 days $453 \pm 51^0/_{00}$. The main method of formation of fragmented nuclei is by amitosis. Approximately 150 and $170^0/_{00}$ of cells with fragmented nuclei in a 14- and 18-day old AH22A were in the reversible resting R_1 stage (or in a very protracted G_1-period, extending over 4 days), whereas the remaining 50 and $230^0/_{00}$ of cells respectively had left the mitotic cycle irreversibly and were evidently undergoing involution, which takes place more rapidly during passage-stimulated division.

KEY WORDS: ascites hepatoma; fragmented nuclei; mitotic cycle.

As a result of a few investigations, evidence has been obtained that during growth of certain ascites tumors the number of cells with fragmented nuclei (FN) in them increases, [2, 3]. Two types of fragmentation have been observed: multinuclear and multilobate (nuclei with deep invaginations). Many of these cells are viable: during stimulation of division by passage, DNA synthesis takes place in the FN [2] and these cells pass through normal mitosis [3]. The authors cited above suggested that FN are formed in ascites tumor cells as a result of disturbances of mitosis. During a long study of ascites hepatoma 22A (AH22A) the present writers

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 10, pp. 445-447, October, 1979. Original article submitted February 9, 1979.

0007-4888/79/8810-1166$07.50

also discovered cells with FN in the late stages of development of the tumors, but a different method of their formation was postulated.

The object of this investigation was to determine the relative number of cells in FN in AH22A of different ages, the mechanism of their formation, and the extent to which cells with FN participate in growth of the tumor.

EXPERIMENTAL METHOD

The work was done on an AH22A maintained by passages of 0.25 cm^3 ascites fluid (about 40×10^6 cells) at intervals of two weeks through C3HA mice weighing 20-24 g. The longest period of survival of the animals was 14-15 days, and in the last few days they were in the terminal stage. A delayed (18 days) AH22A was obtained by the method described previously [1]. The frequency of multipolar anaphases and telophases and of phases of reconstruction was determined by counting 50 (25 in the case of the 12-day AH22A) of these late stages of mitosis in each animal (3 or 4 at each time). The number of cells with FN, the number of small cells, and the index of labeled nuclei (ILN) were determined by counting 1000 cells in each animal (3 or 4 cells at each time) and expressed in promille. Thymidine-^3H was injected intraperitoneally in a dose of 0.3 $\mu Ci/g$ body weight into animals with terminal and delayed AH22A and in a dose of 0.15 $\mu Ci/g$ during the first few days after passage. The specimens were coated with type N liquid emulsion and exposed for 10-20 days at 4°C.

EXPERIMENTAL RESULTS

In the course of growth of the AH22A heterogeneity of its cells appeared with respect to the shape of the nucleus: cells with FN (chiefly multilobate) appeared. In a 6-day tumor there were $15 \pm 9.3‰$ cells with FN (mean for 4 animals), and in a 14-day old tumor there were $196 \pm 53‰$ (mean for 8 animals). To discover how FN arise, the frequency of multipolar anaphases and of phases of reconstruction of the nucleus was determined in AH22A at the early (2 and 4 days) and late (10 and 12 days) stages of development. In young and old tumors multipolar mitoses (in the overwhelming majority, with three poles) were found with practically equal frequencies: 2 ± 1.4 and $2.25 \pm 1\%$ respectively (mean for 3 or 4 animals at each time), and all stages of reconstruction, moreover, were accompanied by clearly defined cytotomy. The number of cells with FN increased particularly in the delayed (18 days) AH22A to $453 \pm 51‰$ (mean for four animals), although during the four days of delay mitotic activity in the tumor remained at a very low level [1].

The facts described above indicate that the principal method of formation of cells with FN in AH22A is by amitosis (fragmentation and formation of constriction rings), and division by mitosis (multipolar mitoses without cytotomy) was evidently completely absent. In the late stages of tumor development the proliferative pool is known to be considerably reduced, i.e., the number of cells left in the mitotic cycle falls. Determination of the relative number of labeled and unlabeled cells with simple and fragmented nuclei in the delayed AH22A (after administration of thymidine-^3H for 4 days at 12-hourly intervals) showed that most cells with FN were unlabeled (368 ± 8.2 unlabeled cells with simple nuclei, 401 ± 48.1 with FN; the number of labeled cells was 168 ± 21.3 and 47 ± 1.0 respectively). This is evidence that they had left the mitotic cycle in the resting state. During stimulation of divisions by inoculation of a new host with terminal and delayed AH22A, during emergence of the tumor many of the cells with FN returned to the mitotic cycle. Data showing incorporation of label by different tumor cells in the early stages after passage are given in Table 1. They show that cells with FN switched to DNA synthesis simultaneously with cells having a simple type of nucleus; the total number of cells with FN incorporating label 15 h after passage was $150\text{-}160‰$, but the number of unlabeled cells with FN was sharply reduced (as was shown previously [1], by this time most cells have switched to DNA synthesis). Consequently, in the terminal and delayed AH22A approximately $150‰$ of cells with FN were in the reversible resting R_1 stage (or in a very protracted G_1-period, the duration of which exceeds 4 days). The remainder of the cells with FN (numbering about 50 and $230‰$ respectively in terminal and delayed ascites hepatomas evidently left the mitotic cycle irreversibly and were undergoing involution: the cells were sharply reduced in size and their nuclei were shrunken and pycnotic. During the first 15 h after passage a gradual increase in the number of these degenerating small cells could be observed (Table 1). The process of involution of the cells (among which there were probably many cells in FN), which commences in the terminal stage, takes place more rapidly during stimulation of cell division. This was clear from the following experiment: 0.25 cm^3 of ascites fluid was taken from each of two mice with terminal AH22A and injected into intact mice. The donors and recipients were killed 18 h later and the relative number of small (degenerating) cells was determined. In the 14-day AH22A there were $3.5 \pm 1.5‰$, in the 14 day +18 h tumor there were $44.0 \pm 7.8‰$, and 18 h after passage of the 14-day tumor there were 155.0 ± 7.0. Clearly during stimulation of division the number of small cells was much greater than in the

TABLE 1. Changes in ILN (in $^0/_{00}$) after Passage of 14-day and 18-day AH22A

Time after passage and time of injection of thymidine-^{3}H, 14-day AH22A	Labeled		Unlabeled		
	simple nuclei	FN	simple nuclei	FN	small cells
0	64	4	726	201	5
3	99±10,3	17±3,6	656±22,4	197±24,8	31±3,6
6	177±25,2	41±13,6	586±51,0	173±27,5	23±11,4
9	304±4,3	80±22,2	461±32,4	108±20,7	47±14,6
12	432±15,6	107±10,6	316±16,3	76±14,8	69±4,0
15	408±10,4	147±21,6	266±14,0	90±21,2	89±31,7
18-day AH22A					
0	20	4	523	393	60
3	40±10,1	4±2,1	550±8,4	264±27,3	142±20,3
6	107±26,7	18±7,2	502±103,0	254±87,8	119±37,4
9	285±13,3	119±15,0	321±13,9	186±33,8	89±21,2
12	365±43,7	130±27,1	194±20,3	56±18,1	255±35,4
15	345±47,3	169±4,0	213±49,5	39±9,5	234±55,4

terminal AH22A (in this case the donor mouse was killed on the 15th day of tumor development). The total number of cells with nucleus of the proper shape (labeled and unlabeled) remained roughly constant during the first 12-15 h after passage, i.e., the sudden change in the condition after passage did not lead to normalization (by amitosis) of the shape of FN. Mitotic activity was still low at this time [1]. The number of cells with FN fell sharply towards the end of the first day after passage, after establishment of intensive proliferation [1]: in the 24-h AH22A (after passage of the terminal AH22A) there were 55±10.1$^0/_{00}$ cells with FN (mean for three animals). The decrease in the number of cells with FN during growth of AH22A evidently took place by mitotic cell division, as the result of which the shape of the nuclei returned to normal. Whereas the mode of formation of FN, the relationship of cells with FN to the mitotic cycle, and their behavior during stimulation of division are sufficiently evident, only suggestions can be put forward regarding the causes of their appearance in the late stages of tumor development. The change in shape of the nuclei is perhaps a morphological expression of changes in cell metabolism, connected both with the slowing or cessation of synthesis required in preparation for division and with changes in the environment of the cell (increasing hypoxia, for example).

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