100. Epstein-Barr Virus and Human Chromosomes

Growth Characteristics in Culture and Nude Mice of the Virus-Positive, Chromosome 14-Containing Human-Mouse Hybrid Cells^{†)}

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Introduction. The Epstein-Barr virus (EBV) can readily convert normal human lymphocytes into lymphoblasts with infinite replicative capabilities, and is highly suspected of having a causal relationship to human tumors such as Burkitt lymphoma and nasopharyngeal carcinoma (cf. Epstein and Achong, 1979a).

Our previous paper (Yamamoto *et al.*, 1978) has indicated that the resident EBV genome is to be closely associated with human chromosome 14 and the presence of this particular chromosome alone seems sufficient for the maintenance and the expression of the EBV genetic information in the virus-transformed lymphocytes. It is also documented that changes involving chromosome 14 are highly remarkable in Burkitt tumors (Manolov and Manolova 1972; Jarvis *et al.*, 1974; Zech *et al.*, 1976) and in many other lymphoreticular malignancies (cf. Klein, 1979; Epstein and Achong, 1979b).

The present study was then undertaken to inquire into whether or not the close association of EBV genome with chromosome 14 could be related to the mechanism of EBV-induced oncogenesis.

Materials and methods. The human FV5/mouse MCB2 hybrid clones and subclones (Yamamoto *et al.*, 1978) have previously been isolated and characterized in relation to the presence of EBV genome, the expression of the virus-determined nuclear antigen (EBNA) (Reedman and Klein, 1973), and the presence of human chromosomes (Table I). The cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and 10% tryptose phos-

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Table I. EBV genome, EBNA, and human chromosomes in FV5/MCB2 hybrid clones and subclones

FV5/MCB2 clones and subclones	EBV genome*	EBNA**	Human chromosomes
7	<1	0	2, 3
10	<1	0	3, 4, 5, 9, 11
12	<1	0	13, 21, 22
16	<1	0	1, 2, 3, 15, 17, 19, 20
17	1-2	95-100	2, 3, 7, 14, 15, 17
18	1-2	95-100	1, 2, 3, 7, 14, 15, 17, 21
19	1-2	95-100	14
19-15	1-2	95-100	14
19-18	1-2	95-100	14

* Number of EBV genome equivalents per cell. ** Percentage of EBNA-positive cells.

phate broth.

The growth characteristics in culture of the hybrid cells were studied referring to the colony-forming efficiency in semisolid agar medium (Mizuno *et al.*, 1976), the maximum saturation density in a given culture dish (50 mm), and the doubling time in the logarithmic growth phase. The neoplastic growth of the hybrid cells was assessed in BALB/c nude mice (nu/nu), when inoculated subcutaneously.

Results. *High growth potential in culture of EBV-positive, chromosome 14-containing FV5/MCB2 hybrid cells.* The FV5/MCB2 hybrid clones and subclones containing EBV DNA, EBNA, and human chromosome 14 (positive clones 17, 18, 19 and positive subclones 19-15, 19-18) were compared with the hybrid clones without containing EBV markers and chromosome 14, but containing some

Table II. Growth in culture of FV5/MCB2 hybrid clones and subclones

FV5/MCB2	Positive* ¹					Negative* ²			
	17	18	19	19-15	19-18	7	10	12	16
Colony formation* ³	16.8	33.0	51.2	49.3	50.1 (40.1)	13.0	11.0	6.0	2.8 (8.2)
Saturation density* ⁴	6.0	8.2	11.8	7.3	9.7 (8.6)	8.0	3.8	3.6	3.8 (4.8)
Doubling time* ⁵	18.0	22.8	15.0	16.8	16.5 (17.8)	32.4	29.2	28.8	20.4 (27.7)

*¹ FV5/MCB2 clones and subclones containing EBV DNA, EBNA, and chromosome 14. *² FV5/MCB2 clones without containing EBV DNA, EBNA, and chromosome 14, but containing some other human chromosomes. *³ Percentage of colony formation in semisolid agar medium. Numbers in parentheses are average values of 5 positive hybrid cultures and 4 negative counterparts, respectively. *⁴ Maximum number of cells ($\times 10^6$) grown in a 50-mm dish. Numbers in parentheses are average values of 5 positive hybrid cultures and 4 negative counterparts, respectively. *⁵ Doubling time of cells in the logarithmic growth phase. Numbers in parentheses are average values of 5 positive hybrid cultures and 4 negative counterparts, respectively.

other human chromosomes (negative clones 7, 10, 12, 16), with the attention to the mode of growth in culture (Table II). First, when inoculated into semisolid agar medium, the colony-forming efficiency of the 5 positive hybrid cultures was on an average of 40.1%, while the average value was 8.2% in the 4 negative cultures. Second, the average maximum number of cells per dish from the 5 positive cultures was 8.6×10^6 , and 4.8×10^6 in the 4 negative cultures. Third, the average cell doubling time of the 5 positive hybrid cultures was 18.2 h, while the average time in the 4 negative counterparts was 27.7 h.

Neoplastic growth in nude mice of EBV-positive, chromosome 14-containing FV5/MCB2 hybrid cells. Subsequently the above-mentioned 5 EBV DNA-positive, EBNA-expressing, chromosome 14-containing hybrid clones and subclones were assessed for their neoplastic growth capacity in nude mice in parallel condition to the 4 negative clones (Table III). Tumors grew very rapidly by inoculation with a much smaller number of the positive hybrid cells, in comparison with the negative cells.

Table III. Growth in nude mice of FV5/MCB2 hybrid clones and subclones

FV5/MCB2	Positive* ¹					Negative* ²			
	17	18	19	19-15	19-18	7	10	12	16
10 ³ * ³	0/4* ⁴	0/4	2/4	3/4	3/4	0/4	0/4	0/4	0/4
10 ⁴	3/4	4/4	4/4	4/4	4/4	0/4	0/4	0/4	0/4
10 ⁵	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4

*¹ FV5/MCB2 clones and subclones containing EBV DNA, EBNA, and chromosome 14. *² FV5/MCB2 clones without containing EBV DNA, EBNA, and chromosome 14, but containing some other human chromosomes. *³ Number of cells inoculated per mouse. *⁴ Number of mice developing tumors/number of mice inoculated.

Discussion. Previously Yamamoto *et al.* (1978) have assigned the resident EBV genome onto chromosome 14. Also, it has been shown that changes in chromosome 14 are very remarkable in B-cell neoplasias (cf. Klein, 1979; Epstein and Achong, 1979b) including Burkitt tumors (Manolov and Manolova, 1972; Jarvis *et al.*, 1974; Zech *et al.*, 1976). On the above bases the suggestion was possibly made that the close association of EBV genome with chromosome 14 could possibly be related to the mechanism of EBV-induced oncogenesis in human B-lymphocytes (Yamamoto *et al.*, 1978).

Then, data presented in this paper seem to indicate that the resident EBV genome-positive, EBNA-expressing (i.e. EBNA antigen gene-functioning), human chromosome 14-containing hybrid clones and subclones have a much higher neoplastic growth potential

as compared with hybrid clones without containing EBV markers and chromosome 14. Growth in culture and in nude mice of the latter negative clones probably reflects growth of the background MCB2 tumor cells which were used as mouse parental cells in our hybrid-line study (data not given).

Generally speaking, a basic mechanism of EBV-induced oncogenesis in human lymphocytes is possibly explicable on previously proposed views (Yamamoto *et al.*, 1977; Osato, 1979). Upon infection of lymphocytes with EBV, the virus genome, particularly EBNA antigen gene, will be integrated in a specific site of chromosome 14, which may probably be responsible for the growth regulation of B-lymphocytes. Then the integrated EBV genome will impair such regulatory function, and then it results in oncogenesis in EBV-infected lymphocytes. Other mechanisms for the occurrence of Burkitt tumors have also been proposed recently in connection with the possible function of chromosome 14 (Klein, 1979; Epstein and Achong, 1979b).

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