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Cyto-Molecular Comparison Between Human Primary Cancer and its Derived Cancer Cell Line

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=Abstract=

In order to evaluate which cyto-molecular genetic evolutionary patterns take place during the in vitro establishment of permant squamous cell carcinoma cell line of urinary bladder, cyto-molecular genetic follow up was performed on primary culture for two weeks and on cancer cell line after continued culture for three years in vitro. Near-diploid cells present on primary culture and near-hypertriploid or hypotetraploid cells, in contrast, present on cancer cell line. Chromosomal gains or losses are random from primary cancer to cancer cell line. There are two kinds of structural cytogenetic abnormalities through progress in culture time. One is maintaining abnormal clone from original cancer to derived cancer cell line. Others are cytogenetic alteration; In creasing and decreasing abnormal clones are co-existed in both group. Activations of oncogenes are different from primary cancer to cancer cell line. It is concluded that the eventual chromosomal compositions of squamous cell carcinoma of bladder derived cell line are the production of cyto-molecular evolutions from original tumor.

Key Words : Cyto-molecular, Abnormalities, Hypotetraploid, Urinary bladder

Introduction

The utilization of cultured cell lines in experimental studies is dependent on the propagated cells remaining representative of the tumors from which they were derived. A variety of phenotypic markers has been followed from the original tumors in many instances of established human neoplasms such as leukemia (Venaut et al., 1978), lymphoma (Beinheim et al., 1981) and melanoma (Becher et al., 1983; Trent et al., 1984) through propagation in vitro.

Permanent cultured cell lines established from human neoplasms are useful in a variety of studies because large numbers of viable cells can be repeatedly obtained. These characteristics apply this source to useful for immunization in monoclonal antibody production, screening of monoclonal antibody, and in vitro and in vivo therapeutic testing (Bigner et al., 1987). It is essential that the cultured cell lines retain as closely as possible genetic characteristics of the tumor which they were originally derived. The data obtained so far from squamous tumors (Heim et al., 1988a, 1989; Jin et al., 1988, 1989, 1990a,b,; Mertens et al., 1989) give the impression of being fundamentally different from those of bone marrow and mesenchymal neoplasms. In these epithelial tumors, multiple cytogenetically unrelated clones seem to be the rule rather than the exception.

Purpose of this study is that does cancer cell line reserve its original genetic material from primary cancer and does cancer cell line be able to be utilized for substitution of its primary cancer in epithelial origin carcinoma?. We carried out the cyto-molecular studies from squamous carcinoma of urinary bladder for this question.

Materials and Methods

A. Cytogenetic studies

The tumor was a moderate and invasive squamous carcinoma of urinary bladder by histopathological diagnosis (Fig. 1, 2). The fresh tumor sample was minced with scissors, disaggregated overnight in a collagenase solution, and plated flaskettes in F10 medium supplemented with 10% fetal calf serum, glutamine and antibiotics. After two weeks and three years in culture, the cultured cells were harvested by colcemid exposure followed by hypotonic treatment in 0.075M KCl and fixation in methanol/acetic acid. The slides were incubated overnight at 60°C. A total of 48 metaphases in primary cancer cells and 114 metaphases in cancer cell lines were counted and karyotyped.

B. Southern blot hybridization

DNA was extracted according to a Modified Blin method, extracted DNA was digested with Bam HI, EcoRI, Hind III and Xbal (Biolabs, New England). Ten micrograms of digested DNA was then size fractionated by electrophoresis on a 0.8% agarose gel and transferred to a nitrocellulose membrane (Amersham-USA). Membranes were dried and prehybridized 16hrs at 42°C in 50% formamide, 5XSSC, 50mM sodium phosphate (pH 6.8), 0.1% SDS, 0.1mg/ml sonicated sperm DNA and 5xDenhardt's reagent (1% BSA, 1% Ficoll-type 400, 1% polyvinyl pyrrolidone). The blot was subsequently hybridized in the same mixture at 42°C for 24hrs to 50ng DNA probe which had been ^{32}P labeled by nick translated oncogenes. The filters were washed in 1XSSC and 0.5% SDS at 42°C for three times, 10 minutes each, and were autoradiographed.

Results

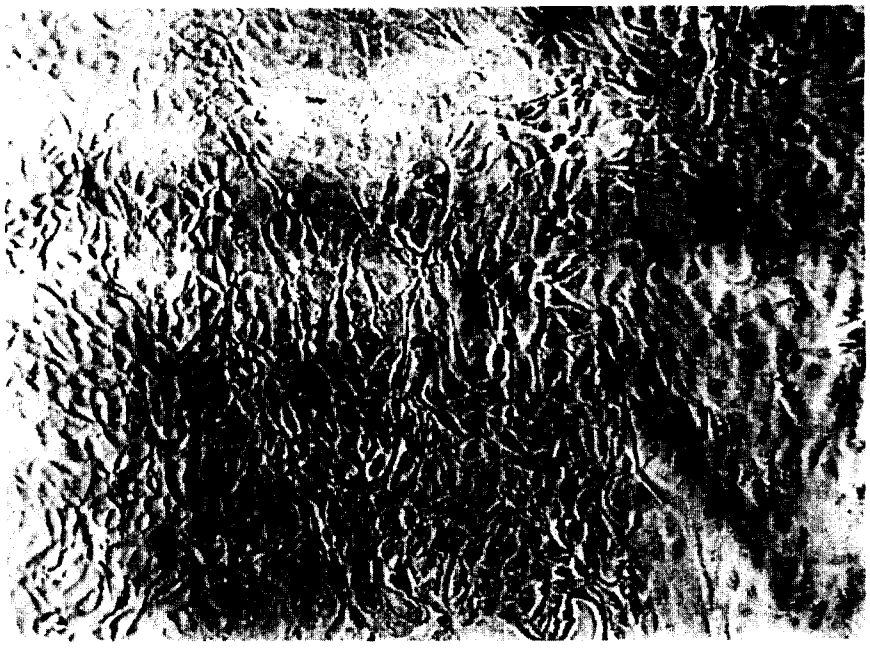

Fig. 1. Histological section of the primary squamous cell carcinoma of urinary bladder (inverted microscop, X100).

Fig. 2. Established cell line, of squamous carcinoma of urinary bladder which showed keratin peal (H&E, X200).

A. Karyotypes in primary cancer

a) numerical abnormality

A total of 48 metaphases were analyzed. All cells were pseudodiploid and the modal karyotype was 52-56, XY (Table 1).

Chromosomal gains were frequently found in chromosome 1, 3, 20, 21, 19, 16, 9, 13, 22 and 5 in order. However, chromosomal losses were also found in chromosome 8, 6, 10, 15 and 17 in order as like less frequent on chromosome gain (Table 2).

Table 1. Distribution of chromosome number in the primary squamous cell carcinoma of the urinary bladder according to ploid state

Ploid state (Chromosome range)	Cell number	(Percentage)
Hypodiploid (36 - 45)	4	(8.33)
Diploid (46)	0	(0.00)
Hyperdiploid (47 - 57)	34	(70.83)
Hypotriploid (58 - 68)	5	(10.42)
Over triploid (over 69)	5	(10.42)
Total	48	(100.0)

Table 2. Analysis of numerically chromosomal changes in the primary cancer cell

Chromosome number	Chromosome gains	Chromosome losses	Percentage#	Percentile number
1	23	0	+ 109.5	71.2
2	11	4	+ 33.3	53.7
3	22	0	+ 104.8	70.1
4	9	5	+ 19.1	50.4
5	10	1	+ 42.9	55.9
6	1	13	- 57.1	32.9
7	5	4	+ 4.8	47.1
8	2	16	- 66.7	30.7
9	13	1	+ 57.1	59.1
10	1	11	- 47.6	35.1
11	0	5	- 23.8	40.5
12	8	7	+ 4.8	47.1
13	13	3	+ 47.6	57.0
14	5	5	0	46.0
15	7	10	- 14.3	42.7
16	14	3	+ 52.4	58.0
17	6	10	- 19.1	41.6
18	7	1	+ 28.6	52.6
19	17	1	+ 76.2	63.5
20	22	0	+ 104.8	70.1
21	19	0	+ 90.5	66.8
22	12	4	+ 38.1	54.8
X	6	0	+ 28.6	52.6
Y	1	2	- 4.8	44.9

Total cell number: 21 (within modal chromosome number)
Standard chromosome number: 46 (0 percent)
Percentage#: sum of chromosome gains and losses / 21 X 100

b) Structural abnormality

The following abnormal tumor karyotype in clone emerged: del(1)(q31), der(2)t(2;?)(qter;?), del(3)(p21), iso(13q), iso(15q), iso(18q), marker chromosome and ring chromosome. The various abnormalities were found in these proportions; der(2) in 15 cells, del(3) in 11 cells, M1 and ring chromosome in 18 cells of the 48 karyotyped metaphases. Besides, Nonclonally occuring structural rearrangments are given in Table 3.

Table 3. Structural abnormalities of chromosome analysis in the primary cancer cells

Clonal abnormality	Cell No.	(%)	Nonclonal abnormality	Cell No.	(%)
del (1)(q31)	4	(18.2)	del (1)(p31)	2	(9.1)
der (2)t(2;?)(qter;?)			del (1)(q21)	1	(4.5)
	15	(68.2)	3q+	1	(4.5)
del (3)(p21)	11	(50.0)	del (5)(p14)	1	(4.5)
iso (13q)	3	(13.6)	del (6)(q21)	1	(4.5)
iso (15q)	7	(31.8)	7p+	1	(4.5)
iso (18q)	3	(13.6)	del (8)(p21)	1	(4.5)
M 1	18	(81.8)	8p+	2	(9.1)
M 2	9	(40.9)	8q+	1	(4.5)
M 3	5	(22.7)	9p+	2	(9.1)
M 4	3	(13.6)	del (10)(p13)	1	(4.5)
M 5	5	(22.7)	10p+	1	(4.5)
ring chromosome	18	(81.8)	iso (14q)	2	(9.1)
			t (13;15)(q;q)	1	(4.5)
			t (14;15)(q;q)	1	(4.5)
			iso (21q)	1	(4.5)
			M 6	2	(4.5)
			M 7	1	(4.5)
			M 8	2	(4.5)
			M 9	1	(4.5)
			M10	1	(4.5)

B. Karyotype in cancer cell line

a) numerical abnormality

A total of 114 metaphases were analyzed. Although no distinct stem karyotype was seen, the modal karyotype was 81-84, XXY (Table 4).

Table 4. Distribution of chromosome number in derived cancer cell lineaccording to ploid state

Ploid state (Chromosome range)	Cell number	(Percentage)
Hypodiploid (36 - 45)	1	(0.88)
Diploid (46)	0	(0.00)
Hyperdiploid (47 - 57)	5	(4.39)
Hypotriploid (58 - 68)	15	(13.16)
Triploid (69)	2	(1.75)
Hypertriploid (70 - 81)	33	(28.95)
Hypotetraploid (82 - 91)	43	(37.72)
Tetraploid (92)	5	(4.39)
Over tetraploid (over 92)	10	(8.77)
Total	114	

Chromosomal gains were dominantly found in chromosome 1 in 47 cells of the 114 karyotyped. Chromosomal gains were also found in chromosome 20, 3, 16, X, 5, 19 and 7 in order. However, chromosomal losses found in chromosome 6, 8, 15, 13, 10, 14, 22 and 4 in order as like more frequent as chromosomal gains (Table 5).

Table 5. Analysis of numerically chromosomal changes in derived cancer cell line

Chromosome number	Chromosome gains	Chromosome losses	Percentage#	Percentile number
1	47	6	+ 132.3	122.4
2	5	12	- 22.6	86.8
3	21	11	+ 32.3	99.4
4	4	29	- 80.6	73.5
5	12	10	+ 6.5	93.5
6	0	88	- 283.9	126.7
7	11	11	0	92.0
8	0	70	- 225.8	40.0
9	7	25	- 58.1	78.6
10	2	42	- 103.2	68.3
11	7	16	- 29.0	85.3
12	0	20	- 64.5	77.2
13	3	43	- 129.0	62.3
14	1	38	- 119.4	64.5
15	0	65	- 209.7	43.8
16	20	16	+ 12.9	95.0
17	6	23	- 54.8	79.4
18	6	21	- 48.4	80.9
19	12	17	- 16.1	88.3
20	29	7	+ 71.0	108.3
21	7	14	- 22.6	46.8
22	4	35	- 100.0	69.0
X	17	3	+ 45.2	102.4
Y	0	18	- 58.1	78.7

Total cell number: 31 (within modal chromosome number)
Standard chromosome number: 92 (0 percent)
Percentage#: sum of chromosome gains and losses / 31 X 100

b) Structural abnormality

The following abnormal tumor karyotype in clone emerged: del(1)(p31), der(2)t(2;?)(qter;?), del(3)(p21) and marker chromosome were appeared in over 50%. Various nonclonally structural rearrangements are given in Table 6.

Table 6. Structural abnormalities of the chromosome analysis in derived cancer cell line

Clonal anomalies	Cell No.	(%)	Nonclonal anomalies	Cell No.	(%)
del (1)(p22)	4	(12.9)	del (1)(q21)	1	(3.2)
del (1)(p31)	20	(64.5)	3q+	1	(3.2)
del (1)(q31)	18	(58.1)	4q+	1	(3.2)
del (1)(q41)	7	(22.6)	del (6)(q25)	1	(3.2)
der (2)t(2;?)(qter;?)	23	(74.2)	del (8)(p21)	2	(6.5)
del (3)(p21)	21	(76.7)	8q+	1	(3.2)
del (3)(q21)	3	(9.7)	del (10)(p13)	1	(3.2)
5q+	3	(9.7)	10p+	2	(6.5)
iso (13q)	5	(16.1)	13p+	1	(3.2)
iso (14q)	4	(12.9)	t (13;14)(q;q)	1	(3.2)
iso (15q)	4	(12.9)	t (14;15)(q;q)	1	(3.2)
t (13;15)(q;q)	5	(16.1)	iso (17q)	1	(3.2)
iso (21q)	4	(12.9)	iso (22q)	2	(6.5)
M 1	8	(25.8)	M 6 - M 30	1	(3.2)
M 2	20	(32.3)			
M 3	22	(71.0)			
M 4	8	(25.8)			
M 5	3	(9.7)			
ring chromosome	4	(12.9)			

c) Southern blot analysis

Two major xbal fragments (18.0 and 10.4kb in size) of H-ras were detected in control and primary cancer. In contrast, deletion of 10.4kb band of H-ras were detected in cancer cell line. K-ras amplification was observed in cancer cell line contrast to primary cancer Rearrangement of Erb B2 was observed in both the primary cancer and cancer cell line. However status of rearrangements was different in both group (Fig. 3. 4. 5). Other oncogenes (N-ras, c-myc, c-fos, p53, v-sis) were nonspecific in this study.

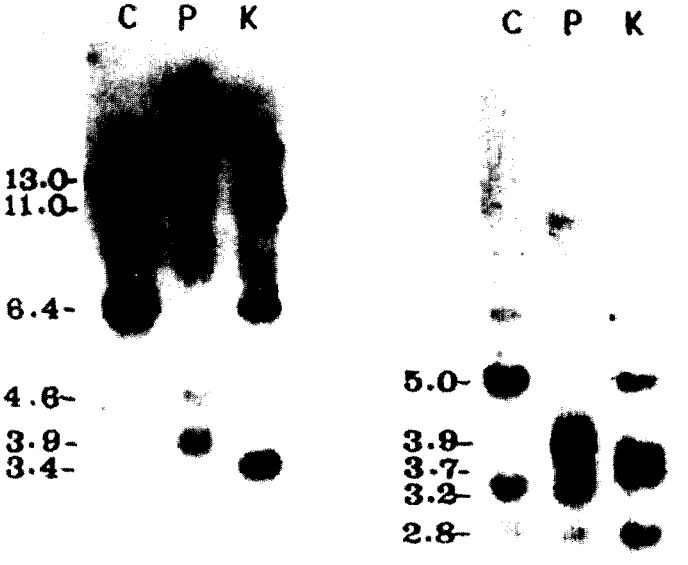

Fig. 3. Southern blot analysis of Erb B2 gene

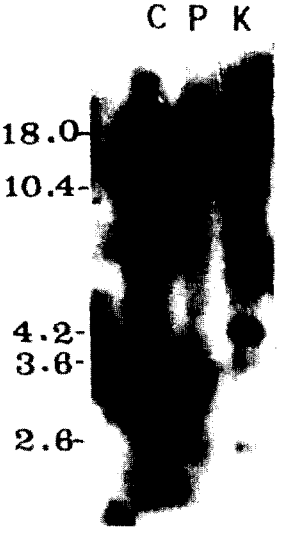

Fig. 4. Southern blot analysis of H-ras gene

Table 7. Reservations and alternations of structural cytogenetic analysis in the process from primary bladder cancer to derived cancer cell line

Cytogenetic reservation	Primary cancer	Cancer cell line
Maintaining abnormal clone		
der(2) t(2;?) (qter;?)	68.2%	74.2%
del(3) (p21)	50.0%	76.7%
iso(13q)	13.6%	16.1%
iso(15q)	31.8%	12.9%
Cytogenetic alternation		
Increasing abnormal clone		
del(1) (q31)	18.2%	58.1%
Decreasing abnormal clone		
ring chromosome	81.8%	12.9%
Cells from nonclonal to clonal		
del(1) (p31)	9.1%	64.5%
del(1) (q41)	0.0%	22.6%
t(13;15) (q;q)	4.5%	16.1%
iso(21q)	4.5%	12.9%
Cells from clonal to nonclonal		
iso(18q)	13.6%	0.0%

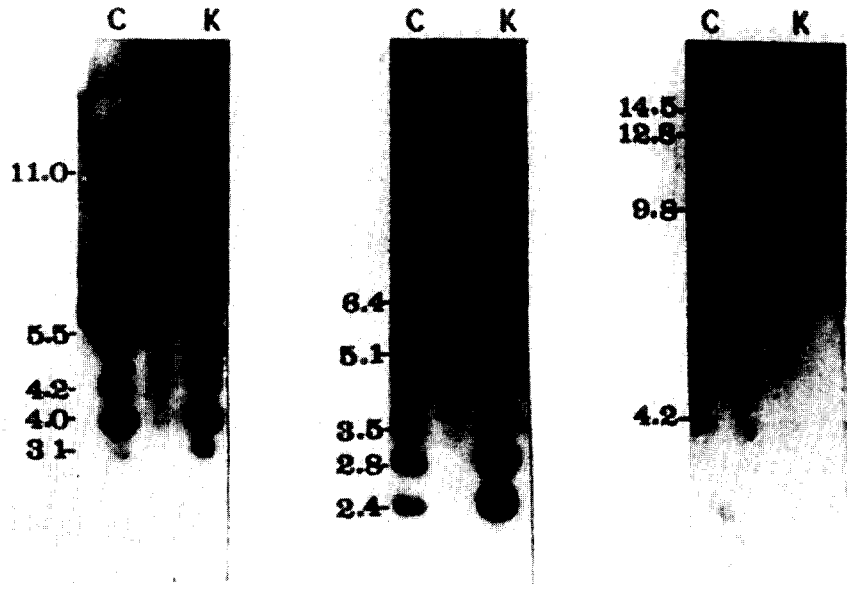

Fig. 5. Southern blot analysis of K-ras gene

Discussion

Karyotypes of established carcinoma derived cell lines are usually near-triploid or near tetraploid with numerous structural rearrangements (Becher et al., 1983; Trent et al., 1984). Human carcinomas in short term culture, in contrast, have mainly near-diploid stem lines. These discrepancy could be explanted that established cell lines may result from selection of a minor popuplation of primary cells by the culture conditions, or serially passaged cell lines may undergo chromosomal progression as they establish in culture. In the present report the karyotypes of the original tumor was hyperdiploid. Cancer cell line after 3 years in culture, in contrast, revealed hyper-triploid or hypo-tetraploid. Chromosomal gain or loss was random in both group. We concluded that the eventual karyotypes of established cell line of squamous carcinoma of bladder arised by in vitro evolution and not by simples selection of pre-existing minor population of polyploid cells. Data from Rey et al.,(1987) and a previous paper from this laboratory (Rey et al., 1983) dealing with the analysis of glioma cell lines at low passage levels and followed cytogenetically over long ranges of passages demonstrated that the duplication of the chromosomal complement of near-diploid cell populations leads to near-tetraploid modal numbers. Then, chromosomal progression through chromosomal losses occured and the establishment of near triploid glioma cell lines was obtained at advanced passages.

It was note worthy that there were two kinds of sturctural cytogenetic abnormalities through progress in culture time. One was maintaining abnormal clone from original cancer to derived cancer cell line. Others

were cytogenetic alterations; Increasing abnormal clones and decreasing abnormal clones were existed together in both primary cancer and cell line (Table 7). It was observed in a few that cells from nonclonal to clonal abnormalities were found, reversely, cell from clonal to nonclonal abnormalities was also found. It was interested that why these different findings could occur during progress of original cancer to cancer cell line. Unfortunately, there was no reports to compare with this. It was pointed out at least that cancer cell lines as materials in experiments were not substituted for the primary cancer in the study of carcinogenesis because of genetic alteration. Also we found interesting data that H-ras and K-ras was activated in progressing cancer and not in primary cancer. Erb B2 was activated in both primary cancer and cancer cell line. However, status of gene rearrangements was different in both groups. Concerning oncogene studies in this report, we could not find any reports to compare with. Activation of oncogenes were not quite the same as in primary cancer as in cancer cell lines. We performed culture on serial passages of gastric carcinoma for more than two years of in vitro propagation, 32 passages were cyto-molecularly studied. It was very interesting that near-diploid was revealed on original cancer and continued to passage 25. Near-diploid and near-tetraploid were coexisted on passage 30. After passage 31, near-tetraploid was lasted permanently. Activation of oncogens were changed by serial passages (unpublished data). These findings concluded that there was possible gene for duplication of chromosome or chromosomal evolution. Bigner et al.,(1987) previously discussed the possibility that chromosomal progression could take place in vivo. If this were the case of our study, then what we have observed would only be the cancer cell line of chromosomal progression in vitro and thus we would not be able to discuss the different passways of the in vitro evolution, but the different in vivo/in vitro phases within the same pattern in cyto-molecular studies. All these results seem to be promising, and futher work on the subject should add interesting data on the in vivo/ in vitro cyto-molecular genetic evolution of malignany that will enable us to better understand the factors involved in their origin and development.

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