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CYTOGENETIC STUDIES OF CELL LINES; MDCK AND VERO

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The established cell lines; MDCK and Vero were studied to find out transformation of chromosome number. The various techniques like G-Banding and C-banding were also studied. The changes in chromosome number were noticed which could be related to number of passages given to cell lines. Present studies observed 76 chromosomes minimum and 81 maximum in MDCK cells, whereas 54 chromosomes minimum and 61 maximum in case of Vero cells. Hence the inconsistency in chromosome number implies that the MDCK cells have been moderately transformed, which could be because of 15 and 21 passages respectively.

The 'cell line' implies for the cells that arise from a primary culture at the time of the first subculture and these cell lines can be subcultured indefinitely *in vitro*. Primary cell lines preserve many of the characteristics of the cells from which they were derived whereas established cell lines often diverge from these. All primary cell lines initially have the normal number of chromosomes whereas established cell lines almost invariably have an unusual number. (Paul, 1975). Although the number of chromosomes may vary greatly in cultured cells but many of the chromosomes of the original tissue retain their morphology even after year of subculture. The major application of cell lines is for viral research and vaccine production. Cell lines can also be prepared from cancer cells, but they are different from those prepared form normal

cells in several ways. Cancer cell lines often grow without attaching to a surface and they proliferate to a much higher density in a culture dish.

The established cell lines, Madian Darby Canine Kidney (MDCK) and Vero, were included for the chromosomal studies. These cell lines were received from the IIL, Hyderabad, are routinely used for cultivation of animal viruses. The chromosomal studies were performed on these cell lines with the objectives to standardize the chromosomal preparation from these two cell lines and find out the transformation of the chromosome numbers which could be related to the number of passages given to the cell lines.

Madian Darby Canine Kidney cell line is known as MDCK cell line. Cells were originally derived from a kidney of an apparently normal adult female Cocker Spaniel canine in1958 by S. H. Madian and N. B. Darby (Madian & Darby, 1958). MDCK cell lines support growth of wide range of animal viruses; VSV (Indiana strain), infectious canine hepatitis, Vaccinia, Coxsackie B5, Adeno and reo viruses, SVEV. These are also being used for over expressing the human insulin receptor (Yeh and Roth, 1994) A variant of this cell line MDCK-L cell) has also been established by Jin et al. (1996) that is uniquely resistant to infection with influenza A and B viruses yielding 3 to 4 orders lower amount of progeny virus compared with MDCK cells. These are the epithelial cells showing the normal karyotypy, 78,XX (2n=78).

Vero epithelial cell line was established in 1962 by Yasumura and Kawakita at Chiba University in Japan. These are derived from

the Kidney of African Green Monkey. These cells are used for virus studies of Polio, Rubella, arbovirusand reovirus. The Vero cells also often utilized in the detection of virotoxins, interrelated toxin produced by some trains of E. Coli that are a key cause of hemorrhagic colitic and hemolytic uremic syndrome in humans. The normal karyotpye is 60,XY or XX (2n = 60).

MATERIALS AND METHODS

The MDCK and Vero cell lines were brought from Hyderabad to Anand in transportation medium. In one experiment these cell lines were subcultured in RPMI-1640 medium from Himedia, supplemented with antibiotics and 13% fetal calf serum. Finally the pH of medium was adjusted to 7.2 by adding sodium bicarbonate. In another experiment these cell lines were subcultured in M-199 from Sigma, supplemented with antibiotics and 13% goat serum. Finally the pH of medium was adjusted to 7.2 by adding sodium bicarbonate. Cultures were incubated at 37 ^{o}C in TC flask for 72 hours. Colchicine @ 2 µg/ml was added to the culture one hour prior to harvesting. In one set of cultures, the Eethedium bromide @ 10 µg/ml was also added two hours prior to harvesting, in addition to Colchicine, for elongation of chromosomes (Hsu et al., 1973), Before transferring cells from TC flask to centrifuge tubes for harvesting, 0.25% trypsin were used for detachment of cells. As described by Patel (1999) the cells were separated by centrifugation at 150 g for 10 minutes followed by hypotonic treatment with 0.56% KCL for 30 & 20 minutes, and fixed in 3:1 ratio of methanol and acetic acid glacial Finally suspension drops on slides were air dried. Routine GTG banding with little modification (Patel et al. 1995) and CBG-banding as described by Patel and Khoda (1998) were performed.

RESULTS AND DISCUSSION

Chromosomal preparations were obtained by using RPMI-1640 with 13% fetal calf serum, M-199 with goat serum and Ethedium bromide. Best result of chromosomal preparation was obtained by using RPMI-1640 with 13% fetal calf serum. However, chromosomal preparation was also found good by using M-199 with 13% goat serum but the chromosomal index was less as compared to RPMI medium. The hypotonic treatment for 20 minutes was found more suitable for cell lines as compared to 30 minute usually given to the lymphocyte culture. Using Ethidium bromide for elongation of chromosomes of MDCK and Vero cells showed adverse effect on the morphology of chromosomes. Giemsa stained chromosome slides of both cell lines; MDCK and Vero, were screened under light microscope. Total 50 metaphase fields were screened for each cell lines to count the chromosome numbers. All metaphase fields were first screened under 20 X objective (200 times magnification) and then 100 X objective (1000 times magnification) under oil emulsion. Beside, Giemsa staining (figure-1 and 3), the conventional G-bandings were observed on the chromosomes of both the cell lines (figure-2 and 4). It was difficult to get C–bands on the chromosomes of MDCK cell line, whereas distinct C- bands were observed on the chromosomes of Vero cell lines (Figure-5).

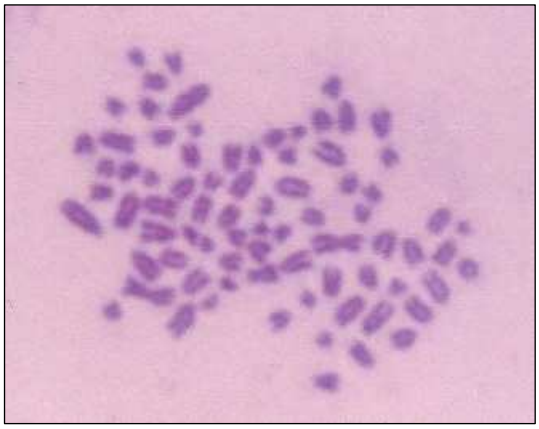

Figure 1: Conventional Giemsa stained metaphase chromosomes of MDCK cell line.

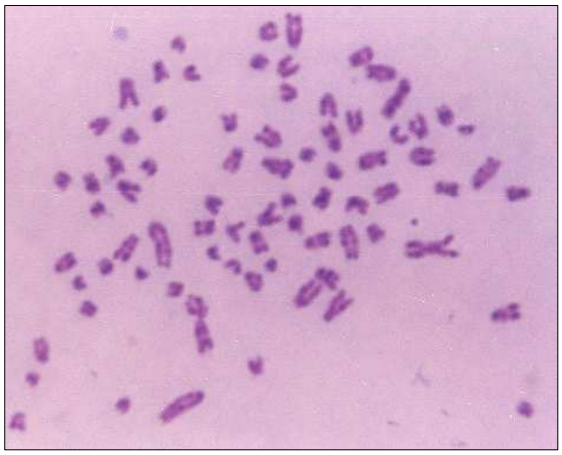

Fig 2: G-banded metaphase chromosomes of MDCK cell line.

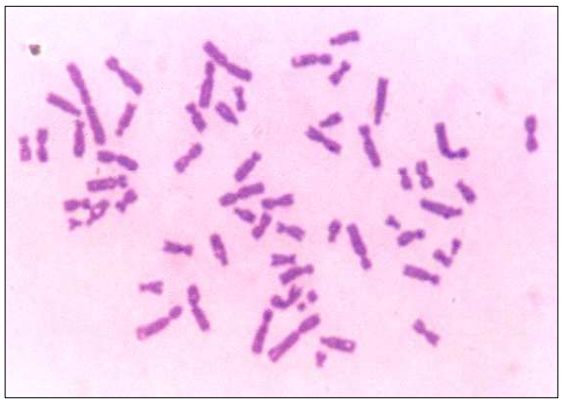

Fig 3: Conventional Giemsa stained metaphase chromosomes of Vero cell line.

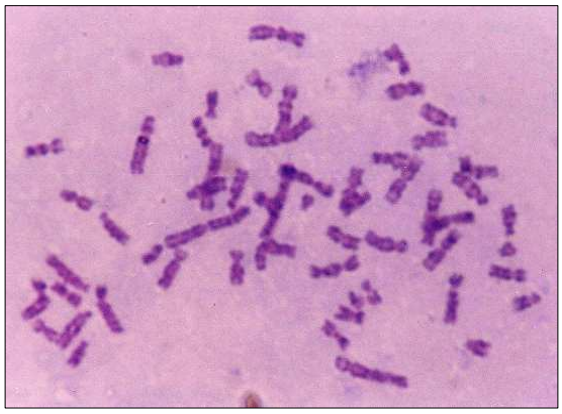

Fig 4: G-banded metaphase chromosomes of Vero cell line.

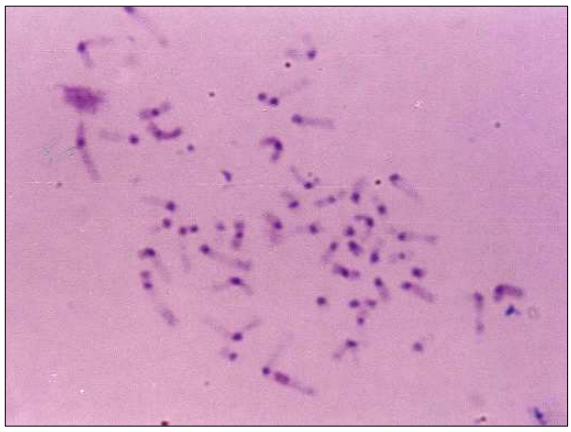

Fig 5: C-banded metaphase chromosomes of Vero cell line.

It has already been emphasized that primary cell lines usually retained their diploid karyotype. Transformed cell lines on the other hand show a great variation in karyotype. Typically, shortly after transformation the incidence of tetraploid cell increases; aneuploid cells then make their appearance and stable established cell lines commonly have an aneuploid karyotype with a wide spread of chromosome numbers (Paul, 1975). The emergence of aneuploidy is apparently due to mitotic non-disjunction during cell division.

In the early stages of an established cell line the numbers of chromosomes may be abnormal but the morphology of individual chromosome remains normal. Subsequently, as a result of chromosomal breaks, fusions and translocations the morphology of chromosomes may become unrecognizable (Paul, 1975). For example, many mouse cell lines contain metacentric chromosomes whereas it is typical of the mouse that all the chromosomes are normally acrocentric.

The diploid chromosomes in canine are 78 (2n = 60). All autosomes are acrocentric. The sex chromosomes (X and Y) are submetacentric. However, MDCK cells exhibited different chromosome number in each field which are tabulated below:

Table 1: Chromosome Number in MDCK cells:

Cells with various Chromosome Number 76	77	78	79	80	81	Total cells scored
No. of cells 2	3	24	5	7	9	50
Percentage 4	6	48	10	14	18	

Above table shows inconsistency in chromosome number in more than 50 cells, which indicates the MDCK 50% cells have been moderately transformed. Because of cell line transformation, correct karyotype of MDCK cell was not possible to prepare. However, present studies observed 76 chromosomes minimum and 81 maximum in a cell. Hence the inconsistency in chromosome number implies that the MDCK cells have been moderately transformed, which could be because of 15 passages.

The diploid chromosomes in green African monkey are 60 (2n = 60). Most of autosomes and sex (X and Y) chromosomes are submetacentric and metacentric. The chromosome numbers in Vero cell are tabulated below;

Table 2: Chromosome Number in Vero cells:

Chromosome Number	54	56	57	58	59	60	61	Total
No. of cells	2	3	5	4	12	22	2	50
Percentage	4	6.25	10	8.3	25	45.8	4	

Above table shows inconsistency in chromosome number in more than 50 cells, which indicates the Vero cells similar to MDCK, have been moderatly transformed. Because of cell line transformation, correct karyotype of MDCK cell was not possible to prepare. However, present studies observed 54 chromosomes minimum and 61 maximum in a cell.. Hence the inconsistency in chromosome number implies that the Vero cells have also been moderately transformed, which could be because of 21 passages.

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