CYTOGENETIC EVIDENCE OF GENE AMPLIFICATION IN NATURAL POPULATIONS OF BLACK RATS FROM RADIOACTIVE AREAS

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ABSTRACT

Bone marrow cells of black rats, *Rattus rattus* from two radioactive areas (Chhatrapur and Quilon) after routine cytological staining exhibited a peculiar type of anomaly i.e. paired dot-like structures or double minutes, which contain nucleic acids. However, they were not found in rats from non-radioactive area (Bhubaneswar). The frequency of double minutes in individuals and in cells was scored. The frequency was higher in Quilon rats than in Chhatrapur rats. The double minutes are regarded as gene-amplifying elements or amplicons, which manufacture extra protein to counter the cell from harmful challenges. The appearance of double minutes in the progenitive tissue of rats is supposed to defend the animal from the background radiation shock.

INTRODUCTION

It has been well-documented that selective gene amplification is often manifested cytogenetically as double minutes (DMs), homogenously staining regions (HSR) or C-bandless chromosomes (CM)[1-3] and these structures are generally held as cellular responses to harmful challenges[4]. In the extensively studied experimental systems it has been proved definitely that DMs and HSR are visible signs of amplification of genes for dihydrofolate-reductase (DHFR), which is the target enzyme for the cytotoxic effect of methotrexate (MTX)[5,6].

MTX-resistant SEWA mouse ascites tumor cell lines also indicate that DMs have originated as selective amplification of the *dhfr* gene, leading to over-production of the enzyme and protection against the killing action of MTX[7]. It seems reasonable that other instances of cellular resistance, associated with the same cytogenetic aberration, should also reflect gene amplification. Such instances include resistance to actinomycin-D (AMD) and vincristine (VCR)[8]. In some of these cases amplification of the gene, or excess of a gene product, has been established; in other cases the evidence for gene amplification is more circumstantial. Furthermore, the 'spontaneously' occurring DMs and/or HSR in tumors have been shown in at least two instances to be associated with amplification of oncogenes, *myc*[9] and *Ki-ras*[10]. From the literature, occurrence of DMs has been detected in a total of 63 cases, 38 of which were human and 25 animal malignancies[11]. The fact that DMs have been restricted to tumors and in certain cases have been maintained or even increased in frequency during serial *in vivo* transplantation[12], indicates that they play a role in the evolution of malignant stem lines towards ever greater efficiency in breaking down the defences of the host.

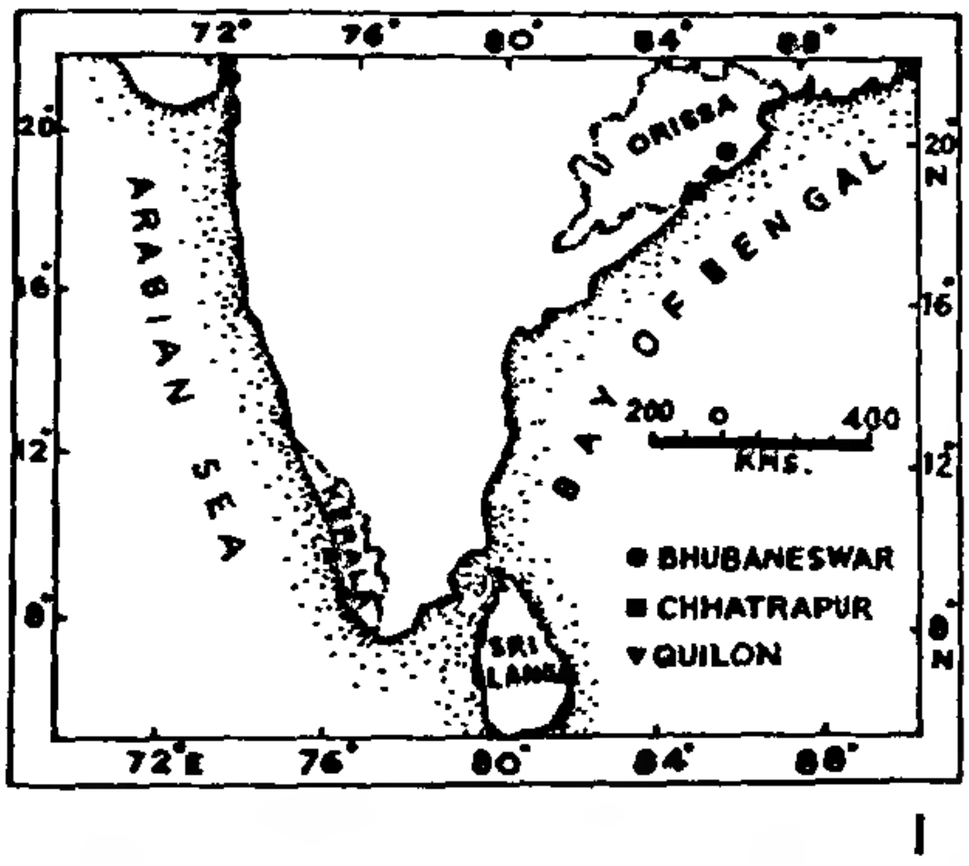
A preliminary report on the occurrence of DMs in the somatic cells of animals from natural populations was made earlier[13]. In the present communication, our results on the occurrence and frequency of DMs in the bone marrow cells (BMC) of black rats, *Rattus rattus*, trapped from high background radioactive areas is reported.

MATERIALS AND METHODS

A total number of 345 black rats, *Rattus rattus* were trapped at random from time to time covering a period of four years (1982–86), irrespective of sex and age from Chavara in Quilon district (Kerala State), Chhatrapur in Ganjam district (Orissa State) which are radioactive areas[14] and from Bhubaneswar (Orissa State) a non-radioactive area (table 1 and figure 1). Rats were maintained in animal room for a week before cytogenetic investigations were carried out. All the animals were healthy. Chromosomes from the BMC of 199 rats were harvested following colchicine-citrate-air-drying technique[15] and stained with buffered Giemsa (pH 6.8).

RESULTS

Thorough examination of the bone marrow preparation of all rats from Bhubaneswar showed no sign of DMs whereas 22.3% of Chhatrapur rats and 37.1% of Quilon rats were with DMs in the interphases (figures 2 and 3) and metaphases (figure 4).



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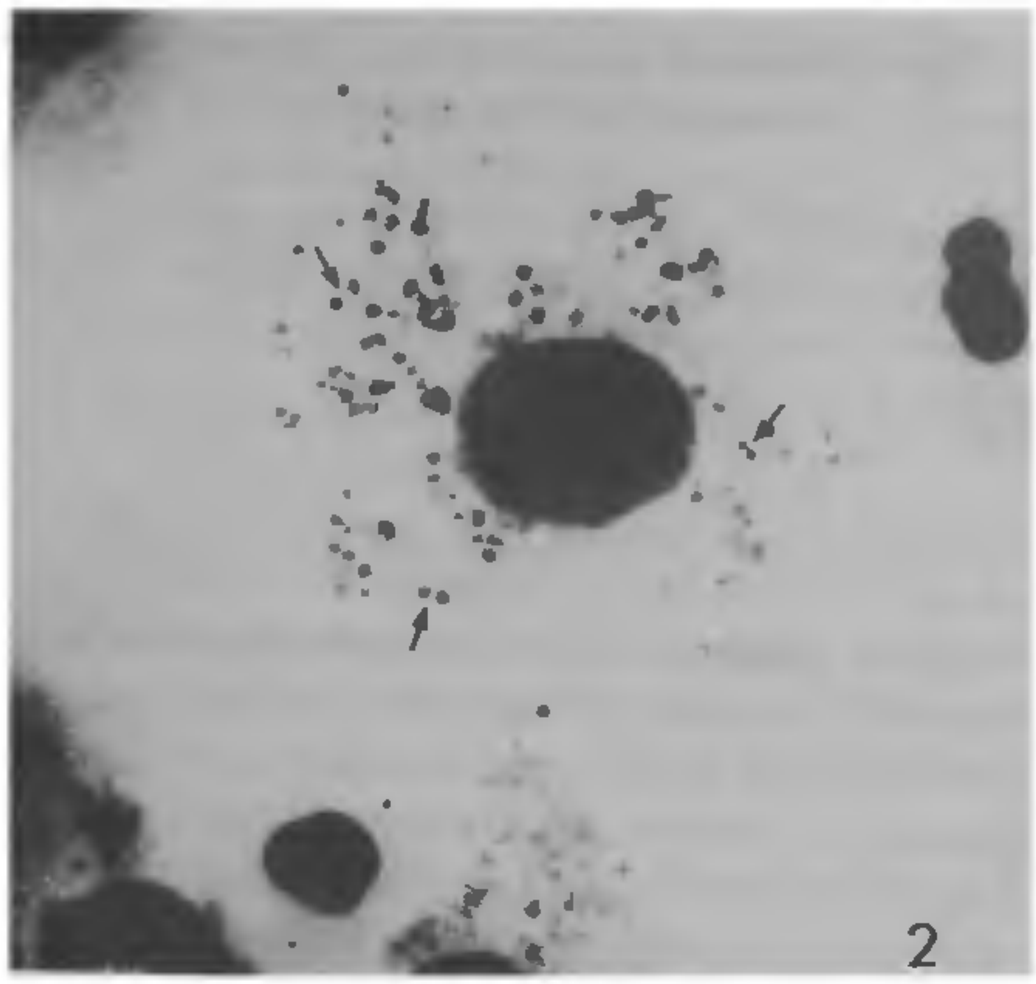

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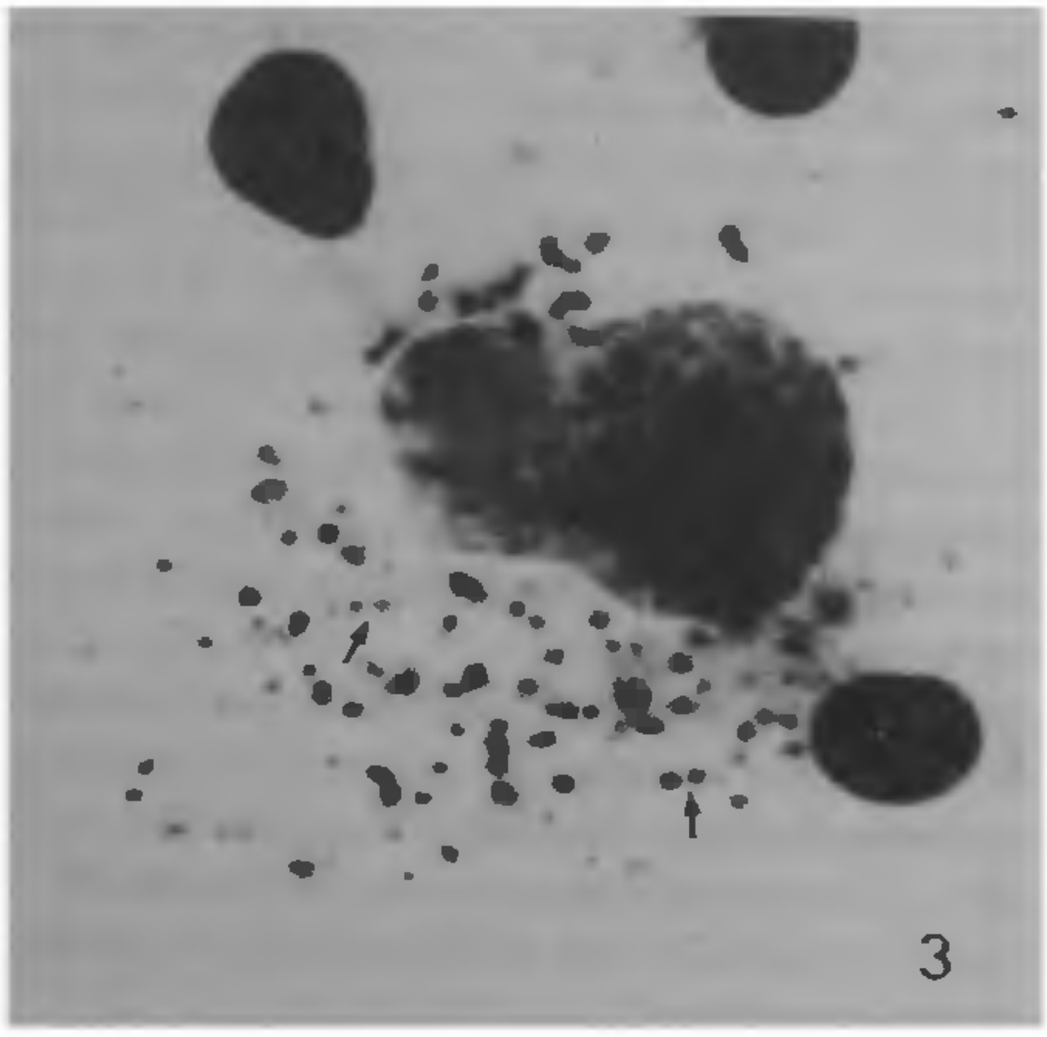

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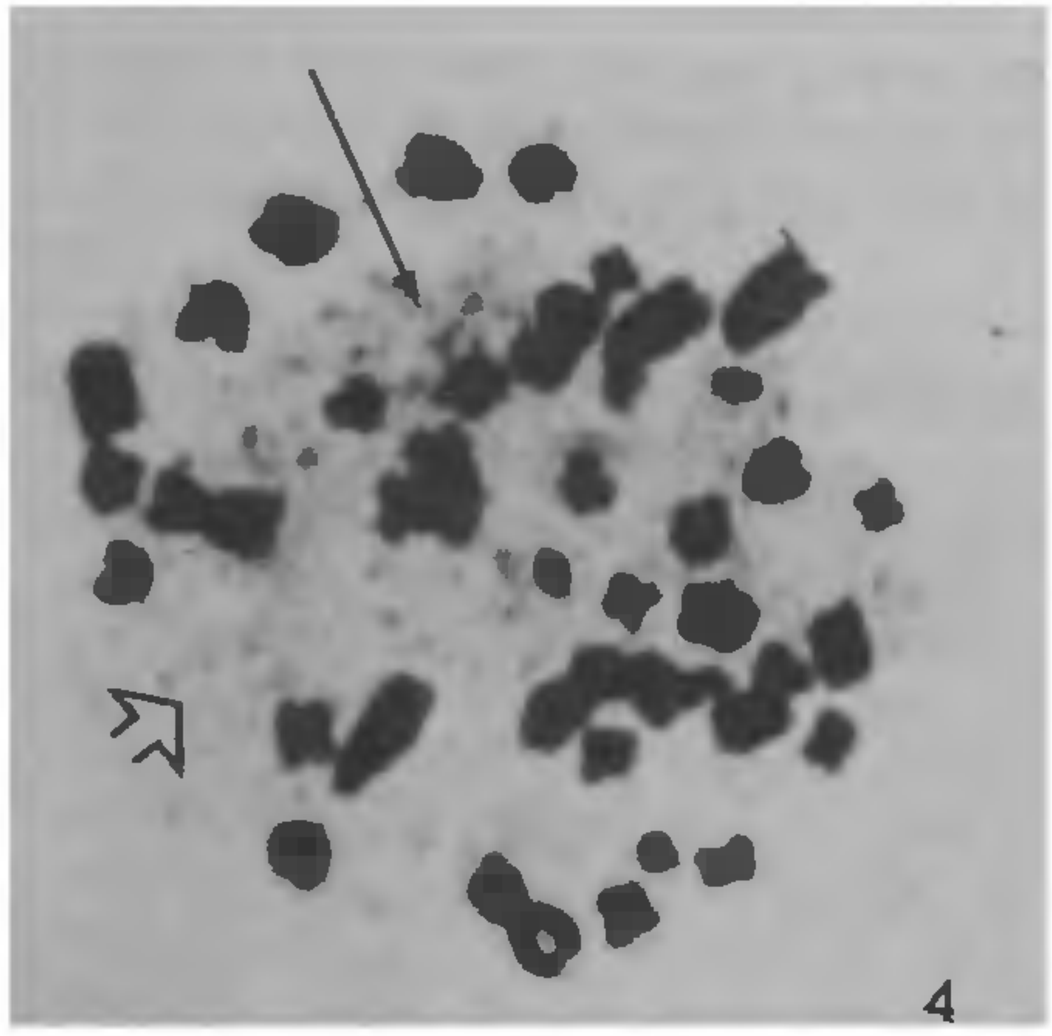

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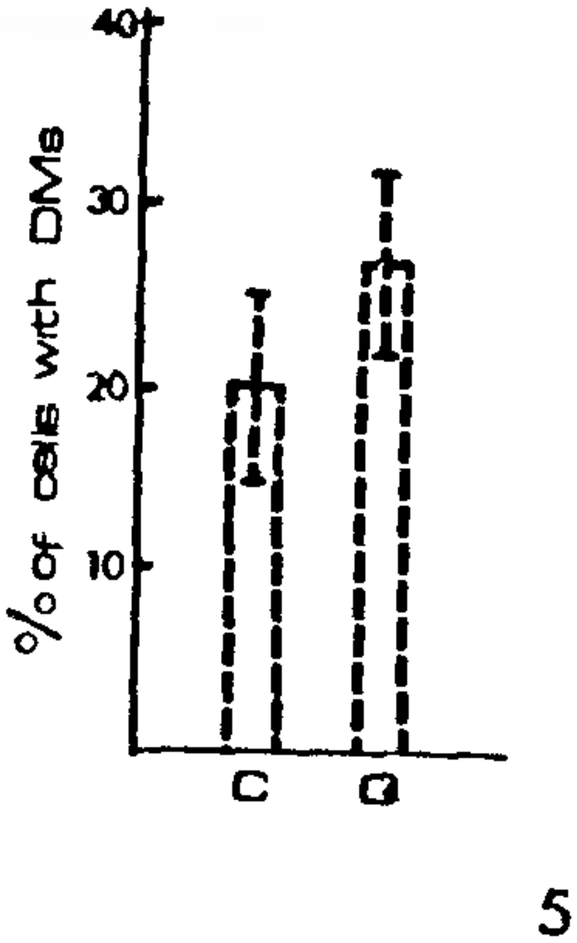

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Figures 1–5. **1.** Trapping sites of black rats; **2.** Small double minutes in interphase; **3.** Large double minutes in interphase; **4.** Double minutes in metaphase, and **5.** Histogram showing the frequency of cells with DMs (C = Chhatrapur; Q = Quilon).

Table 1 *Three population groups of black rats analysed for detection of double minutes*

Population	No. of rats trapped	No. of rats analysed	No. of rats with DMs (%)
Bhubaneswar	85	53	–
Chhatrapur	125	76	17 (22.3)
Quilon	135	70	26 (37.1)

For detailed analyses 10 individuals from each population were studied. 500 cells were scored from each individual to determine the frequency of DMs. The frequency of DMs in different individuals varies from 19 to 34.6% in Quilon population and 11 to 28% in different individuals in Chhatrapur population. In Quilon population out of 5000 cells studied 1344 cells (26.9%) were with DMs (table 2, figure 5). In Chhatrapur population, out of 5000 cells 1031 (19.54%) cells were with DMs (table 2, figure 5).

DISCUSSION

The Indian Rare Earths Limited has established two plants at Quilon and Chhatrapur to extract thorium (emitter of gamma rays) from the monazite sands available in the nearby sea coasts. The thorium content of monazite in Quilon ranges from 8 to 10.5% and is the highest in the world[14] and the mean radiation intensity of the radioactive belt as a whole is about 7.5 times higher than the control belt[16]. In Chhatrapur the monazite sand contains about 2.5% of thorium. However, the present authors are not aware of the mean radiation intensity of the area. The WHO Expert Committee on Radiation in 1959 regarded it as rather improbable that the investigation of any of the high background areas known to them would, by itself, lead to the demonstration of significant genetic changes due to chronic low level exposure. In 1965 Professor Gruneberg and his colleagues failed to discover positive evidence for genetic effects from the skeletal and dental studies on black rats of Quilon. However, they felt that additional mutations due to low level radiation might be masked by an increase in natural selection or a decrease in environmental variance[16]. They had not then looked into the chromosomes of the animal.

Student's *t* test conducted on the frequency of DMs in Chhatrapur and Quilon rat populations (table 2) showed no significant difference. However individual variations existed in DM frequency implying that the production of DMs, which are unstable structures, is independent of chronic low doses of radiation from the background and time of exposure since the birth of the animal.

Vertebrates are vulnerable to radiations because of their dependence on proliferative tissue for lines of both defence and supply. The stem cells in progenitive bone marrow tissues must bear irradiation sequelae which lead to cellular transformation passed through retroplasia to neoplasia. The transformation is likely to be initiated at the submicroscopic level, that is gene mutation-alteration in its gene sequences may lead to inactivation of a crucial protein and result in cell death. On the other hand, very rarely, a mistake in base pairing leads to an improved gene in manufacturing 'better enzyme' contributing to a more effective structure like DMs— the storehouse of amplifying elements— amplicons/oncogenes.

Thus the black rats in the radioactive background can modulate a specific amount of protein to overcome the environmental hazards. The appearance and disappearance, increase and decrease in the number of DMs in BMC exhibit the state and amount of protein production required by the cell in question for its survival. The adaptive mechanism of the cell may be attributed to defend the animal from the radiation shock in perspective.

This paper forms part of the Ph.D. thesis submitted by one of the authors (GM)[17].

30 November 1987

Table 2 *Mean percentage of cells with DMs in black rats from two radioactive areas (5000 bone marrow cells from 10 rats of each population analysed)*

Population	Cell % with DMs (mean ± SE)
Chhatrapur	19.54 ± 5.533
Quilon	26.9 ± 5.484

t test shows no significant difference.

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NEWS

NEA/OECD REPORT ON RADIOLOGICAL IMPACT OF CHERNOBYL ACCIDENT

"As a consequence of the Chernobyl nuclear plant accident in 1986, people living in member countries of the Organization for Economic Co-operation and Development (OECD) are not likely to have been subjected to a radiation dose significantly greater than that received from one year of exposure to the natural background radiation", a new report from the Nuclear Energy Agency (NEA) of the OECD concludes. As a result, the report says, the lifetime average risk of radiation-related harm for the individual members of the public has not changed to any noticeable extent by the accident. It further states that the number of potential health effects (cancers and genetic effects) that can be derived by calculating collective doses will not constitute a detectable addition to the natural incidence of similar effects within the population. The NEA report—entitled *The radiological impact of the Chernobyl accident in OECD countries*—follows an earlier study and evaluates the radioactive fallout recorded in OECD countries from the Chernobyl accident, based on extensive data obtained from national monitoring programmes. The report's data shows that the average individual dose ranged from 2.5 microsieverts in Canada to 660 microsieverts in Austria. Member States of the OECD include 19 European countries and Australia, Canada, Japan, and the United States.—Further information about the report or how it can be obtained is available from the NEA/OECD, 38 boulevard Suchet, 75016 Paris, France. (*IAEA Newsbriefs*, No. 22, (Vol. 3, No. 1), p. 3; Published by the Division of Public Information, International Atomic Energy Agency, P.O. Box 100, 1400 Vienna, Austria.)