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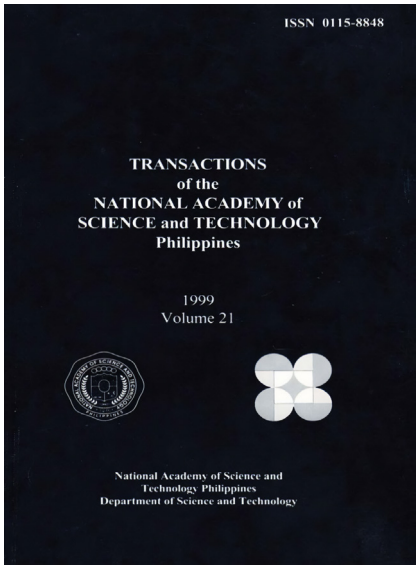

Research Note: Association Between Chromosome Fragile Site and Oncogene Location: A Profile of Filipino Head and Neck Cancer Patients

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RESEARCH NOTE:

ASSOCIATION BETWEEN CHROMOSOME FRAGILE SITE AND ONCOGENE LOCATION: A PROFILE OF FILIPINO HEAD AND NECK CANCER PATIENTS

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ABSTRACT

Chromosome instability is often associated with predisposition to cancer. Peripheral blood lymphocytes from 25 cancer pateients and 25 control individuals were cultured for 72 h. Chromatid and chromosome breaks were induced by treating lymphocytes with bleomycin, a known mutagen. Chromatids and chromosomes were scored in50 spreads for each individual. Frequency of breaks was tallied per chromosome number. A correlation was made between frequency of breaks and differential sensitivity of chromosomes to mutagen. Association between location of oncogene and fragile sites on chromosome was determined.

Keywords: mutagen, head and neck cancer, bleomycin, lymphocytes, chromatid and chromosome breaks, fragile site, oncogene

INTRODUCTION

A number of assays have been developed to assess the susceptibility of genetic material to mutagen-induced genetic lesions. Specifically, chromosomal aberrations are considered the most readily discernable manifestations of genetic

damage in cells. The bleomycin sensitivity test is one assay which induces mutagen-induced chromosomal lesion in cultured cells. The assay has been proven to play a significant role in predicting carcinogenesis in tissues that are in contact with the external environment (Hsu et al., 1989).

Bleomycin is a an antitumoral glycopeptide, described to be a radiomimetic agent because it can cause single and double strand breaks and chromosome damage in all phases of the cell cycle, similar to ionizing radiation.

Chromosome sensitivity to mutagen-induced chromosomal damage is positively associated with the incidence of upper aerodigestive tract cancers. These cancers are known to be caused by environmental carcinogens particularly found in tobacco and alcohol (Spitz *et al.*, 1989; Spitz and Hsu, 1994). The concept behind this method rests on the idea that chromosomes of high risk individuals when treated with bleomycin will likely accumulate more mutations than low risk individuals.

Fragile sites (FS) in human chromosomes are regions prone to breakage which result when cells are exposed to specific chemical agents or conditions of tissue culture (Sbrana and Musion, 1995). Some studies suggest a relationship between FS and cancer as indicated by the preferential clastogenic action on these sites by many mutagens and carcinogens known to act through different molecular mechanisms. It is believed that FS may be general targets of mutagenic action (Yunis *et al.*, 1987).

PATIENTS AND METHODS

Subjects

Thirty (30) patients who were diagnosed with head and neck cancer in a government hospital in Manila and who have had no prior treatment for cancer were included in the study. All patients signed an informed consent form and answered a basic questionnaire specifically prepared for the study. Ten milliliters (10 mL) of peripheral blood were extracted from each subject using a heparinized vacutainer tube.

Thirty (30) non-cancer individuals comprised the control group. As a rule blood samples from the control group were cultured simultaneously with the cancer specimen.

Culture Conditions

Microculture (whole blood culture) technique was performed in RPMI 1640 supplemented with 10 fetal bovine serum (FBS) with 2% phytohemagglutinin (PHA) for 72 hours at 37°C in 5% CO_2 atmosphere. For each subject, two culture conditions were carried out: one treated with bleomycin and the other without bleomycin. The mutagen was added during the last five hours of culture. Colcemid (0.2 μg/ml), an arresting agent was added to the cultures two hours before harvest. Chromosome preparations were obtained by standard techniques: through 0.075M KCl hypotonic shock, methanol: acetic acid fixation, and air-dry slide preparation.

Cytogenetic Analysis

Slides were allowed to age for 5-10 days prior to staining. G-banding was done by subjecting the chromosomes to a mild treatment of trypsin followed by Giemsa staining (5% solution). When adequate mitoses were obtained, 50 cells were screened per subject. Chromatid gap and breaks, as well as chromosome gap and breaks were scored for each subject and the average number of aberrations per cell was computed. Results were expressed as breaks per cell (b/c).

Results

The ages of the cancer group (A) ranged from 18 to 77 years old and the mean age was 48. There were 19 males and 11 females. Table 1 shows the profiles of sensitivity in all 30 cancer subjects and 30 controls. One thousand five hundred (1,500) cells were screened from the cancer patients and the total aberrations scored was 1,836. The mean value was 1.22 break per cell (b/c).

In the control group, ages ranged from 17 to 66 and the mean age was 28. There were 15 males and 15 females. A total of 980 chromosome breaks was recorded from among 1,500 metaphase cells analyzed. The mean number of break per cell was 0.653 (Table 1).

In the cancer group, a good number of aberrations was localized on chromosome 3. An abnormal metaphase cell from a cancer patient showing various structural aberrations like chromatid gaps (ctg), chromatid break (ctb), acentric fragments (ac).

DISCUSSION

Results of this study show a higher mean number of breaks per cell in the cancer group compared to the control subjects. The mean value of b/c in the cancer group was 1.22 while that in the control group was 0.653. The bleomycin assay is a mutagen-sensitivity assay, in which bleomycin, a mutagen, induces chromosome breaks. It may be used to identify individuals with less efficient DNA repair mechanisms, an indication of them having a much higher risk of developing tumors than the normal controls. This concept of chromosomal fragility has been linked to cancer development.

Analysis of G-banded chromosomes reveals that certain chromosomes or chromosome regions are more susceptible to damage than the others. These damage-prone areas of the chromosomes called fragile sites, are often implicated in chromosomal rearrangements present in malignant disease. Though quite controversial, this hypothesis is supported by the observation that there is an association between FS localization, cancer breakpoints and oncogenes (Sbrana and Musio, 1995). The findings of these fragile sites in cancer are further supported by studies which claim that FS may be general targets of mutation (Yunis and Soreng, 1987).

Table 1. Distribution profiles of bleomycin sensitivity in 30 head and neck cancer patients and 30 controls.

Breaks per cell (B/c)	Subjects	Controls
0.00-0.20	1	6
0.21-0.40	7	4
0.41-0.60	5	5
0.61-0.80	2	5
0.81-1.00	3	3
1.01-1.20	0	3
1.21-1.40	2	2
1.41-1.60	1	2
1.61-1.80	2	0
1.81-2.00	2	0
2.01-2.20	1	0
2.21-2.40	0	0
2.41-2.60	0	0
2.61-2.80	2	0
2.81-3.00	0	0
3.01-3.20	1	0
3.21-3.40	0	0
3.41-3.60	0	0
3.61-3.80	0	0
3.81-4.00	0	0
4.01-4.20	0	0
4.21-4.40	0	0
4.41-4.60	0	0
4.61-4.80	0	0
4.81-5.00	0	0
5.01-5.20	0	0
5.21-5.40	0	0
5.41-5.60	0	0
5.61-5.80	0	0
5.81-6.00	1	0
no. of individuals	30	30
mean b/c value	1.22	0.653
standard deviation	1.20	0.464
coefficient of variation	98.0%	71.1%
% > 0.80	46.7%	33.3%
% > 1.00	36.7%	23.3%

Our data provide substantial biological rationale to expand this study in terms of sample size as well as to include other high risk groups. These may include not only the patients with environmentally-induced cancers, but also those who are constantly exposed to harmful substances like industrial workers, pesticide handlers, drivers, painters, sidewalk vendors or even the ordinary commuter who walks the streets and breathe the polluted air.

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