6:3:1(1)

6:3: Leave/Deputation.

(i) Grant of Sabbatical leave for a period of 1 year w.e.f. 15.9.99 to 14.9.2000 in respect of Prof. A. Chatterjee.

Prof. A. Chatterjee, Lecturer, department of Zoology, has submitted an application dated 4.5.99 for the grant of Sabbatical leave for 1 year w.e.f. 15.9.99 to 14.9.2000, for taking up the project awarded by the UICC, Columbia University, USA. The letter of award is at Annexure I. The request for the said leave was forwarded and recommended by the Head, of the department and the Dean, School of Life Science.

He joined this University on 25.11.88, and has completed 10 years of sefvice. Earlier, Prof. A. Charterjee had availed study leave for 1 year w.e.f. 1.9.94 to 31.8.95 and has already rendered 3 years of service after availing the study leave.

Having examined the case, Dr. Chatterjee fulfils the conditions laid down in the University Ordinance under Clause 11 (i) of OE-6, subject to execution of the Bond under Clause 11(iii) of OE-6 of the ordinance.

In view, the matter is placed before the EC for its decision on the matter.

No.96-150/Estt-II/89-83.

Project Description Annexure: I

Title: Molecular genetic studies to identify a tumour suppressor gene at 5p 15.1-15. 2 in cancers of Testis and Cervix Uteri.

Objectives, princ. plôs and relevance to cancer problem.

The proposed project is aimed to understand the genetic basis of two tumour systems, viz. male germ cell tumours (GCT/testicular cancer) and cervical carcinoma. GCT a common malignancy in young males, is a complex tumours where germ cell transform and exhibit embryonal like differentiation patterns. Carcinoma of the cervix uteri is a common malignancy among women world w wide and most cervical cancers are preceded by distinct preneoplastic epithelial changes called dysplasias providing opportunities to study genetic alterations at an early stage of transformation. The genetic basis of origin, progression and differentiation of both the cancers are not fully understood. Recent molecular genetic analysis of GCT and carcinoma cervix uteri have identified important clues to genetic basis of transformation and progression. In a genome wide search for genetic alterations, two novel sites of deletions at 4q and 5p[1,2] have been identified in cervix cancer and deletions at 12q13, 12q22[3] and 5p[4] in GCTs.

The deleted region at 5p15. 1-15.2 defined by the markers D5S208, D5S12, D5S432 and D5S416 was seen in both GCT and cervical cancer. These markers are separated by approximately 7 cM[5]. It has already been reported that a high frequency of loss of heterozygosity(LOH) at the markers D5S406 and D5S208 mapped to 5p15.1-15.2 in early cancerous lesions and invasive cancers of cervix uteri[2] Alllic deletions on 5p have also been shown to be frequent in hepatocellular carcinoma with cirrhosis[6] and lung cancer[7]. Molecular analysis aimed at identifying candidate tumour suppressor genes (TSG) have mapped allelic deletions at 5p 5p15.1-15.2 in over 50% of cases suggesting a site of putative TSG commonly involved in these two tumour types[1,8,9]. These data strongly suggest that a candidate TSG lies in the interval of D5S406 and D5S416, which may play role in male GCTs, certical carcinoma and possibly other tumour typôs.
Identifying of the putative gene at this site may provide new i insights in the development of GCT and cervical carcinoma. The goal of this proposal is to identify the gene involved at 5p 15.1-15.2 and validate its usefulness in understanding the biological relevance in cell transformation and tumour progression with the following specific aims.

....2/-

1. Further narrowing the minimal deletion at 5p15.1-15.2 to 1 Mb size to see whether a common deleted gene is involved in both GCT and cervical carcinoma.

2. To search for the tumour suppressor gene at 5p15.1-15.2 by positional cloning methods.

Materials, Methodologies and Procedures

Tumour specimens, normal cells and cell lines: Tumour tissues and the corresponding peripheral blood mononuclear cells, already available at the host institution, will be utilized for these studies. Several cell lines from both GCT and cervical cancer are available at the host laboratory in an ongoing research in these tumour systems.

Narrowing of the common region of deletion at 5p15.1-15.2: A small insert genomic contig in the deleted region will be generated by isolating BAC and PAC clones that will provide reagents to generate new polymorphic markers and estimate the size of the region accurately. The newly generated polymorphic DNA probes will be integrated into the contig map and analyzed for LOH on the tumour DNAs to further narrow the boundaries of the common region of deletion at 5p15.1-15.2 to 1Mb.

LOH studies in search of TSG: LOH is an assay used to determine tumour specific deletion of genetic material compared to constitutional alleles using locus specific polymorphic markers such as restriction fragment length ploymorphisms(RFLPs), variable number of tandem repeats and microsatellites. Several such markers are available in genome data bases.
Analysis of LOH by PCR will be performed using primer pairs for several di- and tetranucleotide repeat polymorphism. PCR will be carried out in 25ul reaction volume containing 40-100 ng of genomic DNA with 20 pmol of primers, in which one fifth of the forward primer was end labelled with γ^{32}PATP and amplified for 25-30 cycles. The amplified PCR products will be denatured in sequencing stop solution and electrphoresed on 6% denaturing polyacralemide gels containing 10% formamide. The dried gels will be autoradiographed for 5-48h, and allelic deletions will be scored visually. Reduction of signal intensity by more than 50% of one allel over the other allele in tumour DNA compared with the intensity of constitutional alleles will be considered LOH[10].

...3/-

Relevance to activities in the home institute and for the cancer patient.

Carcinogenesis caused by alkaloids, polyphenols and tannic acid in raw betel nut (RBN: having more alkaloids) and lime (could act as promoting factor) are of concern among hill tribes in this northeastern (NE) region of India. We have measured the length of exposure of the oral and esophageal mucosa to chemicals which are released from the betel nut, lime and leaf during the course of a day. Assuming an average of 15 min per chewing period, then the oral mucosa is exposed daily for about 3.5h to chemicals released from the quid. Among betel nut chewers of 35yrs of age and older, the frequency of oral carcinoma rises significantly. The average age of onset of chewing was about 12yrs. Thus, in the period between onset of chewing and diagnosis of leukoplakia/ tumours, the oral mucosa was exposed for about 28,000h(3.2yrs) to betel quid extracts. Stich et al[11] had shown that the saliva of betel nut and betel quid chewers of NE region of India having potent clastogenic activities.

We had started our work in 1992 with an aim to know the molecular mechanisms of carcinogenesis by toxic components of RBN. We have seen the cytotoxic effct of arecoline (ARC: alkaloid component of betel nut) is enhanced in glutathione(GSH) depleted cells[12]. Lethal mutation (LM) could be a relevant indicator of genetic damage in cells exposed to RBN-extracts and their delayed expression may have relevance to the time course of carcinogenesis. The expression of LMs in the progeny of cells surviving after exposure of RBN extracts is being estimated in mammalian cell lines with different GSH status. For LMs study, we are using excision assay technique [13*14*]. We have observed that oral administration of ARC(for 15-30 days) shows more DNA damages in mouse bone marrow cells than peritoneal treatment and interestingly this damage could be minimised by feeding N-acetyl cysteine(precursor of GSH) during the course of ARC treatment [15]. Our ongoing cytogenetic studies on RBN chewers(with or without leukoplakia/tumour) showing increased frequency of deletion in chromosome 5(25% of cases) 11(35%) and 17 (12%) and in other cases very few chromosomal rearrangements have also been observed. Studies are in progress for establishing these cytogenetic effect with their endogenous GSH status.

....4/-

At this juncture, these cytogenetic clues could lead to an extensive search for genetic alteration in betel-nut and lime chewers in NE region of India with the help of extensive mole cular genetic analysis methods. Therefore, the proposed work and my practical exposure will have a great impact on the expansion of our research interest at home institute and enable us to understand the precise mechanisms of RBN induced carcinogenesis. Such molecular investigation on betel-nut related oral and oropharyngeal cancer will provide information about the role of TSGs in different stages of evolution of oral cancers and may provide clues for early diagnosis besides cytogenetic surveilance which serve as an early indicator, enabling prevention of adverse effects.

* Ref. No. 12,13 and 14 have enclosed herewith as Project related publications.

Relationship to my past, current and future activities & choice of host Institute.

Last 12 years, besides teaching, my research work has been concerned mainly on cellular factors determining inherent radio and chemo-sensivity in mammalian cells(pl. see the list of publications). In the above section I have already mentioned one of our current activities. Below I am mentioning about few projects on which our work are in progress:

a. Induction of DNA damages and lethal mutation by arecoline and raw betel-nut extract in relation to endogenous glutathione status in mammalian cells.

b. Cellular GSH status and its response to radiation induced cell cycle arrest, p53 expression, lethal mutation induction and apoptosis in mammalian cells.

c. Genotoxic effects of a new organotin compound $Et_2SnCl_2.L^4$ in normal and Dalton's lymphoma cells. On the basis of chemical structure this new organotin compound could be a potent anticancer drug in place of cisplatin.

Therefore, the proposed training will be helpful to do molecular analysis of deletion mutations in the betel-nut chewers having leukoplakia/tumours(as mentioned in the previous section). Accurate DNA diagnosis becomes feasible once a polymorphic (RFLP) makker has been located within 5cM of the desease gene. These deleted markers and/or the TSG could be utilized in evaluating high risk precancerous lesions of oral and oropharyngeal regions of betel quid chewers to invasive cancer. During my stay and work I could also closely observe the methodology and techniques by which mutations in any TSG gene could be analyzed. All these newly learnt molecular techniques could also be useful to know the machanism by which

....5/-

GSH, cellular intrinsic factor, defining cellular radio and chemosensivity with respect to end points like cell cycle, apoptosis and repair. This is important since the possible relevance of GSH in cancer chemotherapy and the development of resistance during the course of treatment was emphasized by t the findings that tumour cells made resistant to some anticancer drggs have increased cellular GSH level[16,17].

Dr. V.V.V.S. Murty and his group at College of Physians & Surgeons of Columbia University, USA, are carrying out extensive cytogenetic, molecular cytogenetic and molecular genetic studies to understand the genetic basis of GCTs and cervical carcinoma. In pursuit these goals, they are utilizing techniques such as physical mapping, genome analysis, DNA sequencing, tumorigenicity assays and generation of knock out mice. Therefore, my work and exposure with this group will be extremely helpful and relevant for performing molecular genetic studies of deleted or rearranged chromosomal regions of chromosome 5, 11 and 17 which we have observed in betel-nut chewers with leukoplakia/tumour in North Eastern region of India.

I am confident that the newly acquired skills will disseminate successfully in this department whose infrastructure from the research point of view is adequate.

Justification of Project duration

First 10 months: Narrowing the minimal deletion at 5p15.1-15.2 to 1 Mb size and looking for TSG by positional cloning.

Nest 2 months: Analysis of data and finalization of report.

References:

1. Mitra AB, Murty VVVS, Li R-G, Pratap M, Luthra UK, Chaganti RSK(1994) Allelotype of cervical carcinomaa, Cancer Research, 54: 4481-4487.

2. Mitra AB, Murty VVVS, Singh V, Li R-G, Pratap M, Luthra UK, Chaganti RSK(1995) Genetic alterations at 5p15:A petential markers for progression of precancerious uterine cervix lesions. J. Nati. Cancer Inst., 87: 742-745.

3. Murty VVVS, Houldsworth J, Baldwin S, Reuter V, Hunziker W, Besmer P, Bosl GJ, Chaganti RSK (1992) Allelic deletions in the long arm of chromosome 12 itendify sites of candidate tumour suppressor genes in male germ cell tumours. Proc. Nati. Acad. Sci., USA 89:11006-11010.

4. Murty VVVS, Bosl G, Houldsworth J, Meyers M, Mukherjee AB, Reuter V, Chaganti RSK(1994a) Allelic loss and somatic differentiation in human male germ cell tumours, Oncogene, 9:2245-2251.

5. Gyapay G, Morissette J, Vignal A et al.(1994) The 1993-1994 Genethon human genetic linkage map. Nature Genetics, 7:246-339.

....6/-

6. Ding S-F, Habib NA, Dooley J, Wood C et al (1991) Br. J. Cancer, 64:1083-1087.

7. Weiland I, Bohm M (1994) Cancer Res., 54:1772-1774.

8. Murty VVVS, Li R-G, Houldsworth J, Bronson DL, Reuter VE, Bosl GJ, Chaganti RSK(1994). Frequenct allelic deletions and loss of expressions characterize the DCC gene in male germ cell tumours. Oncogene, 9:3227-3231.

9. Murty VVVS, Li RG, Reuter VE et al.(1996) Deletion mapping identifies loss of heterozygosity at 5p15.1-15.2, 5q11 and 5q33-34 in human male germ cell tumours. Oncogene,12: 2719-2723.

10. Murty VVVS, Li RG, Mathew S et al. (1994) Replication error type genetic instability at 1q42-43 in human male germ cell tumours. Cancer Res., 54:6225-6260.

11. Stich HF, Bohm BA, Chatterjee K, Sailo JL(1983). The role of saliva borne mutagens and carcinogens in the etiology of oral and esophageal carcinomas of betel nut and tobacco chewers. In: Carcinogens & Mutagens in the environment. vol III Eds. HF Stich. CRC Press Inc., USA.

12. Deb S, Chatterjee A(1998) Influence of buthionine sulfoximine and reduced glutathione on arecolone induced chromosomal aberrations and sister chromatid exchanges in mouse bone marrow cells in vivo. Mutagenesis, 13:243-248.

13. Chatterjee A, Hodgkiss RJ, Rojas A(1995) Contribution of lethal mutations to excision assays for tumour cell survival. Acta Oncologica, 34:493-498.

14. Chatterjee A,Hodgkiss RJ, Rojas A(1998) Induction of lethal mutations in experimental tumours after single and fractionated irradiations in vivo. Int. J. Radiat. Biol. (IN PRESS).

15. Chatterjee A, Deb S(1998) Genotoxic effect of arecoline treated either by peritoneal or oral route in murine bone marrow cells and influence of N-Acetyl cysteine on this. (Submitted in Cancer Letters).

16. Green JA, Vistica DT, Young RC, Hamilton TC et al(1984) Potentiation of melphalan cytotoxicity in human ovarian cancer cell lines by glutathione depletion, Cancer Res., 44:5427-5431.

17. Hamilton TC, Winker MA, Lowe KG(1985) Augmentation of adriamycin, melphalan and cis platin cytotoxicity in drug resistance and sensitive human ovarian carcinoma cell lines by buthionine sulfoximine mediated glutathione depletion. Biochem. Pharmacol.,34:2583-2587.

(vi) Termination of service in respect of Dr. R. Lalthangliana.

EC:103:99:6:2:(vi): The Council considered the termination notice served on Dr. R. Lalthangliana on 26.3.99 and as he has completed 10 years as MLA and failed to report for duty on 1.4.99,the Council unanimously RESOLVED to terminate his services.

(vii) Filling up of the post of Professors / Technical Officer - III (USIC).

EC:103:99:6:2: (vii): The Council considered the filling up of the post of Professor / Technical Officer -III in USIC and RESOLVED that temporary appointments be made until the posts are filled up by regular process.

(viii) Confirmation in respect of Dr. R. Khongsdier Lecturer, Anthropology, NEHU, Shillong.

EC:103:99:6:2: (viii): The Council considered the confirmation of service in respect of Dr. R. Khongsdier as Lecturer in Anthropology w.e.f. 1.5.97 and RESOLVED to approve the same.

(ix) Confirmation of service in respect of Dr. BK Mahapatra, Lecturer, Political Science, NEHU, Shillong.

EC:103:99:6:2: (ix): The Council considered the confirmation of service in respect of Dr. BK Mahapatra as Lecturer in Political Science w.e.f. 16.1.97 and RESOLVED to approve the same.

(x) Unauthorised absence of Dr. VK Gautam.

EC:103:99:6:2: (x): The Council considered the unauthorised absence of Dr. VK Gautam w.e.f. 26.10.1990 to 28.2.1992 and again from 25.5.92 till date and unanimously RESOLVED that his services be terminated.

6: 3- Leave / Deputation

(i) Grant of Sabbatical leave for a period of 1 (one) year w.e.f. 15.9.99 to 14.9.2000 in respect of Dr. A. Chatterjee.

EC:103:99:6:3: (i): The Council considered the grant of Sabbatical leave to Dr. A. Chatterjee, Lecturer in Zoology, for a period of one year w.e.f. 15.9.99 and RESOLVED to approve the same.