

**STUDY OF MOLECULAR CHARACTERISTICS OF CHROMOSOMES
DURING CHEMICALLY INDUCED CELLULAR TRANSFORMATION**

ABSTRACT

LAKHAN KMA

**NORTH-EASTERN HILL UNIVERSITY
SHILLONG, MEGHALAYA,
INDIA**

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DURING CHEMICALLY INDUCED CELLULAR TRANSFORMATION**

ABSTRACT

BY

**LAKHAN KMA
DEPARTMENT OF BIOCHEMISTRY**



**SUBMITTED IN
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**NORTH-EASTERN HILL UNIVERSITY
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The fundamental objective of science is to make the human life qualitatively better, free of diseases and sufferings. Despite the fact that modern industrialized world has successfully eliminated infectious diseases, the major cause of death of last couple of centuries, fear of cancer as a killer disease has not subsided. Even in the new millennium with all encompassing developments in science and technology related to medical field including publication of human genome sequence, the epoch making feat of human endeavors, cancer continues to be one of the most deadly disease threatening mankind today. The scientific community worldwide is putting their mental, physical and financial resources to combat cancer. With economic development, increasing prosperity, better life expectancy and changing lifestyle this disease has firmed its grip on humans further. Cancer is a progressively fatal form of disease making it particularly different from other diseases. No specific drug has either been discovered for cancer nor seems likely in the near future. This is why cancer has always challenged the scientists worldwide and continues to do so. Cancer has been associated with the living world, particularly humans, since time immemorial. The problem of cancer lies in several special characteristics associated with it. For instance, (a) the causes of the diseases are vast and uncertain, (b) it is a progressive disease with long gestation time spreading over several decades in certain cancers, (c) no medicine has yet been discovered to successfully treat it and (d) even though it originates in a localized region, it spreads to otherwise healthy parts of the body even before one knows its existence. Cancer statistics of most of the advanced countries are available. In our country unfortunately, no accurate statistics on cancer incidence or death is available. The cancer registries in India are estimated to cover only about 4-5 % of the population (less than 1 % rural and about 15 % urban population). Nonetheless, the emerging trend in India is no different than anywhere in the world, irrespective of the socio-economic factors. This suggests that the intensity of problem is much more in India since its population is significantly bigger than most developed/developing countries.

Obviously, a great deal of research and investigation are needed to combat the menace effectively. It is estimated that improved screening of population suspected to be exposed to carcinogens and consequent early detection of carcinogenesis can lead to a significant reduction (up to 35 %) in cancer death with the present knowledge of cancer therapy. Research aimed directly at cancer prevention and promoting use of available knowledge for cancer prevention is highly desirable in the present state of cancer control. Attempts are being made to identify suitable biomarkers, locate and map gene so that it may serve as critical tools in different areas of biological and medical research covering carcinogenesis. Gene or chromosome mapping can provide valuable information, which, in turn, can provide tools for diagnosis, understanding and treatment of cancer.

Mapping of chromosomes, however, is an expensive and elaborate process and its application requires sophisticated and expensive laboratory set-ups. This limits the possible application of such information to mitigate the problem of a large segment of human population in developing and under-developed countries including India. An alternative to these limitations is to characterize the chromosomes and the genes located on them in terms of their biochemical activities controlling cellular metabolisms. There are several advantages of this approach. To start with, by looking at an appropriate biochemical parameter, the effective consequence of any change in

gene in terms of mutation, over- or under-expression can be directly measured. The information provides the actual biological consequence of change(s) in the gene. Secondly, biochemical characterization of a gene of interest gives information on its activity irrespective of type of change(s), thereby, providing the real *in situ* status of the gene(s) and its biological consequences. Furthermore, it becomes a quick way of looking at the status of gene(s) while genetic mapping is time consuming and requires sophisticated laboratory set-up. In addition, this kind of molecular marker of carcinogenesis has possible applied potentials for diagnosis of cancer as well as for monitoring progression of cancer therapy.

Considering the advantages associated with biochemical characterization of chromosomes, the work was designed to look into the activities of gene(s) associated with poly-ADP-ribosylation (PAR) reaction by monitoring PAR of cellular proteins in mouse tissues. Mouse chromosome 14 houses the main anabolic enzyme for PAR, poly ADP-ribose polymerase (PARP) gene located at 14q11.2-q12. The main catabolic enzyme of PAR, poly-ADP-ribose glycohydrolase (PARG), has also been tentatively mapped on the same chromosome but its precise position on chromosome 14 has not been worked out.

Using a hepatocarcinogen, dimethylnitrosamine (DMN), and a naturally occurring general carcinogen, aqueous extract of betel nut (AEBN), in a chronic *in vivo* administration protocol on Swiss albino mice, this work has attempted to characterize changes on the genome or chromosomes of the mice during initiation stage of carcinogenesis. DMN has been shown to initiate cancer in mice in about 4 weeks of exposure. The characterization of the chromosomes was done by monitoring structural changes in the mouse genome using DNase I. Further characterization was done at biochemical level by monitoring poly ADP-ribosylation (PAR) of cellular proteins by an ELISA-based immuno-probe assay developed in the laboratory. Total PAR of cellular proteins as well as PAR of individual proteins, including isolated histone proteins, were monitored in liver, spleen cells (SC), bone marrow cells (BMC) and blood lymphocytes. The extent of total PAR of cellular proteins as well as that of histones was quantified densitometrically.

The mouse SC genome underwent progressive relaxation starting from 2nd week of DMN or AEBN administration as indicated by results of DNase I digestion of the genome. Analysis done by agarose gel electrophoresis and pulse-field gel electrophoresis produced similar, confirmatory results. DMN caused relatively more relaxation of the genomic DNA than AEBN under similar conditions. Concurrent administration of 3-aminobenzamide (3-AB), an inhibitor of PAR reaction, with DMN further relaxed the genome suggesting involvement of PAR in the process.

For biochemical characterization, PAR of cellular proteins was monitored using a novel ELISA-based immuno-probe assay developed in the laboratory. The assay is simple, sensitive, reliable, cost-effective and environment friendly since it does not involve use of any radioisotope. Total PAR of cellular proteins was assayed by slot-blot immuno-probing while PAR of individual proteins was assayed by Western-blot immuno-probing. The hepatocarcinogen DMN affected the total PAR of cellular proteins in liver, the target organ, as well as other tissues such as SC, BMC and blood lymphocytes. AEBN also showed similar results in all cases. Statistically significant and progressive lowering of total PAR of cellular proteins in the four tissues studied

was caused by DMN or AEBN almost immediately after exposure of mice to the carcinogen in a chronic oral administration protocol. DMN in combination with 3-AB, on expected lines, further inhibited the total PAR of cellular proteins in all tissues examined.

Tissue specific differences were apparent and the extents of inhibition of total PAR of cellular proteins were different in different tissues and for the two carcinogens used in this investigation. Histone proteins were found to be the main target proteins for PAR in all tissues beside some other high and low molecular weight proteins. Histone proteins were grouped as H1, H3/H2b and H2a for the analysis. PAR of these histones were variable in different tissues. However, in all cases, DMN or AEBN lowered the extent of their PAR progressively during initiation stage of carcinogenesis. Concurrent presence of 3-AB with DMN further lowered the PAR of histone proteins on expected lines. This suggests that DMN or AEBN affected gene activities or enzyme activities of PAR reaction in a way that PAR was lowered.

Blood lymphocytes mirrored the effect for both DMN and AEBN in terms of total PAR of cellular proteins as well as PAR of individual proteins, particularly histones. Lowering of PAR was statistically significant in all cases beyond 2nd week of administration. The effect of two different carcinogens, DMN and AEBN, on the PAR of blood lymphocyte proteins may provide us with a handy tool for monitoring biochemical or physiological status of individuals exposed to carcinogens. This may possibly be a good biomarker for early detection of cancer or for monitoring progress of carcinogenesis since (1) it is a common post-translational modification associated with many proteins and (2) the assay, employed in this investigation, detects only the ADP-ribose moieties and not a particular protein.

All tissues examined in this investigation have shown a negative, nearly straight line, correlation between period of DMN or AEBN exposures and PAR of proteins from different tissues of Swiss albino mice. The most significant observation made in this study that has direct implication on its use in medical practice is that even blood lymphocytes exhibited significant reduction in PAR modification of cellular proteins with progression of DMN or AEBN exposure periods. This was in line with the trend exhibited by liver, SC and BMC. Both total PAR of cellular proteins and PAR of individual histone proteins showed similar trends. Different tissues or proteins did show variability in terms of PAR, but the general message was clear. Tissue specific variations were found which is normally expected. Though obtaining liver, SC or BMC from mice is no problem but the same is not true for human situation. The results from mouse blood lymphocytes give convincing clues that the strategy can be easily extrapolated to human situation. Further, immuno-probe assay of total PAR of cellular proteins is relatively simple as compared to the assay of PAR of individual proteins. The former involves slotting the sample directly on a NC membrane (slot-blot) and immuno-probing while, the latter, requires electrophoresis, transblotting the proteins from gel onto a NC membrane (Western blotting) and then immuno-probing. Since our results show a very clear, reproducible and statistically significant correlation between total PAR of blood lymphocyte proteins and initiation of carcinogenesis induced by two different classes of chemical carcinogens, DMN and AEBN, (Figs. 11, 13 and 14), it is logical to postulate that this slot-blot immuno-probe assay can be employed in human situation also. It is postulated, based on the results

presented in the thesis using mouse model that this strategy shall apply to any type of human cancer as well.

The biochemicals required for the whole slot-blot immuno-probe assay can, in principle, be packaged into a kit making it very convenient for transport. Most of the constituents of this kit have good shelf-life and require ordinary refrigeration, *etc.* The 1st Ab is the critical constituent, which actually detects the PAR modifications of proteins. In the investigation, the 1st Ab was raised against heterogeneous ADP-ribose polymer obtained from mouse SC. Preliminary investigation on human blood lymphocytes using the same 1st Ab against mouse ADP-ribose polymer shows that the 1st Ab was also able to detect PAR of proteins in human blood lymphocytes. This already supports the basic premise of application of the assay for detection of human cancer and for its use as a tool of mass screening. However, all procedures shall need to be standardized for assay involving human samples before final conclusion is drawn. Much remains to be done before it is applied to detect human cancer. Nonetheless, the work carried out and elaborated in the thesis takes us closer to the goal of making cancer detection simple, sensitive and reliable in the near future.

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