STUDY OF INFLUENCE OF POLY-ADP-RIBOSYLATION AND REGRESSION OF CHEMICALLY INDUCED CARCINOGENESIS *IN VIVO*

ABSTRACT

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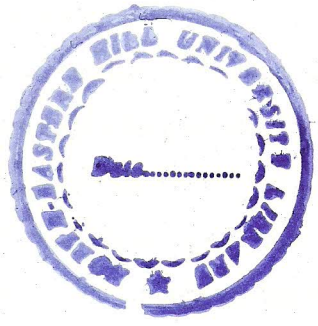

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Several macromolecules, especially proteins, undergo some modifications subsequent to their biosynthesis. These events are necessary to determine their functions. The modifications, particularly of nuclear origin, are important in terms of their degree of association with the fundamental structure of life i. e. DNA. poly-ADP-ribosylation (PAR) is one such post-translational modification widely studied and yet, enigmatic about its implications in several molecular processes including carcinogenesis. This post-translational modification involves addition of ADP-ribose moieties from endogenous NAD^+ substrates to target proteins forming linear and branched chains of ADP-ribose polymers. The reaction is catalyzed by chromatin associated enzyme poly ADP-ribose polymerase (PARP). The protein bound polymers are simultaneously degraded by enzyme poly ADP-ribose glycohydrolase (PARG). These two enzymes are physiological counterparts to each other. Therefore, poly-ADP-ribosylation reaction condition of a physiological system is determined by the equilibrium state between the activities of the two main enzymes involved. It is now known that normal cells escape chain of sequential control mechanisms at molecular level when they become progressively transformed to cancer cells or malignancy. The causes include interactions with exogenous agents such as ionizing radiation, UV radiation and chemical carcinogens. The earlier events during the process include alterations in gene expression pattern, expression of neogenes, shut down of differentiation genes, etc. Carcinogenesis begins with initiation stage in which genome is irreversibly altered. It usually takes a long intervening period before these initial events manifest themselves as detectable cancers. The reaction of PAR, which modifies primarily DNA bound histones, brings about changes in chromatin superstructure by essentially modifying charge interactions between histones and DNA. Therefore, this modification would have implications in carcinogenesis. In this perspective, the work envisaged to monitor level of PAR of total cellular proteins as well as histone proteins during experimentally established two biological systems in Swiss albino mice. In the first model, carcinogenesis was induced by a hepatocarcinogen compound, dimethylnitrosamine (DMN) at a chronic dose rate of 10 mg/ kg. b. wt. The study was restricted to the initial phase in multistage carcinogenesis. Bi-weekly observations were made in the study. The effect of 3-aminobenzamide (3-AB), an inhibitor of poly-ADP-ribose synthesizing enzyme, PARP, was examined at a dose of 2 mM either alone or during simultaneous exposure of the mice to DMN. In the second system tumerogenesis was induced by Dalton's lymphoma ascites. Ten million ascites cells were used for the initial intraperironeal injection in mice abdomen. The observation for the study was limited to 15

days after transplantation of ascites cells. In both experimental model level of PAR was monitored from different tissues along with simultaneous examination of physiological changes. The work also aimed to develop a suitable, sensitive, convenient and optimized method to monitor the endogenous level of PAR. For this purpose, polyclonal ADP-ribose polymer antibody was raised in the laboratory. The specificity of the poly ADP-ribose was confirmed using snake venom phosphodiesterase (SVP), a degradative enzyme which cleaves pyrophosphate bonds in poly ADP-ribose, besides checking with standard Ouchterlony immunodiffusion assay. The techniques of slot- and Western blots were used for monitoring level of PAR of total cellular proteins and individual histone proteins, respectively. Another focus of the work was to correlate chromatin structural organization and PAR from the results. The work brings out the following main points.

- A polyclonal antibody against heterogenous ADP-ribose polymer antigen isolated from normal mouse spleen cells has been raised in the laboratory. An ELISA based immunoprobing method employing the polyclonal antibody has been established and optimized. The method specifically detects PAR of proteins by immune interaction between the raised polyclonal antibody and ADP-ribose polymer antigens.

- In the assay developed and optimized in this work, level of PAR of total cellular proteins and individual histones have been monitored by slot blot immunoprobing for total cellular protein while Western blot immunodetection for individual histone proteins. The novel assay developed has been found to be simple, sensitive and the assay can be applied to varied tissue without involving tedious sample preparations like those in isotopic assay. Thus, the assay provides an advantageous step ahead of the conventional method.

- In DMN induced carcinogenesis, the physiological parameters such as body weight, cell number in spleen and BMC, protein content in liver and spleen, and histone content in spleen remained essentially unaffected during the treatment period. Similar was the case in 3-AB treatment, so also in combined regime of DMN + 3-AB. However, in ascites Dalton's lymphoma induced tumorogenesis, the growth of tumor cells was proportional to the increase in body weights of mice. Protein contents in liver and spleen showed declining tendencies.

- The slot blot immunoassay reveals that the method is limited obscuring a clear insight. However, general lowering of PAR in liver and spleen under DMN influence was observed in the later part of treatment. Level of PAR generally declined for histones. During carcinogenesis and tumorogenesis, the extent of PAR of total cellular proteins of liver, spleen and bone marrow cells were relatively higher than histones isolated from spleen and ascites suggesting ribosylation of other proteins besides histones.

- Under the influence of DMN, the protein expression pattern did not change markedly in liver and spleen homogenates and isolated histones from spleen cells except for slight over- and under expression of some proteins. Influence of Dalton's lymphoma showed similar results during tumorogenesis.

- Histones were primary targets of poly-ADP-ribosylation as revealed by Western blot assay. Most non-histone proteins were not ADP-ribosylated in liver. Polyclonal anti ADP-ribose polymer antibody also detects higher molecular weight proteins particularly in spleen in both the systems suggesting that proteins other than histones were ribosylated in spleen.

- The general lowering of PAR, especially of histones H1 and some core histones is accompanied by relaxation of chromatin superstructure as revealedby DNase I fragmentation. The result suggests that a negative correlation exists between level of PAR and cellular transformation by DMN.

- Lowering of level of PAR was evident in histones H1, H2b + H3 and H4 under DMN influence especially in liver as shown by the Western blot analysis. 3-aminobenzamide (3-AB), an inhibitor of PARP enzyme, potentiated the effect of inhibition of PAR in the combined regime of DMN and 3-AB. In spleen, inhibition was observed in H1 only in the later part of DMN exposure. The inhibition of PAR was extensive and significant, especially for H1, H2a and H2b + H3 in liver under 3-AB influence. In spleen, the inhibition of PAR of histones was not so pronounced. However, the lowering of PAR by combined treatment was usually less pronounced than that caused by 3-AB exposure. During tumorogenesis, general lowering of PAR in most histones in liver, spleen, histones isolated from spleen and ascites cells were more evident. Therefore, the extent of PAR in different histones varied.

- The results obtained from the work undertaken in this investigation suggest that lowering of PAR is a hallmark during both initiation phase of carcinogenesis induced by DMN as well as tumorigenesis induced by Dalton's lymphoma ascites. Therefore, it can be proposed that employing Western blot immunoprobing assay for measuring endogenous poly-ADP-ribose, lowering of level of PAR can be used as predictive assay for detecting carcinogenesis and tumorigenesis.