The Bioscan 4(4) : 621-624, 2009

RESTRICTION ENDONUCLEASE BASED DNA POLYMORPHISM ANALYSIS OF CARCINOGENESIS

DEOKANT*, B. S. RAIPAT[1] AND M. P. SINHA[2]
Department of Biotechnology, St. Xavier's College, Ranchi - 834 001, Jharkhand, INDIA
[1]Department of Zoology, St. Xavier's College, Ranchi - 834 001, Jharkhand, INDIA
[2]Department of Zoology, Ranchi University, Ranchi - 834 008, Jharkhand, INDIA
E-mail: m_psinha@yahoo.com

KEY WORDS
Blood
Agarose gel
Electrophoresis
Restriction
Endonuclease
DNA polymorphism
Cancer

Received on :
09.10.2009
Accepted on :
19.12.2009

***Corresponding author**

ABSTRACT
W.B.C. of blood is a good source of genomic DNA present in an individual, which contains different sequences acting as cleavage sites for different restriction endonucleases. These cleavage sites may be altered due to mutation, addition, deletion or methylation of even a single nucleotide in cancer. The cleavage sites of two restriction endonucleases Nco I and Pvu II has been used for analysis of such alterations in DNA sequences (DNA polymorphism) from different type of cancers.

INTRODUCTION

Alteration in gene(s) of a normal cell i.e. mutation is responsible for initiation of cancer. Cancer is genetic in origin and starts from somatic mutation (Sinha *et al.*, 1998).

Cancer is the biggest killer next to heart disease has multifactorial aetiology viz. direct genotoxic effect on DNA, hormone induced cell proliferation, induced oxidative damage of DNA (Kasai *et al.*, 2009), ROS induced DNA damage (Ferk *et al.*, 2009).

Alterations in genetic sequences lead to alteration in encoded proteins and as a result some novel proteins may be produced, which act as biomarker for early detection of cancer (Deokant *et al.*, 2009).

Such alterations in DNA sequences are DNA polymorphism and can be analysed by monitoring cleavage sites of various restriction endonucleases. Change in nucleotide sequence even at a single position may shift or remove the existing restriction endonuclease cleavage site(s) or create new ones.

Changes in DNA methylation have been found in the large majority of tumors, which includes both genome- wide hypermethylation and gene specific hypermethylation of cytosine residues which leads to introduction of 5-methyl cytosine in genomic DNA. There is a high variability in their methylation levels of genomic DNA from different types of cancer (Stach *et al.*, 2003).

The L-myc EcoRI polymorphism in a noncoding variation in the second intron of the L-myc gene, resulting in *S* and *L* allele s. Individual carrying the *S* and *L* allele tend to have poor prognosis and increased risk of several tumor types, The meta analysis suggests that the L-myc EcoRI polymorphism is a marker of tumor prognosis in lung cancer and possibly in other types of cancer (Spinola *et al.*, 2004).

The size of human genome is about $3 \delta 10^9$ bp. The structural organization of genes in large genomes, such as the human genome can be elucidated by making use of restriction fragment length polymorphisms (RFLP) (Botstein *et al.*, 1980).

In humans, RFLP were first identified in the vicinity of the globin gene and have been used for diagnosing sickle cell anaemia (Kan and Dozy, 1978).

An important application of restriction fragment length polymorphisms has been the detection of marker for a disease known as Huntigton's chorea (Gusella *et al.*, 1984).

The present communication deals with the analysis of different cleavage sites of Nco I and Pvu II restriction endonucleases from genomic DNA found in different carcinogenesis as a measure of DNA polymorphism.

MATERIALS AND METHODS

Collection of blood samples: The blood samples were collected (with anti-coagulant added) from patients suffering from different types of cancer and named according to the type of cancer like C.C. = Cervix cancer samples, B.C. = Breast

cancer samples, L.C. = Lung cancer samples, A.C. = Anal canal cancer samples, U.C. = Urinogenital cancer samples and patient number was given.

Isolation of genomic DNA from collected blood: Genomic DNA was isolated from collected blood samples (Chomcyznski, 1997).

Qualitative and quantitative analysis of the isolated DNA by Spectrophotometric analysis and Agarose-Gel Electrophoresis: Quality and quantity of the isolated DNA can be analysed by spectrophotometer (Gallagher, 1994) and Agarose gel electrophoresis (Brody, 2004).

Restriction digestion of the isolated DNA: Isolated DNA is subjected to restriction digestion by type II restriction endonucleases Nco I and Pvu II (Botstein, 1980) and digested fragments are analysed by Agarose-gel electrophoresis.

RESULTS AND DISCUSSION

The genomic DNA isolated from different test samples contains genetic information that is translated in the form of proteins. These genetic sequences are altered due to mutation even at a single nucleotide level and are the beginning point of cancer development. Such alterations are examined by analyzing type II restriction endonuclease Nco I (C^!CATGG) and Pvu II (CAG^!CTG) cleavage sites similar tests were done during the present study.

Agarose gel electrophoreses were run with different combinations to test DNA samples isolated from different types of carcinogenesis. In all cases controls were taken from DNA of normal healthy person. The test DNA samples showed marked variation in comparison to the control.

Cancer is any abnormal proliferation of cells due to loss of cell cycle control, which depends on different cyclin dependent kinases, cyclin molecules, cdc molecules or molecules of signal transduction pathway (e.g. src, ras, raf etc.). The normal cellular genes from which retroviral oncogenes originated are called proto-oncogenes. They are important cell regulatory genes encoding proteins that function in the signal transduction pathways controlling normal cell proliferation. Oncogenes

Figure 1: Agarose-gel electrophoresis of genomic DNA in different types of carcinoma

1 – L.C.10, 2 – L.C.45, 3 – A.C.15, 4 – U.C.21, 5 – B.C.31, 6 – C.C.51, 7 – Control

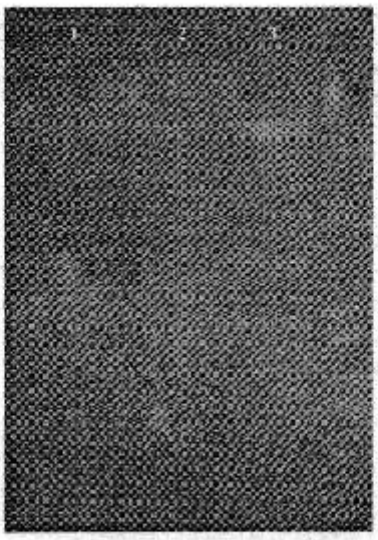

Figure 2: Restriction digestion pattern of genomic DNA from cervix cancer

1 – Nco I digested genomic DNA; 2 – Pvu II digested genomic DNA; 3 – Undigested genomic DNA

are usually expressed at much higher levels than the proto-oncogenes. The Raf poto-oncogene protein consists of an amino terminal regulatory domain and a carboxy terminal protein kinase domain. In the viral Raf oncogene protein, the regulatory domain has been deleted and replaced by viral Gag sequences. As a result, the Raf kinase domain is constitutively active causing cell transformation.

The extent of DNA laddering is less in case of L.C., A.C. and U.C. is more as compared to control but it is less as compared to B.C. and C.C. DNA laddering corresponds to DNA damage by mutation or reactive oxygen species during carcinogenesis (Fig. 1).

The position of restriction endonuclease (Nco I and Pvu II) digested DNA fragments are similar but distant from undigested DNA reflects the close occurrences of restriction sites of both endonucleases at many positions, all over the genomic DNA of cervix carcinogenesis (Fig. 2).

The position of restriction endonuclease (Nco I and Pvu II) digested DNA fragments are slightly different but distant from undigested DNA reflects the slight distant occurrences of restriction sites of both endonucleases at many positions, all over the genomic DNA of anal canal carcinogenesis (Fig. 3).

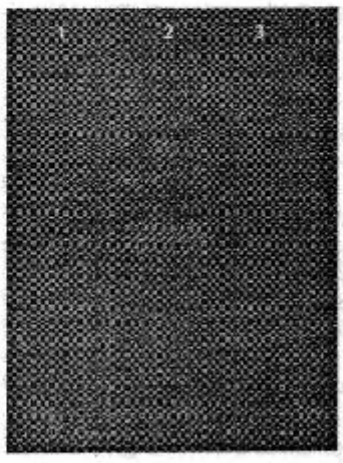

Figure 3: Restriction digestion pattern of genomic DNA from anal canal cancer

1 – Pvu II digested genomic DNA; 2 = Nco I digested genomic DNA; 3 = Undigested genomic DNA

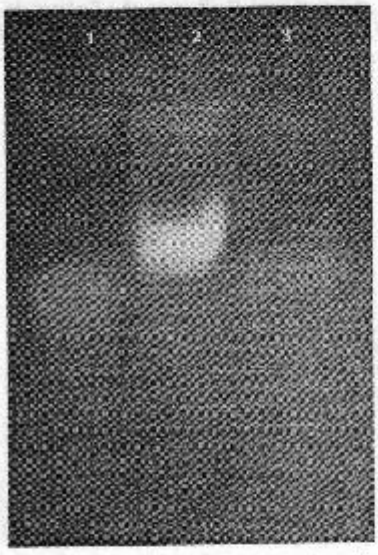

Figure 4: Restriction digestion pattern of genomic DNA from lung cancer

1 – Pvu II digested genomic DNA; 2 – Undigested digested genomic DNA; 3 – Nco I digested genomic DNA

The position of restriction endonuclease (Nco I and Pvu II) digested DNA fragments are slightly different but also relatively very close to undigested DNA reflects the slight distant occurrences of restriction sites of both endonucleases at limited positions, all over the genomic DNA of lung carcinogenesis (Fig. 4).

The position of restriction endonuclease (Nco I and Pvu II) digested DNA fragments are slightly different but also relatively very close to undigested DNA reflects the slight distant occurrences of restriction sites of both endonucleases at limited positions, all over the genomic DNA of breast carcinogenesis (Fig. 5).

From above studies, we can conclude that the presence of restriction endonuclease cleavage sites vary among the genomic DNA of different carcinogenesis types due to mutation, addition or deletion of single nucleotide.

This is a very primitive study for monitoring changes in the restriction sites for Nco I and Pvu II restriction endonucleases on genomic DNA of different types of carcinogenesis. The difference in restriction patterns represents DNA polymorphism among different carcinogenesis except breast and lung cancer.

Figure 5: Restriction digestion pattern of genomic DNA from breast cancer

1 – Pvu II digested genomic DNA; 2 – Undigested digested genomic DNA; 3 – Nco I digested genomic DNA

This study will be helpful in understanding the extent of molecular changes in genomic DNA during different carcinogenesis stages and types. The appearance or disappearance of a restriction site for a particular restriction endonuclease may be used as marker for early detection of cancer, before it starts to develop, even at the fetal level.

The present report can be useful in measureing the degree of susceptibility of a person to different diseases.

This result may further be extended for studying the polymorphism of a particular gene (p53 gene- a tumor suppressor gene) with the help of PCR-RFLP analysis.

ACKNOWLEDGEMENT

Dr. B. R. Shrivastava, Director, Dr. Harshvardhan, Medical Superintendent, Cancer Hospital and Research Institute, Gwalior are Acknowledged for permitting blood sample collection from different types of cancer patients.

REFERENCES

Botstein, R., Eva, Y., Lee, H. P., Young, L. J., Sery, T. W., Hayes, C. R., Friedman, T. and Lee, W. H. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphism. *Am. J. Hum. Genet.* **32:** 314-331.

Brody, J. R., Chihoun, E. S., Galmeir, E., Creavalle, T. O. and Kern, S. E. 2004. Ultra fast high resolution agarose gel electrophoresis of DNA. *Bio Techniques.* **37:** 598-602.

Chomcyznski, P., Mackey, K. and Wifnger, W. 1997. DNA Zol (R): A reagent for the rapid isolation of genomic DNA. *Bio Techniques.* **22:** 550-553.

Deokant, Raipat, B. S. and Sinha, M. P. 2009. Studies on serum protein profiles as biomarker of cancer. *The Bioscan.* **4(1):** 157-160.

Ferk, F., Chakravorty, A., Simic, T., Kundi, M. and Kansmuller, S. 2009. Antioxidant and free radical scavenging activities of sumaee (*Rhus coriaria L.*) and identification of galic acid and its active principle. Abstract in international symposium on genomics and molecular basis of human disease (March 17-19, 2009) organized by Magadh Univ. Bodh Gaya. p. 16.

Gallagher, S. 1994. Quantitation of DNA and RNA with absorption and fluorescence spectroscopy. In: Current protocols in molecular biology. Ausubel, F.M. *et al.*, (Ed.) John Wiley and Sons, New York. pp. A.3.D.1.-A.3.D.8.

Gusella, J.F., Tanzi, R.E., Anderson , Hobbs., W., Gibbons, K., Raschtchian, R., Gilliam, Wallaace, M. R., Wexler, N. S. and Conneally, P. M. 1984. DNA markers for nervous system diseases. *Science.* **225:** 1320-1326.

Kan, Y. and Dozy, A. 1978. Antenated diagnosis of of sickle cell anemia by DNA analysis of amnistic fluid cell. *Lamlet.* **2:** 910-912.

Kasai, H., Kawai, K. and Li Yun-Shan. 2009. Analysis of 8-hydroxydioxyguanosine as a marker of oxidative stress. Abstract in International symposium on genomics and molecular basis of human disease (March 17-19, 2009) organized by Magadh Univ. Bodh Gaya. p. 1

Maniatis, T., Fritsch, E. F. and Sambrook, J. 1982. Molecular cloning: A laboratory manual. Cold Spring Harbour Laboratory, New York.

Sinha, K., Singh, S. R., Gorai, A. C. and Sinha, M. P. 1998. Histological changes during induced hepatocarcinogenesis in wister albino rats through environmentally occurring carcinogen. In: Recent Advances in Ecobiological Research Vol. II. M. P. Sinha (Ed.) Asish Publishing Corporation, New Delhi. pp. 437-456.

Spinola, M., Pedotti, P., Dragani, T. A. and Taioli, E. 2004. Meta-analysis suggests association of L-myc EcoRI polymorphism with cancer prognosis. *Clinical Cancer Research.* **10:** 4769-4775.

Stach, D., Schmith, O. J., Stilgenbauer, S., Benner, A., Dohner, H., Wiessler, M. and Lyko, F. 2003. Capillary electrophoretic analysis of genomic DNA methylation levels. *Nucleic Acids Res.* **31:** 2e2.