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November 17, 2003

From,
Dr. Mariappan Vairapandi
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To,
Biocomplexity Faculty Search Committee,
C/O Prof. Rob de Ruyter van Steveninck,
Biocomplexity Institute,
Indiana University,
Swain Hall West 117,
Bloomington IN 47405-7105

Ref: (NW45910) R: Junior Faculty Position

Dear Sir,

This Letter is in response to the advertisement in Nature, regarding the opening of Junior Faculty position at your Institute. I work as a Research Assistant professor at the school of Medicine at Temple University. My area of expertise is in DNA Damage & Repair in the process of Cancer and to Develop therapeutic compounds for the treatment of Cancer.

My works resulted in the first reported identification of the human DNA Demethylase responsible for removal of 5-methylcytosine from DNA and the basis for the regulation of DNA at the level of Replication, Transcription, and Cell Cycle progression. My Current works involve the role of this Human DNA Demethylase and p300/CBP protein's combined role in Chromosomal Organization through DNA Demethylation and Acetylation. Their combined role of DNA Demethylation and Acetylation are amenable to PCNA and GADD45 proteins mediated regulation at various stages of cell cycle and reflect the stage of Cancer development when their role is not operable.

My recent works have resulted in the development of an RO1 application to NIH for the funding of my proposed work. I would like to apply for the Junior Faculty position opening in your Department. I have attached my Resume, List of References, my significant published works, a two-page write-up about my proposed work along with this letter for your perusal. I request you to consider my application for the openings in your department.

I remain,
With Regards,

Sincerely Yours,

M. Vairapandi

Dr.M.Vairapandi.

Brief Bio-Sketch and Objectives of Dr.M.Vairapandi:-

I have my Masters in Immunology, PhD in Biochemistry and 10+ years of Research Experience in cell and molecular biology. My studies with Dr.Nahum J.Duker of Temple University, involved with DNA damage and led to the identification of thymine-hydrate as a photo-product at 5-Methyl cytosine sites of DNA, after UV irradiation. This Thymine-hydrate is the intermediary in the mutation of 5mC to thymine. This was a novel transition mutation and molecular basis for UV mediated mutations on DNA and carcinogenesis and escaped the DNA repair machinery of the eukaryotic cellular systems. Removal of 5mC from DNA (demethylation) is also the basis of many eukaryotic cell cycle regulating events.

These observations led to the focused effort to identify the enzymes involved in the removal of 5mC from DNA in the human system. Towards this end I was very successful in identifying and characterizing the human Demethylase which was found to be a Glycosylase. This was the first finding about a Human Demethylase. This enzyme was partially purified and later I was able to demonstrate the binding of PCNA and RNA as other co-factors, bound to the core 52kDa. protein. These co-factors associated co-operatively with the core enzyme to enhance the Glycosylic removal of 5mC from DNA according to the regulatory signals of Cell Cycle mechanism.

Later I joined with Dr.Liebermann's lab as an Associate Scientist. Here, I was involved in the Characterization of murine MyD118 protein, which is a primary response gene in the myeloid differentiation program of M1 leukemic cells. Over expression of this protein was shown to mediate cellular death (Apoptosis), in many cancerous cell lines. This protein was found to interact with cell cycle regulatory proteins PCNA and p21. Later, the association of PCNA with Myd118 was to found to moderate the Apoptotic ability of MyD118 protein. Further studies have found the association of cell cycle regulatory proteins CycB1 and p34cdc-2 (cdk1) as other interacting partners for the MyD118 protein. MyD118 protein directly inhibited p34cdc2(cdk1)-cycB1 kinase activity and a role of MyD118 protein in the G2/M phase of the cell cycle is assigned.

My association with Onconova Therapeutics inc. was at the level of testing efficacy of various small molecular Anti-Cancer compounds and studying the Action mechanism of these compounds to make the safety of the compounds pronounced on the human cellular models.

Currently I am working on the protein-protein interactions of DNA Demethylase with different partners like PCNA and CBP/P300 and how their interaction will direct the events of cell cycle regulation. Basically these studies are aimed at finding the interface of PCNA which interacts with a myriad of growth suppressing as well as growth promoting cell cycle proteins. The future study of regulated interactions of various proteins with DNA Demethylase or the derailment of these interactions by viral agents, will unravel the molecular mechanisms of cellular growth, differentiation, carcinogenesis and/or the apoptotic process of cellular death.

Having got a background like this I would like to apply for the Faculty position opening in your department. Certainly my experience will help in the execution of your ongoing programs as well as developing new programs in the near future. I have attached my Bio-Data for your perusal and provided with a list of references to contact when interested.

Sincerely yours,

M. Vairapandi

Dr.M.VAIRAPANDI.

Reasearch Plans for Dr.M.Vairapandi:
Background of the work proposed:

DNA Damage and Repair remained as my research interest for a long time. My works on the UV induced DNA Damage led to the identification of Thymine-hydrate as a photo-product of 5-Methyl cytosine sites on DNA. This Thymine-hydrate is the intermediary in the mutation of 5mC to thymine. This was a novel transition mutation and molecular basis for UV mediated mutations on DNA and carcinogenesis.Removal of 5mC from DNA (DNA- Demethylation) is the basis of many eukaryotic cell cycle regulating events. The mechanism for DNA demethylation remained a controversy for very long. My studies with Dr. N. J. Duker are the first to identify the human DNA-Demethylase activity as a 5MeC-DNA Glycosylase (1)which was contested (2).Later,new mechanisms for demethylation of DNA were proposed. One involved RNA and a possible ribozyme mediated DNA demethylation (3).Another mechanism involved a demethylase activity that transformed methylated cytosine bases to cytosine bases that had been identified and cloned (4).Both of these mechanisms were also contested,and others were not able to reproduce their findings (5-7).Later the transformation of Methylated cytosine bases to cytosine was asserted again in an assay which relied on an incubation time of 48 hours (8). My continued efforts to purify the 5meC-DNA glycosylase activity with well-defined synthetic substrates, demonstrated 700-fold enrichment of the activity that was free from contaminating nucleases (9,10).Later this activity was found in human HeLa cells, chick embryos and differentiating mouse myoblasts (11,12,13).This DNA Demethylase mechanism was the only corroborated mechanism with other published reports where removal of 5-methylcytosine as a free base from DNA was by the enzyme 5-methylcytosine-DNA Glycosylase. We were the first to demonstrate human DNA demethylase activity as 5Methylcytosine-DNA Glycosylase with associated a basic site nicking activity that resembled "Base Excision DNA Repair activity" in its action mechanism. Also we demonstrated the association of this activity with the nuclear protein, Proliferating Cell Nuclear Antigen (PCNA) and Small RNA moieties (10).This PCNA associated DNA-Demethylase Activity may create single-strand breaks on DNA by 5MeC-DNA Glycosylase action mechanism (1,9,10).My studies have also shown that PCNA interacted directly with GADD45 (Growth Arrest and DNA Damage induced)/p21 proteins and provide interface as a moving platform on DNA for the facilitation of growth arrest at G1/G2 phase of the cell cycle.Thus PCNA is functional from G1 exit to G2/M phase of the cell cycle with both positive and negative regulatory roles in cell cycle progression.(14-16) In normal cell cycle progression,G0/G1 exit is brought out by the inactivation of pRB and it 's association with p300-CBP and PCNA protein (17-19).p300-CBP protein mediates Acetylation of DNA histones through its assigned Histone Acetyl Transferase (HAT)activity (20).This Histone acetylation on specific domains on DNA is to promote transcriptional activity of p300/CBP complex.One of the earlier genes transcribed in the process of cellular proliferation is Proliferating Cellular Nuclear Antigen (PCNA)that associated with p300-CBP protein (21). Acetylation/Deacetylation of DNA binding Histones and Methylation/Demethylation of DNA modulate the structural organization of chromatins (22-24).Histone Acetylation and DNA Demethylation are recognized as transcriptional activators whereas Histone de-acetylation and Methylation of DNA are recognized as transcription silencers.Thus the complex of DNA-Demethylase/PCNA/CBP/p300 protein activity may Regulate/Orchestrate Acetylation of DNA Histones/DNA Demethylation that may be followed by the opening and unwinding of DNA in order to provide optimal transcription and replication conditions. Thus DNA Demethylase/PCNA/p300-CBP protein complex has an exemplary role in opening the condensed histones on chromosomes and de-methylating DNA to promote DNA relaxation at G0/G1 exit to promote crossover from G0/G1 restriction point to the ' S ' phase of the Cell Cycle.

Current Un-Published work:

My un-published results demonstrate PCNA associated DNA demethylase activity that targeted hemimethylated DNA as the preferential substrate in normal cell lines. In addition recombinant GADD45 proteins inhibited the PCNA Associated DNA Demethylase activity in normal cell lines. In cancerous and virally transformed cell lines DNA Demethylase activity associated with p300 protein that targeted fully methylated DNA as the preferential substrate and is not inhibited by added recombinant GADD45 proteins.DNA Demethylase activity has the capability to associate with either p300 protein or PCNA protein with variable susceptibility to GADD proteins. Thus Demethylation of DNA and acetylation of histones are linked by a GADD regulatory mechanism that could lead to alterations in chromatin structure and a possible altered association of different factors could lead to reorganization of the chromatin and Oncogenesis.

Significance of the work proposed:

Alterations in DNA Methylation are now widely recognized as a contributing factor in human tumorigenesis.Recent research implicates the failure of DNA demethylation and hypermethylation of DNA promotor regions for the transcriptional silencing of many tumor suppressor genes. The active copy of the imprinted gene H19 is turned off by inappropriate Methylation in several pediatric tumors including Wilms' Tumour and embryonal rhabdomyosarcoma (25).Alterations in chromatin structure have been described as the mechanistic basis for this repression. Demethylation and Acetylation associated chromosomal organization is most probably hampered in cancerous and virally transformed cell lines. Loss of specific G1/G2 checkpoint controls has been implicated in the process of Oncogenesis. Malignant /Cancerous cell lines have accelerated growth rate by devolving varied mechanisms to by-pass these growth restriction points. Viral DNA replication is generally dependent upon circumventing host cell cycle control to force S phase entry in an otherwise quiescent cell. Most often by-passing of these check points permit – ' premature entry ' of the cells into " S ' phase or undergo mitosis regardless of the possible presence of DNA damage. The transforming activity of viral Oncoproteins is due in large part to the ability of these proteins to perturb and suppress the function of Retinoblastoma (pRB)and p53 tumor suppressor proteins.Deregulation of cell cycle progression control ,allows cells to exit G0 and enter S phase. Almost all-viral proteins such as E1A,SV40 large T-antigen,HPV-E7 and HBV-HBx interact with p300-CBP protein as an earlier event in the transformation of eukaryotic cells (26,27).One of the earlier genes expressed in the process of cellular proliferation or viral antigen mediated transformation is Proliferating Cellular Nuclear Antigen (PCNA)(28,29).Thus, viral protein mediated- transformation retains the early transcription and expression of PCNA gene and it ' s role in positive promotion of cell cycle. However, the viral transformation has evolved ways to circumvent the negative regulatory G1/G2 checkpoints provided by the PCNA protein along with Gadd45,,p21,and p16 proteins. So viral protein induced transformed cell lines are a good model system and present a way to compare the loss of check point controls in cancerous cell lines.
My Studies are planned to systematically and analytically study and compare the DNA demethylase activity in normal cell lines versus cancerous and virally infected cell lines to decipher the role of DNA Demethylase in G0/G1/G2 exit of the cell cycle. Also studies are planned to comparatively study the association of DNA demethylase activity with PCNA/p300 in normal cell lines versus cancerous/malignant cell lines and viral antigen transformed cell lines.These studies will help to decipher the role of DNA demethylase in the Organization and Re-organization of chromatin structures and the role of GADD proteins in the regulation of normal cell cycle progression and their failed role in the development of Oncogenesis.

References:

1.Vairapandi M,Duker NJ.1993.Nucleic Acids Res.21(23):5323-7.
2.Steinberg R.A..(1995).Nucleic Acids Research 23,1621-1624.
3.Weiss,A.,et.al.,(1996)Cell,86,709-718
4.Bhattacharya K.S.,et.al.,(1999)Nature.397(6720):579-83.
5.Swisher J.F.A.,et.al.,(1998)Nucleic Acids Res.26,(24), 5573-5580.
6.Ng HH,et.al.,.(1999)Nature Genetics 23:58-61.
7.Wade PA,et.al.,(1999)Nature Genetics 23:62-66.
8.Detich N,et.al.,J Biol Chem 277(39):35791-4 (2002)
9.Vairapandi M,Duker NJ.1996.Oncogene.13(5):933-8.
10.Vairapandi M,et.al.,(2000)J Cell Biochem.79(2):249-60.
11.Jost,J.-P.,et.al.,(1995)J.Biol.Chem.270,9734-9739.
12.Fremont,M.,et.al.,(1997)Nucleic Acids Res.25,2375-80.
13.Jost,J.-P.&Jost,Y-C.(1994)J.Biol.Chem.269,10040-43.
14.Vairapandi M,et.al.,1996.Oncogene 12(12):2579-94.
15.Vairapandi et.al.,.J Biol Chem.2000 275(22):16810-9.
16.Vairapandi M.,et.al.,(2002)J Cell Physiol.192 (3):327-38
17.Dyson,N.1998 Genes Dev.12,2245-2262
17.a.Sherr,CJ.(1996)Science 274,1672-1677.
18.Hasan S,et.al.,Nature (2001)410(6826):387-91
19.Ngan Vo et.al.,(2001)J.Biol.Chem.,276,13505-08
20.Ait-Si-Ali et.al.,(2000)Oncogene 19,2430-2437.
21.Lee BH,et.al.,J Virol 1998 Feb;72(2):1138-45.
22.Shikama,N.et.al.,(1997).Trends Cell Biol.7,230-6.
23.Jones PA,Baylin SB (2002)Nat Rev Genet.3(6):415-28,
24.Cervoni N,Szyf M..(2001)J Biol Chem..276(44):40778-87.
25.Lynch CA,et.al.,(2002)Mol Cancer1(1):2
26.Ait-Si-Ali S,et.al.,(1998).Nature 396,184-186.
27.Wang,et.Al.,J.Virol.69,7917-7924 (1995).
28.Yamaguchi et.al.,1992.Nucleic Acids Res 20(9):2321-5.
29.Zerler B,et.al.,.Mol Cell Biol 1987 Feb;7(2):821-9