

Endosymbiotic Archaeal Generated RNA Viroids Can Regulate Cell Function and Contribute to Disease State – Role in Viral Speciation

Introduction

Endomyocardial fibrosis (EMF) along with the root wilt disease of coconut is endemic to Kerala with its radioactive actinide beach sands. Actinides like rutile producing intracellular magnesium deficiency due to rutile-magnesium exchange sites in the cell membrane has been implicated in the etiology of EMF[1,2]. Organisms like phytoplasmas and viroids have also been demonstrated to play a role in the etiology of these diseases[3,4]. RNA viroids could contribute to the pathogenesis of schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration[2]. The possibility of generation of RNA viroids by actinide based primitive organism like archaea with a mevalonate pathway and cholesterol catabolism was considered[5-8]. An actinide dependent shadow biosphere of archaea and viroids in the above mentioned disease states is described[6]. The role of RNA viroids generated by actinidic archaea in regulation of body functions and the pathogenesis of human disease is discussed.

Materials and Methods

Informed consent of the subjects and the approval of the ethics committee were obtained for the study. The following groups were included in the study: – endomyocardial fibrosis, Alzheimer's disease, multiple sclerosis, non-hodgkin's lymphoma, metabolic syndrome x with cerebrovascular thrombosis and coronary artery disease, schizophrenia, autism, seizure disorder, Creutzfeldt Jakob disease and acquired immunodeficiency syndrome. There were 10 patients in each group and each patient had an age and sex matched healthy control selected randomly from the general population. The blood samples were drawn

in the fasting state before treatment was initiated. Plasma from fasting heparinised blood was used and the experimental protocol was as follows (I) Plasma+phosphate buffered saline, (II) same as I+cholesterol substrate, (III) same as II+rutile 0.1 mg/ml, (IV) same as II+ciprofloxacine and doxycycline each in a concentration of 1 mg/ml. Cholesterol substrate was prepared as described by Richmond[10]. Aliquots were withdrawn at zero time immediately after mixing and after incubation at 37 ^{o}C for 1 hour. The following estimations were carried out: – Cytochrome F420, free RNA and free DNA[11-14]. Cytochrome F420 was estimated flourimetrically (excitation wavelength 420 nm and emission wavelength 520 nm). The statistical analysis was done by ANOVA.

Results

The parameters checked as indicated above were: – cytochrome F420, free RNA and free DNA. Plasma of control subjects showed increased levels of the above mentioned parameters with after incubation for 1 hour and addition of cholesterol substrate resulted in still further significant increase in these parameters. The plasma of patients showed similar results but the extent of increase was more. The addition of antibiotics to the control plasma caused a decrease in all the parameters while addition of rutile increased their levels. The addition of antibiotics to the patient's plasma caused a decrease in all the parameters while addition of rutile increased their levels but the extent of change was more in patient's sera as compared to controls. The results are expressed in tables 1-2 as percentage change in the parameters after 1 hour incubation as compared to the values at zero time.

Table 2.1 *Effect of rutile and antibiotics on cytochrome F420.*

Group	CYT F420 % (Increase with Rutile)		CYT F420 % (Decrease with Doxy+Cipro)	
	Mean	± SD	Mean	± SD
Normal	4.48	0.15	18.24	0.66
Schizo	23.24	2.01	58.72	7.08
Seizure	23.46	1.87	59.27	8.86
AD	23.12	2.00	56.90	6.94
MS	22.12	1.81	61.33	9.82
NHL	22.79	2.13	55.90	7.29
DM	22.59	1.86	57.05	8.45
AIDS	22.29	1.66	59.02	7.50
CJD	22.06	1.61	57.81	6.04
Autism	21.68	1.90	57.93	9.64
EMF	22.70	1.87	60.46	8.06
F value	306.749		130.054	
P value	< 0.001		< 0.001	

Table 2.2 *Effect of rutile and antibiotics on free RNA and DNA.*

Group	DNA % change (Increase with Rutile)		DNA % change (Decrease with Doxy+Cipro)		RNA % change (Increase with Rutile)		RNA % change (Decrease with Doxy+Cipro)	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Normal	4.37	0.15	18.39	0.38	4.37	0.13	18.38	0.48
Schizo	23.28	1.70	61.41	3.36	23.59	1.83	65.69	3.94
Seizure	23.40	1.51	63.68	4.66	23.08	1.87	65.09	3.48
AD	23.52	1.65	64.15	4.60	23.29	1.92	65.39	3.95
MS	22.62	1.38	63.82	5.53	23.29	1.98	67.46	3.96
NHL	22.42	1.99	61.14	3.47	23.78	1.20	66.90	4.10
DM	23.01	1.67	65.35	3.56	23.33	1.86	66.46	3.65
AIDS	22.56	2.46	62.70	4.53	23.32	1.74	65.67	4.16
CJD	23.30	1.42	65.07	4.95	23.11	1.52	66.68	3.97
Autism	22.12	2.44	63.69	5.14	23.33	1.35	66.83	3.27
EMF	22.29	2.05	58.70	7.34	22.29	2.05	67.03	5.97
F value	337.577		356.621		427.828		654.453	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Discussion

There was increase in cytochrome F420 indicating archaeal growth. The archaea can synthesise and use cholesterol as a carbon and energy source[15,16]. The archeal origin of the enzyme activities was indicated by antibiotic induced suppression. The study indicates the presence of actinide based archaea with an alternate actinide based enzymes or metalloenzymes in the system as indicated by rutile induced increase in enzyme activities[17,18]. The archaea can undergo magnetite and calcium carbonate mineralization and can exist as calcified nanoforms.[19]

There was an increase in free RNA indicating self replicating RNA viroids and free DNA indicating generation of viroid complementary DNA strands by archaeal reverse transcriptase activity. The actinides modulate RNA folding and catalyse its ribozymal action. The viroids are evolutionarily escaped archaeal group I introns which have retrotransposition and self splicing qualities[20]. Archaea induced immune activation and redox stress can produce histone deacetylase inhibition resulting in endogenous retroviral (HERV) reverse transcriptase and integrase expression. This can integrate the RNA viroidal complementary DNA into the noncoding region of eukaryotic noncoding DNA using HERV integrase as has been described for borna and ebola viruses[21]. The noncoding DNA is lengthened by integrating RNA viroidal complementary DNA with the integration going on as a continuing event. The archaea genome can also get integrated into human genome using integrase as has been described for trypanosomes[22]. The integrated viroids and archaea can undergo vertical transmission and can exist as genomic parasites[21,22]. This increases the length and alters the grammar of the noncoding region producing memes or memory of acquired characters[23]. The viroidal complementary DNA can function as jumping genes producing a dynamic genome important in storage of

synaptic information, HLA gene expression and developmental gene expression. The RNA viroids can regulate mRNA function by RNA interference[20]. The phenomena of RNA interference can modulate T cell and B cell function, insulin signaling lipid metabolism, cell growth and differentiation, apoptosis, neuronal transmission and euchromatin/heterochromatin expression.

The RNA viroids and its complementary DNA developed into cholesterol enveloped RNA and DNA viruses like herpes, retrovirus, influenza virus, borna virus, cytomegalo virus and Ebstein Barr virus by recombining with archaeal, eukaryotic and human genes resulting in viral speciation.[24,25,26] The RNA viroids can also recombine with endogenous commensal RNA and DNA viruses producing speciation. Viral species are ill defined and fuzzy with all of them forming one common genetic pool with frequent horizontal gene transfer and recombination. Thus the multi and unicellular eukaryote with its genes serves the purpose of viral speciation.

The multicellular eukaryotes are like archaeal biofilms. The archaea with a mevalonate pathway uses the extracellular RNA viroids for quorum sensing and in the generation of symbiotic biofilm like structures which develop into multicellular eukaryotes[27,28]. The endosymbiotic archaea and bacteria with mevalonate pathway still uses the RNA viroids for the regulation of multicellular eukaryote. Pollution is induced by the primitive nanoarchaea and mevalonate pathway bacteria synthesised PAH and methane leading on to redox stress. Redox stress leads to sodium potassium ATPase inhibition, inward movement of plasma membrane cholesterol, defective SREBP sensing, increased cholesterol synthesis and nanoarchaeal/mevalonate pathway bacterial growth[29]. Redox stress leads on to viroidal and archaeal multiplication. Redox stress can also lead to HERV reverse transcriptase and integrase expression. The noncoding DNA is formed of integrating RNA viroidal complementary DNA

and archaea with the integration going on as a continuing event. The archaeal pox like dsDNA virus forms evolutionarily the nucleus. The integrated viroidal, archaeal and mevalonate pathway bacterial sequences can undergo vertical transmission and can exist as genomic parasites. The genomic integrated archaea, mevalonate pathway bacteria and viroids form a genomic reserve of bacteria and viruses which can recombine with human and eukaryotic genes producing bacterial and viral speciation. The change in the length and grammar of the noncoding region produces eukaryotic speciation and individuality[30]. The integration of nanoarchaea, mevalonate pathway prokaryotes and viroids in to the eukaryotic and human genome produces a chimera which can multiply producing biofilm like multicellular structures having a mixed archaeal, viroidal, prokaryotic and eukaryotic characters which is a regression from the multicellular eukaryotic tissue. This results in a new neuronal, metabolic, immune and tissue phenotype leading to human disease.

The archaea and viroids can regulate the nervous system including the NMDA/GABA thalamocorticothalamic pathway mediating conscious perception[2,31]. NMDA/GABA receptors can be modulated by viroid induced RNA interference[2]. The dipolar viroids combined with actinides in the setting of digoxin induced sodium potassium ATPase inhibition can produce a pumped phonon system mediated frohlich model superconducting state inducing quantal perception with nanoarchaeal sensed gravity producing the orchestrated reduction of the quantal possibilities to the macroscopic world[2,31]. The viroids can regulate limbic lobe transmission by RNA viroid mediated RNA interference modulating norepinephrine, dopamine, serotonin and acetyl choline receptors[18]. The higher degree of integration of the archaea and viroids into the genome produces increased digoxin synthesis producing right hemispheric dominance and lesser degree producing left hemispheric dominance[2]. The viroid RNA interference mediated altered monoamine and NMDA transmission

contributes to the pathogenesis of schizophrenia and autism. Archaea and RNA viroid can bind the TLR receptor induce NFKB producing immune activation and cytokine TNF alpha secretion[2,32]. The archaea and viroid induced chronic immune activation and generation of superantigens can lead on to autoimmune disease. Archaea and viroids can induce the host AKT PI3K, AMPK, HIF alpha and NFKB producing the Warburg metabolic phenotype[33]. The increased glycolytic hexokinase activity, decrease in blood ATP, leakage of cytochrome C, increase in serum pyruvate and decrease in acetyl CoA indicates the generation of the Warburg phenotype. There is induction of glycolysis, inhibition of PDH activity and mitochondrial dysfunction resulting in inefficient energetics and metabolic syndrome. The archaea and viroid generated cytokines can lead to TNF alpha induced insulin resistance and metabolic syndrome x. The accumulated pyruvate enters the GABA shunt pathway and is converted to citrate which is acted upon by citrate lyase and converted to acetyl CoA, used for cholesterol synthesis[33]. The pyruvate can be converted to glutamate and ammonia which is oxidised by archaea for energy needs. The increased cholesterol substrate leads to increased archaeal growth and digoxin synthesis leading to metabolic channeling to the mevalonate pathway[34]. The archaea and viroid induced monocyte activation and Warburg phenotype induced increased cholesterol synthesis leads to atherogenesis. Viroid induced RNA interference can modulate the mRNAs concerned with insulin receptor function and lipid metabolism contributing to metabolic syndrome x. The Warburg phenotype induced increased mitochondrial PT pore hexokinase can lead on to malignant transformation. Viroid induced RNA interference can modulate oncogenes producing malignant transformation. The viroid induced RNA interference can modulate the mRNA concerned with the death receptor pathway producing apoptosis and neuronal degeneration. The RNA viroids can recombine with HERV sequences and get encapsulated in microvesicles contributing to the

retroviral state. The prion protein conformation is modulated by RNA viroid binding producing prion disease.

Thus the actinidic archaea generated RNA viroids can regulate cell function and produce neuro-immuno-genetic-endocrine-metabolic integration. The RNA viroids and their complementary DNA can serve the purpose of viral speciation. The RNA viroids also contributes to the pathogenesis of schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration.

References

[1] Valiathan M. S., Somers, K., Kartha, C. C. (1993). *Endomyocardial Fibrosis.* Delhi: Oxford University Press.

[2] Kurup R., Kurup, P. A. (2009). *Hypothalamic digoxin, cerebral dominance and brain function in health and diseases*. New York: Nova Science Publishers.

[3] Hanold D., Randies, J. W. (1991). Coconut cadang-cadang disease and its viroid agent, *Plant Disease*, 75, 330-335.

[4] Edwin B. T., Mohankumaran, C. (2007). Kerala wilt disease phytoplasma: Phylogenetic analysis and identification of a vector, *Proutista moesta*, *Physiological and Molecular Plant Pathology*, 71(1-3), 41-47.

[5] Eckburg P. B., Lepp, P. W., Relman, D. A. (2003). Archaea and their potential role in human disease, *Infect Immun*, 71, 591-596.

[6] Adam Z. (2007). Actinides and Life's Origins, *Astrobiology*, 7, 6-10.

[7] Schoner W. (2002). Endogenous cardiac glycosides, a new class of steroid hormones, *Eur J Biochem*, 269, 2440-2448.

[8] Davies P. C. W., Benner, S. A., Cleland, C. E., Lineweaver, C. H., McKay, C. P., Wolfe-Simon, F. (2009). Signatures of a Shadow Biosphere, *Astrobiology*, 10, 241-249.

[9] Wächtershäuser G. (1988). Before enzymes and templates: theory of surface metabolism, *Microbiol Rev*, 52(4), 452-84.

[10] Richmond W. (1973). Preparation and properties of a cholesterol oxidase from nocardia species and its application to the enzymatic assay of total cholesterol in serum, *Clin Chem*, 19, 1350-1356.

[11] Snell E. D., Snell, C. T. (1961). *Colorimetric Methods of Analysis.* Vol 3A. New York: Van NoStrand.

[12] Glick D. (1971). *Methods of Biochemical Analysis.* Vol 5. New York: Interscience Publishers.

[13] Colowick, Kaplan, N. O. (1955). *Methods in Enzymology.* Vol 2. New York: Academic Press.

[14] Maarten A. H., Marie-Jose, M., Cornelia, G., van Helden-Meewsen, Fritz, E., Marten, P. H. (1995). Detection of muramic acid in human spleen, *Infection and Immunity*, 63(5), 1652-1657.

[15] Smit A., Mushegian, A. (2000). Biosynthesis of isoprenoids via mevalonate in Archaea: the lost pathway, *Genome Res*, 10(10), 1468-84.

[16] Van der Geize R., Yam, K., Heuser, T., Wilbrink, M. H., Hara, H., Anderton, M. C. (2007). A gene cluster encoding cholesterol catabolism in a soil actinomycete provides insight into Mycobacterium tuberculosis survival in macrophages, *Proc Natl Acad Sci USA*, 104(6), 1947-52.

[17] Francis A. J. (1998). Biotransformation of uranium and other actinides in radioactive wastes, *Journal of Alloys and Compounds*, 271(273), 78-84.

[18] Probian C., Wülfing, A., Harder, J. (2003). Anaerobic mineralization of quaternary carbon atoms: Isolation of denitrifying bacteria on pivalic acid (2, 2-Dimethylpropionic acid), *Applied and Environmental Microbiology*, 69(3), 1866-1870.

[19] Vainshtein M., Suzina, N., Kudryashova, E., Ariskina, E. (2002). New Magnet-Sensitive Structures in Bacterial and Archaeal Cells, *Biol Cell*, 94(1), 29-35.

[20] Tsagris E. M., de Alba, A. E., Gozmanova, M., Kalantidis, K. (2008). Viroids, *Cell Microbiol*, 10, 2168.

[21] Horie M., Honda, T., Suzuki, Y., Kobayashi, Y., Daito, T., Oshida, T. (2010). Endogenous non-retroviral RNA virus elements in mammalian genomes, *Nature*, 463, 84-87.

[22] Hecht M., Nitz, N., Araujo, P., Sousa, A., Rosa, A., Gomes, D. (2010). Genes from Chagas parasite can transfer to humans and be passed on to children. Inheritance of DNA Transferred from American Trypanosomes to Human Hosts, *PLoS ONE*, 5, 2-10.

[23] Flam F. (1994). Hints of a language in junk DNA, *Science*, 266, 1320.

[24] Horbach S., Sahm, H., Welle, R. (1993). Isoprenoid biosynthesis in bacteria: two different pathways? *FEMS Microbiol Lett*, 111, 135-140.

[25] Gupta R. S. (1998). Protein phylogenetics and signature sequences: a reappraisal of evolutionary relationship among archaebacteria, eubacteria, and eukaryotes, *Microbiol Mol Biol Rev*, 62, 1435-1491.

[26] Hanage W., Fraser, C., Spratt, B. (2005). Fuzzy species among recombinogenic bacteria, *BMC Biology*, 3, 6-10.

[27] Whitchurch C. B., Tolker-Nielsen, T., Ragas, P. C., Mattick, J. S. (2002). Extracellular DNA Required for Bacterial Biofilm Formation. *Science*, 295(5559), 1487.

[28] Webb J. S., Givskov, M., Kjelleberg, S. (2003). Bacterial biofilms: prokaryotic adventures in multicellularity, *Curr Opin Microbiol*, 6(6), 578-85.

[29] Chen Y., Cai, T., Wang, H., Li, Z., Loreaux, E., Lingrel, J. B. (2009). Regulation of intracellular cholesterol distribution by Na/K-ATPase, *J Biol Chem*, 284(22), 14881-90.

[30] Poole A. M. (2006). Did group II intron proliferation in an endosymbiont-bearing archaeon create eukaryotes? *Biol Direct*, 1, 36-40.

[31] Lockwood M. (1989). *Mind, Brain and the Quantum*. Oxford: B. Blackwell.

[32] Eberl M., Hintz, M., Reichenberg, A., Kollas, A., Wiesner, J., Jomaa, H. (2010). Microbial isoprenoid biosynthesis and human γδ T cell activation, *FEBS Letters*, 544(1), 4-10.

[33] Wallace D. C. (2005). Mitochondria and Cancer: Warburg Addressed, *Cold Spring Harbor Symposia on Quantitative Biology*, 70, 363-374.