

Chapter 10

**Endosymbiotic Actinidic Archaeal Mediated Warburg
Phenotype Mediates Cancer**

Introduction

Endomyocardial fibrosis along with the root wilt disease of coconut is endemic to Kerala with its radioactive actinide beach sands. Actinides like rutile as well as organisms like phytoplasmas and viroids have been implicated in the etiology of cancer.¹⁻⁴ The Warburg phenotype has been related to the pathogenesis of malignancy.⁴ The possibility of Warburg phenotype induced by actinide based primitive organism like archaea with a mevalonate pathway and cholesterol catabolism was considered in this paper.⁵⁻⁸ An actinide dependent shadow biosphere of archaea and viroids in cancer is described.^{7,9}

Materials and Methods

The following groups were included in the study: - non-hodgkin's lymphoma and glioma. 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+ciprofloxacin 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 Cholesterol substrate was prepared as described by Richmond.¹⁰ Aliquots were withdrawn at zero time immediately after mixing and after incubation at 37°C for 1 hour. The following estimations were carried out: - Cytochrome F420 and hexokinase.¹¹⁻¹³ Cytochrome F420 was estimated fluorimetrically (excitation wavelength 420 nm and emission wavelength 520 nm). Informed consent of the subjects and the

approval of the ethics committee were obtained for the study. The statistical analysis was done by ANOVA.

Results

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 rutilone increased their levels. The addition of antibiotics to the patient's plasma caused a decrease in all the parameters while addition of rutilone 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 1. Effect of rutilone and antibiotics on cytochrome F420.

Group	CYT F420 % (Increase with Rutilone)		CYT F420 % (Decrease with Doxy+Cipro)	
	Mean	± SD	Mean	± SD
Normal	4.48	0.15	18.24	0.66
NHL	22.79	2.13	55.90	7.29
Glioma	22.70	1.87	60.46	8.06
F value	306.749		130.054	
P value	< 0.001		< 0.001	

Table 2. Effect of rutilone and antibiotics on hexokinase.

Group	Hexokinase % change (Increase with Rutilone)		Hexokinase % change (Decrease with Doxy+Cipro)	
	Mean	± SD	Mean	± SD
Normal	4.21	0.16	18.56	0.76
NHL	22.53	2.41	64.29	5.44
Glioma	21.66	1.94	67.03	5.97
F value	292.065		317.966	
P value	< 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.^{6,14} The archaeal 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.^{15,16} The archaeal glycolytic hexokinase activity were increased. The part of the increased glycolytic hexokinase activity detected is human. The archaea can undergo magnetite and calcium carbonate mineralization and can exist as calcified nanoforms.¹⁷

Archaea can induce the host AKT PI3K, AMPK, HIF alpha and NFkB producing the Warburg metabolic phenotype.¹⁸ The increased glycolytic hexokinase activity indicates the generation of the Warburg phenotype. The generation of the Warburg phenotype is due to activation of HIF alpha. This stimulates anaerobic glycolysis, inhibits pyruvate dehydrogenase, inhibits mitochondrial oxidative phosphorylation, stimulates heme oxygenase, stimulates VEGF and activates nitric oxide synthase. This can lead to increased cell proliferation and malignant transformation. The mitochondrial PT pore hexokinase is increased leading onto cell proliferation. There is induction of glycolysis, inhibition of PDH activity and mitochondrial dysfunction resulting in inefficient energetics and insulin resistance. Insulin resistance has been related to cancer. The archaea and viroid generated cytokines can lead to TNF alpha induced insulin resistance and cancer. The increase in the glycolytic enzyme fructose 1,6 diphosphatase increases the pentose phosphate pathway. This generates NADPH which activates NOX. NOX activation is related to NMDA activation and glutamate excitotoxicity. This leads onto oncogenesis.¹⁸

The increase in glycolysis activates the enzyme fructose 1,6 diphosphatase which activates the pentose phosphate pathway liberating NADPH. This increases NOX activity generating free radical stress and H_2O_2 . Free radicals have been related to cancer. Free radical stress is also related to insulin resistance and cancer. Free radicals can activate NF κ B producing immune activation and lymphoproliferative disorder. Free radicals can open the mitochondrial PT pore, produce release of cyto C and activate the caspase cascade. This produces mitochondrial dysfunction and oncogenesis. Free radicals can also produce oncogene activation and malignant transformation. Free radicals can produce HDAC inhibition and HERV generation. The encapsulation of HERV particles in phospholipids vesicles can mediate the generation of endogenous retroviral induced oncogenesis.¹⁸

The lymphocytes depend on glycolysis for its energy needs. The increase in glycolysis owing to the induction of Warburg phenotype can lead to immune activation. Immune activation can lead to lymphoproliferative disorder. TNF alpha can activate the NMDA receptor leading to glutamate excitotoxicity and oncogenesis. TNF alpha can induce expression of HERV particles contributing to generation of oncogenesis. Immune activation has also been related to malignant transformation mediated by NF κ B. TNF alpha can also act upon the insulin receptor producing insulin resistance and oncogenesis. NOX activation consequent to the generation of the Warburg phenotype also activates the insulin receptor. Thus there is a hyperinsulinemic state leading on to oncogenesis.¹⁸

Thus the induction of the Warburg phenotype can lead to malignancy. The Warburg phenotype leads to inhibition of pyruvate dehydrogenase and accumulation of pyruvate. 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. 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 further induction of the Warburg phenotype.¹⁸

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