CHAPTER 5

BIOCHEMISTRY

Doctoral Theses

01. DHEMBLA (Chetna)
Characterization of Proteins Essential in Fatty Acid Biosynthesis Pathway of Leishmania Major.
Supervisors: Prof. Alo Nag, Prof. Suman Kundu and Dr.Monica Sundd
Th 25710

Abstract

Leishmaniases imposes devastating impacts on world's population. The increasing prevalence of drug resistance, necessity for long-term treatment regime and unavailability of functional drugs underscores the need for new drugs and drug targets. The type Il fatty acid biosynthetic (FAS) pathway is highly active in blood stream stage of many parasites and is altered in drug resistant strains, directly associating it with virulence and pathogenesis. It is also a validated drug target in pathogenic bacteria and certain viruses and was thus investigated for Leishmania. The first step of FAS involves 4'phosphopantetheinyl transferase enzyme (PPT) that helps in the transfer of pantetheine arm from coenzymeA to conserved serine residue in acyl carrier protein (ACP) thus activating it to the holo-form for downstream reactions. Extensive site-directed mutagenesis and crystal structure of ACP were used to gain insights into the interaction interface of the proteins. The in vivo interaction of PPT and ACP was studied employing co-immunoprecipitation and pull down assays using Leishmania donovani cultur9 system. The mechanism of substrate recognition was investigated using NMR titration analysis, saturation transfer difference NMR, 31 P NMR, SPR, site-directed mutagenesis, HPLC and Native PAGE for octanoyl transferase (LipB) enzyme involved in the lipoic acid biosynthesis pathway, an offshoot of the FAS pathway, which takes up the octanoyl chain from Q-ACP, a by-product of the type Il FAS, and transfers it to a conserved lysine of the lipoyl domain of a dehydrogenase. Further, through whole cell-based screening of more than 500 NCI library compounds, we obtained one molecule that effectively inhibits promastigote as well as axenic and intramacrophagic stage of Leishmania donovani. The molecule was non-toxic and portrayed high selectivity window for the parasite over mammalian cells. An amalgamation of computational and experimental studies deconvoluted LmLipB as the probable target for its anti-leishmanial action.

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02. KHAN (Mohd.Asim)
Structural and Functional Characterization of Novel and Recombinant Hemoglobins, with Implications of the Latter in the Production of Blood Substitutes.
Supervisor: Prof. Suman Kusdu
Th 25711

Abstract

The recombinant hemoglobin-based oxygen carriers (rHBOC) enjoys several advantages as blood substitutes. The wide use of rHBOCs in humans, however, has been limited due to many shortcomings. One of the major issues is rapid heme dissociation from Hbs resulting in toxicity. Thus, correct folding, proper insertion and orientation of the heme within the protein, and long-term stability are key issues that need to be resolved for both the production of HBOCs in bacteria and their efficacy as safe transfusion agents. The primary aim is to solve such issues of globin instability and rapid heme loss, leading to the design of stable HBOCs, using protein engineering approaches. In this context, we successfully engineered recombinant human Hb0.1 (rHb0.1), a prototype of HBOC, to resist heme dissociation, while being resistant to auto-oxidation, and having oxygen affinity equivalent to natural RBC Hb, characteristics that can be exploited to develop rHBOCs. In order to enhance polypeptide stability of rHb0.1, we also investigated a novel globin from a thermophilic organism in comparison to Hbs from mesophilic organism. Contrary to expectations, Hb from G. sulphuraria, the thermophilic organism, was less stable in vitro relative to its mesophilic counterparts and exhibited irreversible thermal denaturation. Structural analysis revealed parameters that might dictate protein stability in globins, for future applications. In addition, research has indicated that Hb in the blood of animals adapted for high altitude survival often carry mutations. We believe that similar change might exist in humans who survive at high altitude for generations. Blood samples of high altitude natives was thus processed and complete amino acid sequences of Hb (α- and β- chains) were identified by employing mass spectrometry. Possibility existed for the presence of mutation in the Hb of humans dwelling at high altitude that increases O2 affinity, which may also be engineered in rHb0.1.

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03. LATHA (Thammineni Krishna)
Study to Assess an Association of Oraganochlorine Pesticide Exposure with Hypoxia and Lymph Node Metastasis in the Patients of Breast Cancer.
Supervisors: Prof. B.D. Banerjee and Prof. Navneet Kaur
Th 25709

Abstract

The incidence of breast cancer in India is on rise, superseding the cervical cancer in urban India. The etiology of breast cancer is multifaceted, involving genetic, epigenetic, lifestyle and environmental factors, however, the exact cause yet remains vague. Despite, the fact that environmental factors increase the risk of breast cancer, the true burden of environmentally induced breast cancer has been grossly underestimated. Among all environmental factors, Organochlorine Pesticides have been mostly linked with the breast cancer as they are xenoestrogens in nature. Therefore, present study was designed to estimate the OCPs level and to correlate them with the genes involved in hypoxia and metastasis in breast cancer patients. The current study has recruited 100 breast cancer patients as cases and 100 benign breast disease patients as controls. Quantification of OCPs was done by Gas Chromatography. Analysis of hypoxia and metastasis promoting genes was done at mRNA and protein level by Real time PCR and Western blot, respectively.Significantly higher levels of γHCH (p value=0.04), β-Endosulfan (p value =0.01), p'p'DDT (p value= 0.01) and o'p'DDT (p value= 0.02) were found in cases with respect to controls. At the expression levels HIF-1α, CA-IX, MMP-9, VEGF-C were upregulated and KAI-1 & ER- β was down regulated at both mRNA and protein levels in cases. Further, β-Endosulfan, pp'DDT were positively correlated with hypoxia genes and β-Endosulfan, & γHCH with metastasis promoting genes.

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04. SHAH (Harendra Kumar)
Identification of High Risk Phenotypes and Their Association with Organochlorine Pesticides Exposure in Epithelial Ovarian Cancer.
Supervisors: Prof. B.D. Banerjee and Prof. Kiran Guleria
Th 25705

Abstract

The present in vivo study demonstrated a high level of OCPs exposure in ovarian tissue of cases of EOC as compared to control. The high level of OCPs particularly β-HCH, p,p'DDE, Heptachlor, and Endosulfan was found significantly associated with the risk of EOC. Further, a microarray transcriptomic study revealed 163 differential expressed genes involved in several cellular and molecular pathways in EOC ovary tissues as compared to control ovary tissues. Among them, high risk phenotypes as CCR2, PTPRC, G3BP2, UBE2V1, TNFRSF11A, FAK, WNT, TCF, TGFβRII, Smad3, MyD88, VEGF-A, MMP7, and IGFBP7 were identified based on the function of differential expressed genes in the cell and their involvement in the etiology of cancer. The high risk phenotypes are associated with cytoskeleton/WNT/TGFβ, inflammatory, and angiogenesis pathways. The expression of selected high risk phenotypes was further validated at mRNA and protein levels by qPCR and western blot respectively. The expression of CCR2, PTPRC, G3BP2, UBE2V1, TNFRSF11A, FAK, WNT, TCF, TGFβRII, Smad3, MyD88, VEGF-A, MMP7 were upregulated, and IGFBP7 was downregulated which showed a similar pattern with microarray gene expression. This confirms that their activity may involve tumor development, growth, angiogenesis, and invasion in EOC. The Pearson correlation demonstrated a significant relationship between the

level of OCPs exposure and the expression of high risk phenotypes in ovarian tissue. These data reveal that persistent OCPs exposure to ovary tissue might associate with the altered gene expression in ovary tissue toward pathogenesis of EOC.

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05. SINGH (Neeru)
Study on Organochlorine Pesticides- Mediated Renal Cell Dysfunction in Vitro: A Possible Cause of Chronic Kidney Disease of Unknown Etiology.
Supervisor: Prof. B.D. Banerjee
Th 25708

Abstract

This study investigated the potential of Orgnanochlorine pesticides (OCP) in causing renal cellular damage (HK-2 cells) and thus contributes to Chronic Kidney Disease of unknown etiology (CKDu). Following treatment of HK-2 cells with an increasing concentration of OCPs (Heptachlor, p,p-DDT and dieldrin), the cellular viability significantly declined whereas the intracellular reactive oxygen species (ROS) augmented significantly in a dose-dependent manner. Pre-treatment with N-acetyl cysteine (NAC) attenuates the OCP induced oxidative stress (OS) in HK-2 cells. In this study, it has been demonstrated that OCPs induced OS regulates the mRNA expression of TGF-β-mediated Smad signalling genes accompanied by increased nuclear localization of phosphorylated Smad-2, phosphorylated Smad-3 and phosphorylated Smad-4. Furthermore, the m-RNA expression and activation of protein level of an epithelial marker, E-cadherin decreased while that of mesenchymal marker, α-smooth muscle actin (α-Sma) increased in OCPs exposed HK-2 cells. In conclusion, OCP induced OS might be responsible for the activation of TGF-β/Smad signalling which ultimately leads to renal damage by means of Epithelial to mesenchymal transition (EMT). OCPs induced oxidative stress (OS) on transforming growth factor-β1 (TGF-β1) mediated epithelial to mesenchymal transition (EMT) in human renal proximal tubular epithelial (HK-2) cells

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06. TAWAR (Neha)
Association of Organochlorine Pesticides with Endoplasmic Reticulum Stress Response in Type 2 Diabetes.
Supervisors: Prof. B.D. Banerjee, Prof. S.V. Madhu and Prof. Sanjay Gupta
Th25712

Abstract

The present study compared the organochlorine pesticides accumulation in visceral adipose tissue between subjects with NGT and T2DM, and their association with genes associated with endoplasmic reticulum stress and inflammatory pathways. There was a significantly high level of ΔHCH, heptachlor, endrin, endosulfan II, p,p' DDT, o,p' DDE, p,p' DDE, and p,p' DDD in T2DM subjects as compared to subjects with NGT. Among all the OCPs detected, endrin was present in the highest concentration in vAT of T2DM subjects. The study provides satisfactory evidence that OCPs may enhance the risk of T2DM through modulating the expression of genes associated with ER stress. The accumulation of ΔHCH and endrin in vAT may pose a greater risk of the development of T2DM. The positive association of these two pesticides with cellular ER stress might be one of the possible mechanisms of diabetes pathogenesis.

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07. THAKUR (Gaurav Kumar)
Gene Encironment Interaction and Epigenetic Reprogramming of EMT Progression in Epithelial Ovarian Cancer.
Supervisors: Prof. B.D. Banerjee and Prof. Kiran Guleria
Th25706

Abstract

Due to fast changing lifestyles has led to accumulation of environmental pollutants at burgeoning pace, which can lead to adverse health outcome. Among plethora of environmental toxins are pesticides that find its way in agricultural practices. The exposure to pesticides has been implicated in many adverse health outcomes like, hormonal misbalance, neurological disorder, renal diseases, adverse reproductive diseases and fatal diseases like cancer as well. Among the class of pesticides are OCPs, which are potent xenoestogens, hence perturbing the vital female reproductive hormonal balance, which can herald emergence of many hormonally derived diseases. Over the years Epithelial Ovarian Cancer has emerged as one of most fatal gynecological malignancies. The etiology and risk factors associated with EOC remains ambiguous which lead to poor diagnosis. Investigation and identification of molecular and genomic paradigm behind onset of EOC can lead to better management of EOC patients. The advent of many cancers remains dubious, but many studies have underlined the complex and intricate crosstalk between the gene and environment behind advent of cancer. Gene environment is intricate web of interaction between immediate environment and genetic makeup. After taking all results and their interpretations, we can safely conclude that this present thesis work highlight that gene environment interaction is a major factor behind etiology of EOC. The synergistic action of OCP exposure results in epigenetic modulation through differential methylation of

genes. The aberrant methlation of EMT promoting genes has been found to augment the metastatic potential of cells. We found altered genomic expression of EMT promoting genes which were influenced by its methylation status, which in turn were associated with OCP levels in tissues. This study was first of its kind which showed association OCP levels in tissues may lead to of aberrant methylation of EMT promoting genes with in EOC. And lastly this study reveals the axis of gene environment dimension, through OCP levels in tissues and methylation status as one of predisposing factor for EOC.

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