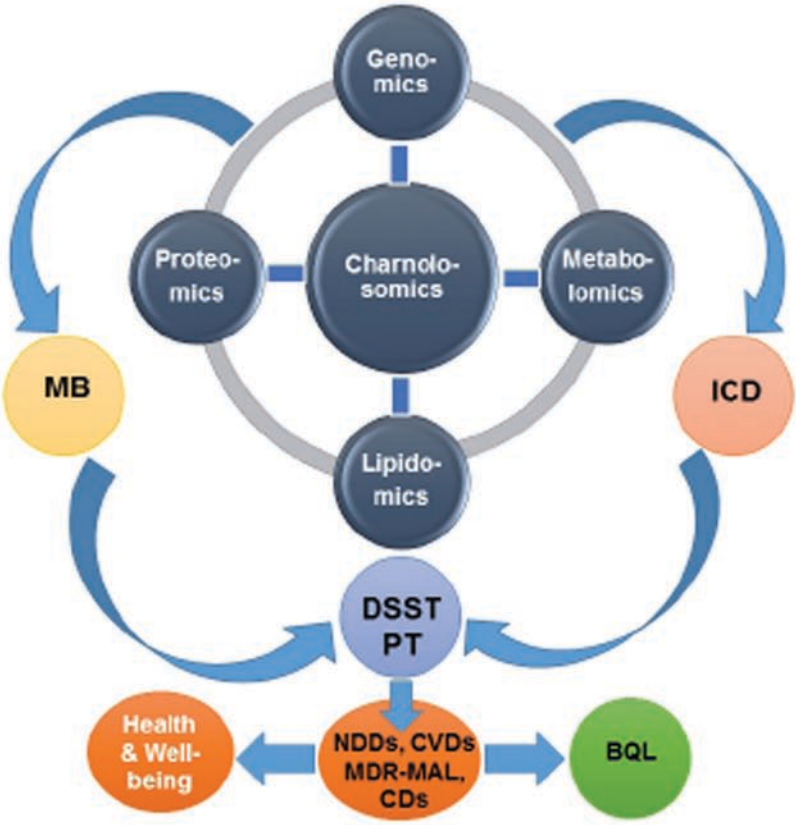
The Charnoly Body

A Novel Biomarker of Mitochondrial Bioenergetics



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A Novel Biomarker of Mitochondrial Bioenergetics

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Acknowledgement

I extend heartiest gratitude to my respected late mother “Charnoly” who motivated me to work hard, face hardships, and be tolerant and patient during social, physical, mental, economical, and psychological crisises in life. It was indeed my mother who guided me to nursery school for the first time. I discovered “Charnoly body” as a doctoral student at the All India Institute of Medical Sciences (A.I.I.M.S.), New Delhi in 1982 when she was alive. I am also highly thankful to my friends, relatives, and professional colleagues who conferred their moral support and persuaded me to discover “Charnoly body”.

My respected teacher, Prof. Christian De Duve from Belgium was awarded the Nobel Prize for his original discovery of lysosomes and peroxisomes in 1974. He was the one to introduce the term “autophagy.” Last year, Prof. Yoshinori Ohsumi from Japan was awarded the Nobel Prize for elucidating the basic molecular mechanism of autophagy. I have been constantly following the footsteps of these legends to discover Charnoly body (CB) and its clinical significance in mitochondrial bioenergetics and intracellular detoxification (ICD) in chronic diseases during my doctoral and postdoctoral research.

I express my sincere thanks to google and the global scientific community for recognizing my original discovery of Charnoly body (CB) as a universal biomarker of physicochemical injury due to free radical-induced compromised mitochondrial bioenergetics (CMB) and charnolophagy as a basic molecular mechanism of intracellular detoxification (ICD) in the most vulnerable cells. Recently, I introduced charnolosome (CS) and disease-specific spatiotemporal (DSST) charnolosomics to develop novel charnolopharmacotherapeutics for the targeted, safe, and effective personalized theranostics of chronic diseases.

I express my sincere thanks to my mentors Dr. Baldev Singh, Dr. Krishnamurti Dakshinamurti, and Dr. Manuchair Ebadi for their moral support and encouragement during my professional and scientific career to discover CB as a novel biomarker of compromised mitochondrial bioenergetics (CMB) in progressive neurodegenerative diseases, cardiovascular diseases, and multidrug resistant malignancies as described elegantly in this book.

I sincerely hope that this manuscript will attract the global biomedical community due to its original, novel, and thought-provoking concepts and mechanisms to discover DSST charnolopharmacotherapeutics for the safe and effective evidence-based personalized theranostics (EBPT) of chronic diseases. Doctors, professors,

researchers, basic scientists, nurses, and the public will enjoy going through its most interesting and motivating contents to enhance their basic knowledge regarding the mitochondrial bioenergetics (MB) and ICD in health and disease.

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Preface

Generally, therapeutic drugs have been designed and targeted to nucleic acid (DNA/RNA) and/or protein synthesis. There are only few drugs based on the mitochondrial bioenergetics (MB) and intracellular detoxification (ICD) in the pharmaceutical industry. This book describes CB as a pre-apoptotic biomarker of compromised mitochondrial bioenergetics (CMB) to develop novel charnolopharmacotherapeutics for the targeted, safe, and effective EBPT of chronic diseases. The book introduces for the first time charnolosomics and disease-specific spatio-temporal (DSST) charnolopharmacotherapeutics for the EBPT of chronic MDR diseases. This edition is an extension of my recently published books: Personalized Medicine (Beyond PET Biomarkers), Progress in PET RPs (Quality Control and Theranostics), and ZIKV Disease (Prevention and cure), Fetal Alcohol Spectrum Disorder (Concepts, Mechanisms, and Cure), and "Nicotinism and Emerging Role of Electronic Cigarettes" by Nova Science Publishers, New York, U.S.A.

It is well-established that free radicals are generated as a byproduct of oxidative phosphorylation in the electron transport chain during ATP synthesis in the mitochondria. The requirements of energy (ATP) are significantly elevated during DPCI in the most vulnerable cell. Thus, free radical-induced CMB induces CB formation involved in impaired ICD and cellular dysfunction. DPCI trigger CB formation as a pleomorphic, electron-dense, quasi-crystalline, multi-lamellar stack of degenerated mitochondrial membranes in the most vulnerable neural progenitor cells (NPCs) and cardiac progenitor cells (CPCs) derived from induced pluripotent cells (iPPCs) in the developing brain and heart, respectively, during the first trimester (gastrulation period) of pregnancy, which causes embryopathies in ZIKV, cytomegalovirus, rubella, and toxoplasma infections, and in nicotine addiction and fetal alcohol exposure. Various anesthetics, anti-epileptics, antidepressants, antipsychotics, and environmental pollutants can also induce charnolopathies, involving microcephaly and chronic diseases such as depression, diabetes, obesity, PD, and AD.

CB is a universal biomarker of cell injury whereas charnolophagy-induced CPS and CS formation serve as novel drug discovery targets to evaluate ICD in the oocyte during the pre-zygotic phase and in the NPCs, EPCs, CPCs, and OPCs, derived from iPPCs, during the post-zygotic phase of embryonic development.

DPCI compromise mitochondrial bioenergetics (MB) in the most vulnerable cells of the developing embryo, particularly during the first trimester of pregnancy (gastrulation period), causing charnolopathies involved in diversified embryopathies. Hence, drugs targeting MB by inhibiting CB formation, augmenting charnolophagy, and stabilizing CPS and CS will be promising charnolopharmacotherapeutics for the targeted, safe, and effective EBPT of chronic diseases, as described in this book.

Accumulation of CB at the junction of axon hillock impairs axoplasmic transport of various ions, enzymes, hormones, neurotrophic factors, neurotransmitters, and mitochondria to cause initially synaptic silence followed by synaptic atrophy, whereas accumulation of CS at the junction of axon hillock releases highly toxic mitochondrial metabolites including: cytochrome-C, iron, 8-OH, 2dG, 2,3, dihydroxy nonenal, acetaldehyde, ammonia, H_2O_2, GAPDH, and several other toxins to cause synaptic degeneration which induce cognitive impairments accompanied with early morbidity and mortality as noticed in FASD, autism, Down's syndrome, PD, AD, ALS, HD, and several other chronic diseases beyond the scope of this manuscript.

Non-specific induction of CB induces alopecia, myelosuppression, neurotoxicity, hepatotoxicity, GIT symptoms, nephrotoxicity, and infertility in MDR malignancies. Hence, drugs may be developed to prevent/inhibit CB formation and augment MB to enhance charnolophagy and CS exocytosis as a basic molecular mechanism of ICD to prevent or treat chronic diseases. Particularly, drugs may be developed to prevent CS destabilization, permeabilization, sequestration, and fragmentation by augmenting antioxidant drug delivery in the diseased organ. Although charnolostatic drugs will be clinically-beneficial to control acute diseased states, disease and organ-specific charnolocidals will be required for chronic MDR diseases as illustrated in this book.

The most unique feature of this edition is that it provides basic molecular mechanisms and the cure of chronic diseases by developing MB-based charnolopharmacotherapeutics as CB antagonists, charnolophagy agonists, and CS stabilizers. The book enhances our basic knowledge regarding safe and effective clinical management of chronic diseases by rejuvenating MB and microRNAs involved in ICD. The book motivates researchers to discover novel DSST charnolopharmacotherapeutics for the safe and effective EBPT of chronic diseases. The primary objective is to regulate MB at the transcriptional and translational level by preventing/inhibiting cell and tissue-specific free radical-induced CB formation involved in post-transcriptional microRNA deregulation.

The original concepts and mechanisms of charnolophagy, CPS, and CS as specific drug discovery targets of CMB for the clinical management of chronic diseases are presented for the first time in this book.

Antioxidants (glutathione, metallothioneins, CoQ_{10}, melatonin, selegiline, resveratrol, sirtuin, rutin, lycopene, and catechin) inhibit CB formation as free radical scavengers. Although, antioxidants can easily pass through blood brain barrier without inducing any adverse effects, their reduced potency necessitates bulk consumption. Hence, ROS-scavenging antioxidant-loaded nanoparticles (NPs) may be developed to enhance CNS drug delivery and augment charnolophagy for ICD as elegantly highlighted in this book.

This manuscript will serve as a text book for biomedical students and nurses, and a reference book for doctors, researchers, professors, and public. Women of reproductive age would like to read its most interesting and thought-provoking concepts for a better quality of life for their progeny. This book will attract the attention of old persons with CMB as well.

Basic biomedical students, researchers, M.D., Ph.D., D.Sc. students, doctors, teachers, and professors at the college and university level, and the public will be interested in studying the novel and thought-provoking contents of this book. Particularly, undergraduate, postgraduate, doctoral, and post-doctoral students,

research associates, and research scientists will find this book interesting and motivating to discover novel therapeutic intervention for the safe and effective EBPT of fetal alcohol spectrum disorders (FASD), Zika viral (ZIKV) disease, AD, PD, major depression, diabetes, obesity, multiple drug addiction, schizophrenia, CVDs, and MDR malignancies.

This newly-released edition of "Charnoly body" by CRC Press, Boca Raton FL, U.S.A. will be interesting, easy to follow, and will facilitate the development of innovative DSST charnolopharmacotherapeutics based on MB for ICD to accomplish the ultimate-goal of targeted, safe, and effective EBPT of chronic diseases. Particularly, basic molecular mechanism(s) and concepts of CB formation, charnolophagy, and CS exocytosis/endocytosis in relation to post-transcriptional regulation of microRNAs promise to provide unique opportunities to discover safe and effective drugs for NDDs, CVDs, MDDs, MDR malignancies, and other chronic inflammatory diseases with currently limited success.

Sushil Sharma

Contents

Abbreviations

AAD	:	Alcohol Abuse Disorder
Alpha-SynMT$_{tko}$	:	Alpha-Synuclein-Metallothionein Triple Knock Out
Ach	:	Acetyl Choline
AD	:	Alzheimer's Disease
ADD	:	Average Daily Dose
ADHD	:	Attention Deficit Hyperactivity Disorder
ADI	:	Acceptable Daily Intake
ADNFLE	:	Autosomal Dominant Nocturnal Frontal Lobe Epilepsy
ADNI	:	Alzheimer's Disease Imaging Initiative
AGEP	:	Advanced Glycation Products
AHR	:	Aryl Hydrocarbon Receptor
AIS	:	Acute Ischmic Stroke
ALARA	:	As-Low-As-Reasonably-Achievable
ALI	:	Air-Liquid Interface
Alpha-Syn$_{ko}$	:	Syn Knock Out
ALS	:	Amyotrophic Lateral Sclerosis
AMPK	:	Adenosine Monophosphate (AMP)-Activated Protein Kinase
ANDS	:	Alternative Nicotine Delivery Systems
ANOVA	:	Analysis of Variance
ANS	:	Autonomic Nervous System
AOC	:	Area Under the Curve
AOPP	:	Advanced Oxidation Products (AOPP)
Apo E	:	Apolipoprotein E
ATR Index	:	Antidepressant Treatment Response Index
ATTUD	:	Association for the Treatment of Tobacco Use and Dependence
Avenic Cigarettes	:	Average Nicotine Cigarettes
Aβ	:	Amyloid-β
B[a]p	:	Benzo [a] Pyrene
BC	:	Biochemical Confirmation
BDDCS	:	Biopharmaceutics Drug Disposition Classification System
BDI	:	Beck Depression Inventory
BDNF	:	Brain Derived Growth Factor
BMI	:	Basal Metabolic Index
BOEs	:	Biomarkers of Exposure
BRITE-MD	:	Biomarkers for Rapid Identification of Treatment Effectiveness in Major Depression

CSF	:	Cerebrospinal Fluid
BSA	:	Bovine Serum Albumin
CAD	:	Cannabis Abuse Disorder
CAD	:	Cocaine Abuse Disorder
CAD	:	Coronary Artery Disease
CAP	:	Cholinergic Anti-Inflammatory Pathway
CB	:	(Charnoly Body: Multi lamellar stacks of electron dense degenerated mitochondrial membranes)
CBT	:	Cognitive-Behavioral Therapy
CCs	:	Combustible Cigarettes
CDC	:	Center for Disease Control and Prevention
CHARON	:	Chemical Analysis of Aerosol Online
CHD	:	Coronary Heart Disease
CHTP	:	Carbon-Heated Tobacco Product
CIPN	:	Chemotherapy-Induced Peripheral Neuropathy
CMB	:	Compromised Mitochondrial Bioenergetics
CMBEL	:	Compromised Mitochondrial Bioenergetic Levels (0–3)
CMV	:	Cytomegalovirus
CO	:	Carbon Monoxide
Complex-1	:	Ubiquinone NADH-Oxidoreductase
COPD	:	Chronic Obstructive Pulmonary Disease
CPS	:	Charnolophagosome
CPs	:	Charnolopharmaceutics
CRP	:	C-Reactive Protein
CS	:	Charnolosome
CS	:	Cigarette Smoking
CSE	:	Cigarette Smoke Extract
CSF	:	Cancer Slope Factor
CSF	:	Cerebrospinal Fluid
CT	:	Computerized Tomography
CTE	:	Chronic Traumatic Encephalopathy
CTLA4	:	Cytotoxic T-Lymphocyte Antigen 4
CVDs	:	Cardiovascular Diseases
DAD	:	Drug Abuse Disorder
DAergic	:	Dopaminergic
DAT	:	DA Transporter
DBS	:	Deep Brain Stimulation
DFX	:	Deferoxamine
DIP-EI/MS	:	Electron Ionization Mass Spectrometry by using a Direct Insertion Probe
DLB	:	Dementia with Lewy Body
DOM	:	Domoic Acid
DP	:	Decay Product
DPCI	:	Diversified Physico-Chemical Injuries
DSM	:	Diagnostic and Statistical Manual of Mental Disorders (IV)
DSST-CS	:	Disease-Specific Spatiotemporal Charnolosome
DTC	:	Disseminated Thyroid Carcinoma

E.M.	:	Electron Microscopy
EBC	:	Eye Blink Conditioning
EBPM	:	Evidence-Based Personalized Medicine
EBPT	:	Evidence-Based Personalized Theranostics
ELISA	:	Enzyme-Linked Immunosorbent Assay
FAPA	:	Flowing Atmospheric-Pressure Afterglow Plasma Ion Source
FAO	:	Fatty Acid Oxidation
[^{18}F]FAZA	:	1-(5-[^{18}F]Fluoro-5-Deoxy-α-D-Arabinofuranosyl)-2-Nitroimidazole
[^{18}F]-FdG	:	Fluorodeoxy Glucose
^{18}F-DOPA	:	^{18}F-Dihydroxyphenylaldehyde
^{18}F-FAZA	:	^{18}F-Fluoroazomycin-Arabinoside
^{18}F-FB-VAD-FMK	:	[^{18}F]4-Fluorobenzylcarbonyl-Val-Ala-Asp(OMe)-Fluoromethylketone
^{18}F-FMSIO	:	^{18}F-Fluoromisonidazole
fMRI	:	Functional Magnetic Resonance Imaging
FST	:	Forced Swimming Test
FTND	:	Fagerstrom Test for Nicotine Dependence
FTQ	:	Fonds de Solidarité
GAS	:	Goldberg Anxiety Scale
GATS	:	Global Adult Tobacco Survey
GD	:	Grave's Disease
GDS	:	Geriatric Depression Scale
GEE	:	Generalized Estimating Equations
GFP	:	Green-Fluorescence Protein
GIF	:	Growth Inhibitory Factor
Glut-1	:	Glucose Transporter-1
GO	:	Grave's Orbitopathy
GTS	:	Green Tobacco Sickness
GSD	:	Geometric Standard Deviations
GSH	:	Reduced Glutathione
GUI	:	Graphic User Interphase
GWAS	:	Genome Wide Association Studies
H_2O_2	:	Hydrogen Peroxide
6-OH-DA	:	6-Hydroxy Dopamine
HAM-D	:	Hamilton Scale of Depression
HD	:	Huntington's Disease
HDL	:	High Density Lipoprotein
HDL-C	:	High Density Lipoprotein-Cholesterol
HFD	:	High Fat Diet
HG	:	High Glucose
HIF-1α	:	Hypoxia-Inducible Factor-1α
Hinic Cigarettes	:	High Nicotine Cigarettes
HO-1	:	Heme Oxygenase-1
HONC	:	Hooked on Nicotine Checklist
Hp	:	Helicobacter Pylori
HPC	:	Hypoxic Preconditioning

HPHCs	:	Harmful and Potentially Harmful Constituents
HQ	:	Hazard Quotient
HRE	:	Hypoxia Response Element
HRMS	:	High-Resolution Mass Spectrometry
HSP	:	Heat Shock Protein
HSP-70	:	Heat Shock Protein-70
HSPs	:	Heat Shock Proteins
HS-SPME	:	Headspace Solid-Phase Micro-Extraction
5-HTTLPRP	:	Serotonin-Transporter-Linked Promoter Region Polymorphisms
IAQ	:	Indoor Air Quality
ICD	:	International Classification of Diseases
ICP-MS	:	Inductively-Coupled Plasma Mass Spectrometer
ICTL	:	Intracellular Toxicity Levels (0–3)
IDRS	:	Illicit Drug Reporting System
IL-10	:	Interleukin-10
IRS	:	Infrared Spectroscopy
IUR	:	Inhalation Unit Risk
KA	:	Kainic Acid
KRS	:	Kufor-Rakeb Syndrome
LADA	:	Latent Autoimmune Diabetes of Adulthood
LADD	:	Lifetime Average Daily Dose
LBs	:	Lewy Body
LC-MS	:	Liquid Chromatography-Tandem Mass Spectrometry
LCR	:	Life-Time Cancer Risk
LDCT	:	Lung Cancer Screening with Low-Dose Computed Tomography
LDL	:	Low Density Lipoprotein
LED	:	Light Emitting Diode
LOD	:	Limits of Detection
Lox	:	Lipoxygenase
LPB	:	Lipopolysaccharide Binding Protein
LPB	:	Lipoprotein Binding Protein
lp-ntPET	:	Linear Parametric Neurotransmitter PET
LPS	:	Lipopolysaccharides
LRP	:	Lung Resistance Related Protein
LSUT	:	Lung Screen Uptake Trial
MAD	:	Morphine Abuse Disorder
MB	:	Mitochondrial Bioenergetics
MCAO	:	Middle Cerebral Artery Occlusion
MCI	:	Mild Cognitive Impairment
MDD	:	Major Depressive Disorder
MDMA	:	Methylene Deoxy Methamphetamine
MDR	:	Multi Drug Resistance
MEAD	:	Methamphetamine Abuse Disorder
MEMS	:	Micro-Electromechanical Systems
MIC	:	Metabolism-Informed Care

MicroPET	:	Micro-Positron Emission Tomography
MIPs	:	Molecularly Imprinted Polymers
miRNA	:	microRNA
MMPs	:	Matrix Metalloproteinases
MNCs	:	Mononuclear Cells
MNWS	:	Minnesota Nicotine Withdrawal Scale
MOFs	:	Metal Organic Frameworks
MPP^+	:	1-Methyl, 4-Phenyl, Pyridinium Ion
MPTP	:	1-Methyl, 2-Phenyl, 1, 2, 3, 6-Tetrahydropyridine
MRE	:	Metal Response Element
MRI	:	Magnet Resonance Imaging
MRS	:	Magnetic Resonance Spectroscopy
MS	:	Multiple Sclerosis
MSA	:	Multiple System Atrophy
MT	:	Metallothionein
MT_{dko}	:	Metallothionein Double Gene Knockout
MTF-1	:	Metal Transcription Responsive Factor-1
MTG	:	MitoTracker Green
MTs	:	Metallothioneins
MT_{trans}	:	Metallothionein Transgenic
nAChR	:	Alpha-4 Nicotinic Acetylcholine Receptor
NAFLD	:	Nonalcoholic Fatty Liver Disease
NASH	:	Non-Alcoholic Steatohepatitis
NDDs	:	Neurodegenerative Disorders
Nef2	:	Nuclear Factor Erythroid 2-Related Factor
NIDA	:	National Institute on Drug Addiction
NIMH-RDCI	:	National Institute of Mental Health-Research Domain Criteria Initiative
NIOSH	:	National Institute for Occupational Safety and Health
nm	:	Nanometer
NMR	:	Nuclear Magnetic Resonance
NMR	:	Nicotine Metabolite Ratio
NNAL	:	4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanol (A Pulmonary Carcinogen)
NNC	:	Normal Nicotine Content Cigarettes
NNCs	:	Normal Nicotine Content (NNC) Cigarettes (Nicotine: 1 Mg/Cigarette)
NNN	:	N-Nitrosonor Nicotine
NNK	:	4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanone
NNN	:	N'-Nitrosonor Nicotine
NO	:	Nitric Oxide
NOS	:	Nitric Oxide Synthase
NPs	:	Nanoparticles
NR	:	Nuclear Reaction
NSAIDs	:	Nonsteroidal Anti-Inflammatory Drugs
OGD	:	Oxygen and Glucose Deprivation
$ONOO^-$	:	Peroxynitrite Ion

OPLS-DA	:	Orthogonal Projection to Latent Structures Discriminant Analysis
OSHA	:	Occupational Safety and Health Administration
OTC-NRT	:	Over the Counter-Nicotine Replacement
PA	:	Passive Avoidance
PAH	:	Polycyclic Aromatic Hydrocarbons
PAI	:	Plasminogen Activator Inhibitor-1
PAMAM	:	Poly (Amidoamine) Dendrimer
PC	:	Proxy Confirmation
PCA	:	Principal Component Analysis
PD	:	Parkinson's Disease
PEG	:	Poly Ethylene Glycol
Pegylation	:	Covalent Conjugation of Drug with PEG
PEL	:	Permissible Exposure Limit
PET	:	Positron Emission Tomography
PGAM1	:	Phosphoglycerate Mutase 1
PGLA	:	Poly (Lactic-co-glycolic) Acid
PIB	:	Pittsburg Compound-B
PKs	:	Pharmacokinetics
PLN	:	Polymer Lipid Nanoparticle
PLS-DA	:	Partial Least Squares-Discriminant Analysis
PM	:	Particulate Matter
PNs	:	Parkinsonian Neurotoxins
PNS	:	Peripheral Nervous System
POMS	:	Profile of Mood State
PPA	:	Point Prevalence of Abstinence
PROMIS®	:	Patient Reported Outcomes Measurement Information System
PT	:	Personalized Theranostics (individualized diagnosis and treatment simultaneously: particularly significant for highly proliferative malignant carcinomas where there is a limited time window for diagnosis as well as treatment)
PTPN22	:	Protein Tyrosine Phosphatase
PTR-ToF-MS	:	Proton-Transfer-Reaction Time-of-Flight Mass Spectrometer
PTSD	:	Post-Traumatic Stress Disorder
PTX	:	Paclitaxel
PUFA	:	Polyunsaturated Fatty Acids
PV/VG	:	Propylene Glycol/Vegetable Glycerin
PVD	:	Peripheral Vascular Disease
PWID	:	People Who Inject Drugs
^{62}Cu-PTSM	:	^{64}Cu-Pyrualdehyde Bis-N-Methylthiosemicarbazone
QSPR	:	Quantitative Structure–Permeability Relationships
QSU	:	Questionnaire of Smoking Urges
RDS	:	Radioisotope Delivery System
REL	:	Recommended Exposure Limit
REM	:	Rapid Eye Movement
RES	:	Reticulo Endothelial System

RfC	:	Reference Concentration
RfD	:	Reference Dose
RhO_{mgko}	:	Mitochondial Genome Knock Out
RI	:	Reflection Index
RNS	:	Reactive Nitrogen Species
ROC	:	Receiver Operating Characteristic
ROI	:	Region of Interest
RONS	:	Reactive Oxygen and Nitrogen Species
ROS	:	Reactive Oxygen Species
RPs	:	Radiopharmaceuticals
RPU	:	Regular Psychostimulant Users
rtfMRI	:	Real Time Functional Magnetic Resonance Imaging
SAGE	:	Serial Analysis of Gene Expression
SHS	:	Secondhand Smoking
SI	:	Stiffness Index
SI	:	α-Synculein Index
SIDS	:	Sudden Infant Death Syndrome
SIN-1	:	3-Morpholinosydnonimine
SLE	:	Systemic Lupus Erythromatosus
SNP	:	Single Nucleotide Polymorphism
SOD	:	Superoxide Dismutase
SPD	:	Serious Psychological Distress
SPECT	:	Single Photon Emission Computerized Tomography
SR	:	Self-Reported
SRNT	:	Society for Research on Nicotine and Tobacco
SSRIs	:	Specific Serotonin Reuptake Inhibitors
ST	:	Smokeless Tobacco
STDS	:	Charnolosome: Spatio-Temporal Disease-Specific Charnolosome
STS-CPS	:	Spatio-Temporally-Specific Charnolopharmaceuticals
SUDs	:	Substance Use Disorders
α-Syn	:	α-Synculein
SI	:	α-Synuclein Index (Nitrated α-Syn/Native α-Syn)
α-SynMT_{tko} Mice	:	α-Synuclein Metallothioneins Triple Knockout Mice
^{68}Ga-TETA	:	^{68}Ga-Tetraazamacrocyclic-1,4,8,11-Tetraazacyclotetradecane-1,4,8,11-Tetraacetic Acid
TBI	:	Traumatic Brain Injury
Tg	:	Thyroglobulin
TG	:	Triglyceride
TGA	:	Thermogravimetric Analysis
THS	:	Tobacco Heating System
TIMPs	:	Tissue Inhibitors of Matrix Metalloproteinases
tMCAO	:	Transient Middle Cerebral Artery Occlusion
TMD	:	Total Mood Disturbance
TMRE	:	Tetramethylrhodamine Ethyl Ester
TNFα	:	Tumor Necrosis Factor-α
TP	:	Target Product

TP	:	Transformation Products
TRD	:	Treatment-Resistant Depression
TSNA	:	Tobacco Specific Nitrosamines
TSRH	:	Thyroid Stimulating Hormone Receptor
TUDs	:	Tobacco Use Disorders
UHPLC-QTOF-MS	:	Ultrahigh-Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry
UN	:	Undernutrition
UPLC	:	Ultra-Performance Liquid Chromatography
US	:	Ultrasound
USPSTF	:	US Preventive Services Task Force
UV/VIS Analysis	:	Ultraviolet/Visible Analysis
VaD	:	Vascular Dementia
VEGF	:	Vasoendothelial Derived Growth Factor
VI	:	Vulnerability Index
VLDL	:	Very Low-Density Lipoproteins
VLNC	:	Very Low Nicotine Content Cigarettes
VOCs	:	Volatile Organic Compounds
WHO	:	Word Health Organization
WISDM	:	Wisconsin Inventory of Smoking Dependence Motives
wv/wv Mice	:	Homozygous Weaver Mutant Mice
wv/wv-MTs	:	Metallothioneins Over-Expressing Weaver Mice

Definitions

Vulnerable Cells. The cells derived from induced pluripotent stem cells and mesenchymal stem cells (such as neural progenitor cells, cardiac progenitor cells, endothelial progenitor cells, hepatic progenitor cells, renal progenitor cells, pulmonary progenitor cells, and osteogenic progenitor cells) particularly in the developing embryo are the most vulnerable to physicochemical injury.

Diversified Physicochemical Injury (DPCI). Physicochemical injury can occur due to severe malnutrition, toxic environmental exposure, and in response to microbial (bacteria, viral, and fungal) infection to cause mitochondrial oxidative and nitrative stress and triggers Charnoly body (CB) formation in the most vulnerable cell. **Note:** The term "diversified physicochemical injury (DPCI)" has been used in this book to represent malnutrition, toxic environmental exposure, and/or microbial (bacteria, viral and fungal) infection in the most vulnerable cell.

Charnoly Body (CB). Charnoly body is a pleomorphic, multi-lamellar, electron-dense, quasi-crystalline, inclusion body which is generated in the most vulnerable cell due to free radical-induced degeneration and condensation of the mitochondrial membranes. Free radicals ($\cdot OH$, $NO\cdot$, $CO\cdot$) are generated as a byproduct of mitochondrial oxidative phosphorylation in the electron transport chain. Free radical production is significantly augmented in DPCI. The energy (ATP) requirement is significantly increased during DPCI to eliminate CB as a basic molecular mechanism of ICD to sustain normal function in the most vulnerable cell. Free radicals cause degeneration of the mitochondrial membranes, which condense to form electron-dense penta or hepta-lamellar structures as an initial attempt to contain highly toxic mitochondrial metabolites such as cytochrome-C, 2,3-dihydroxy nonenal, 8-OH 2dG, acetaldehyde, H_2O_2, ammonia, GAPDH, monoamine oxidases (MAOs), TSPO (which serves as a cholesterol transport channel for the synthesis of steroid hormones to stabilize the mitochondrial and other intracellular membranes), and a canonical calcium channel (TRPC) protein. These proteins are delocalized during CB formation which disrupts intracellular homeostasis to initiate cellular demise. Hence, CB can serve as a universal pre-apoptotic biomarker of CMB.

Charnolosomics. A bioinformatic approach to analyze CB, CS, and charnolophagy biomarkers employing state of the art biotechnologies to evaluate mitochondrial bioenergetics (MB) and ICD for normal cellular function to accomplish the targeted, safe, and effective EBPT of chronic MDR diseases for a better quality of life.

Charnolophagy. An energy (ATP) driven phagocytosis of CB.

Charnolophagosome. A lysosome containing phagocytosed CB.

Charnolosome. An intracellular organelle following complete hydrolysis of CB by lysosomal enzymes.

Charnolopharmacotherapeutics. CB-targeted therapeutic drugs designed based on the CMB involving CB prevention or inhibition, charnolophagy induction, charnolophagosome (CPS)/charnolosome (CS) stabilization, and their exocytosis and endocytosis as a basic molecular mechanism of ICD for a normal cellular function.

Charnolophagy Index. A ratio of charnolophagy versus autophagy.

Charnolophagosome (CPS). A highly unstable and functionally-labile intracellular organelle which is formed following charnolophagy. It is electron-dense and almost 2.5 times larger than the size of a lysosome.

Charnolosome (CS). A CS is formed when the phagocytosed CB in the charnolophagosome is completely hydrolyzed by the lysosomal enzymes. It is a single layered, highly unstable, and functionally-labile intracellular organelle containing toxic mitochondrial metabolites.

CS Stability Index (CSSI). It is a ratio of stable charnolosome (CS_s) divided by stable charnolosome (CS_s) + permeable charnolosome (CS_{perm}) + sequestered charnolosome (CS_{seq}) + fragmented charnolosome (CS_{frag}), and can be quantitatively estimated by multiple fluorochrome flow cytometric analysis.

CS Body. A blebbing on the surface of a CS following a secondary or tertiary free radical attack due to lipid peroxidation during mitochondrial oxidative and ER stress. The CS body pinches off from the CS and fuses with the plasma membrane to synthesize the apoptotic body.

Charnolopathy. CB molecular pathogenesis.

Apoptotic Body. A blebbing on the surface of the plasma membrane following a tertiary or quaternary free radical attack. The fusion of CS body with the plasma membrane releases highly toxic mitochondrial metabolites as described above to cause phosphatidyl serine externalization and eventually release of intracellular constituents in the microenvironment to induce chronic MDR diseases as described systematically in this book.

Free Radicals. Free radicals are highly unstable, reactive oxygen and nitrogen species (including $\cdot OH$, $NO\cdot$, $CO\cdot$) which are formed in the mitochondria as a byproduct to oxidative phosphorylation in the electron transport chain. They cause lipid peroxidation of cellular membranes by inducing the structural and functional breakdown of polyunsaturated fatty acids (including: linoic acid, linolenic acid, and arachidonic acid).

Stages of Free Radical Attack. There are primarily four different stages of free radical attack: (i) Primary free radical attack, (ii) secondary free radical attack, (iii) tertiary free radial attack, and (iv) quaternary free radical attack. (i) Primary free radical attack is attenuated by endogenously-synthesized antioxidants such as glutathione, metallothioneins (MTs), heat shock proteins (HSPs), heat shock factor-α, thioredoxin, superoxide dismutase (SOD), and catalase. (ii) Secondary free radical attack requires

endogenously synthesized antioxidants as well as naturally-produced antioxidants such as polyphenols (resveratrol), lycopene, sirtuins, rutins, catechin, and flavonoids to maintain ICD and sustain intra-mitochondrial homeostasis. (iii) Tertiary free radical attack requires endogenously-synthesized antioxidants, naturally synthesized antioxidants as describe above, and pharmacological antioxidants such as all B vitamins, Vitamin-A, D, E, probucol, edaravone, statins (simvastatin, atorvastatin), and several others in the pharmaceutical industry. (iv) Quaternary free radical attack is difficult to attenuate and cannot be prevented by all the above three sources of antioxidants. In general, quaternary free radical attack is associated with degenerative and proinflammatory apoptosis which cause chronic MDR diseases.

Charnolopharmaceuticals. CB-targeted pharmaceutical agents for the safe and effective EBPT of chronic MDR diseases such as malignancies.

Charnolopharmacology. CB-targeted mitochondrial bioenergetics-based therapeutic drugs with well-established pharmacokinetics (PKs), pharmacodynamics (PDs), and pharmacogenomics, beneficial effects, and adverse effects.

Charnoloscopy. A microscopic (usually confocal, atomic force, and TEM) evaluation of CB, charnolophagy, charnolophagosome, CS, and its exocytosis/endocytosis as a basic molecular mechanism of ICD.

Charnolostatic. An agent which inhibits CB formation.

Charnolocidal. An agent which eliminates CB in a physicochemically-injured cell.

Charnolomimetic. An agent which augments CB formation.

Charnologenetics. Genetically-linked CB formation.

Charnolopharmacogenomics. Genomic changes associated with pharmacological induction or inhibition of CB.

CB-PET RPs. These PET RPs are based on CB-induced CMB and primarily targeted to label CB, CPS, and CS, in addition to detecting charnolophagy (CB-autophagy) and CS exocytosis/endocytosis. Disease-specific CB-PET radioligands will be clinically significant for early differential diagnosis of progressive NDDs, CVDs, and cancer. CB, CPS, and CS can be isolated by differential ultracentrifugation in sucrose-density gradient, purified by MACs separation, and characterized by multifluorochrome flow cytometery with a sorting facility for the safe and effective EBPT of MDR diseases.

CB Epigenetics. Methylation of mitochondrial DNA at the N-4 position of cytosine and acetylation of histones at lysine residues.

α-Synuclein Index. A ratio of nitrated α-synuclein versus native α-synuclein.

Detection of CS. A CS can be detected by employing two fluorescent imaging probes including (i) mitotracker, and (ii) lysotracker. The cells or tissues can be labeled with these fluorochromes. The mitotracker can determine the number of mitochondria and the lysotracker can determine the number of lysosomes. The mitochondria and lysosomes can be distinguished based on the red and green fluorescence respectively. These digital images are merged to localize yellow fluorescence-labeled CS. A CS exhibits yellow fluorescence because it has both lysosomal enzymes as well as

metabolites of mitochondria. Moreover, CS are rich in the mtDNA oxidation product, 8-OH 2dG, and plasma membrane oxidation product, 2,3 dihydroxy nonenal, which can be determined by labeling cells with fluorescently-labelled specific antibodies. In addition to tissues and cultured cells, circulating CS biomarkers including 8-OH, 2dG, and 2, 3 dihydroxy nonenal can be estimated from the saliva, serum, plasma, blood, urine, hair, and toenail samples to quantitatively assess CB-based mitochondrial bioenergetics (MB). Various biomarkers of CS can also be determined by performing multiplex ELISA, antibody microarrays, cDNA microarray, and microRNA microarrays.

Introduction

Christian de Duve, the Nobel Laureate from Belgium, discovered lysosomes and introduced the term "Autophagy". He was awarded the shared Nobel Prize for Physiology or Medicine in 1974, along with Albert Claude and George E. Palade for describing the structure and function of organelles (lysosomes and peroxisomes) in biological cells. Recently, the Nobel Prize in Physiology or Medicine was awarded to Prof. Yoshinori Ohsumi from Japan for discovering the basic molecular mechanisms of Autophagy (2016). I discovered "Charnoly body" (CB) as a pre-apoptotic biomarker of CMB in the developing undernourished (UN) rat cerebellar Purkinje neurons and introduced "charnolophagy" (CB autophagy) which occurs by lysosomal activation due to free radical overproduction during physicochemical injury in the most vulnerable cells.

The CB is a universal biomarker of cell injury, whereas charnolophagy is a novel drug discovery target to evaluate CMB and ICD in health and disease. Charnolosome (CS) is a byproduct of CB and is implicated in the molecular pathogenesis of chronic illnesses. Hence, novel charnolopharmacotherapeutics can be developed by analyzing DSST combinatorial and correlative charnolosomics to accomplish the targeted, safe, and effective EBPT of NDDs, CVDs, and MDR malignancies for a better quality of life. By definition CB is a pleomorphic, electron-dense, multilamellar, quasi-crystalline, pre-apoptotic, universal biomarker of physico-chemical injury which is formed in the most vulnerable cell due to free radical-induced down-regulation of MB. Malnutrition, environmental toxins, heavy metal ions Hg^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, polychlorobiphenyls (PCBs), and microbial (bacteria, virus, and fungus) infections induce CB formation in the most vulnerable cells (like NPCs, CPCs, and EPCs, derived from iPPCs) due to free radical-induced mitochondrial degeneration. Nutritional rehabilitation, physiological Zn^{2+}, and MTs prevent CB formation as potent free radical scavengers and boosters of the MB. MTs and several other antioxidants prevent CB formation and augment charnolophagy during the acute phase and stabilize and facilitate CS exocytosis during the chronic phase as a basic molecular mechanism of ICD to prevent disease progression and remain healthy. Hence, CB, charnolophagy, and CS are novel drug discovery biomarkers and targets for NDDs, CVDs, MDR malignancies, and several other infectious and non-infectious diseases.

This book introduces the original discovery of CB as a universal biomarker of cell injury and CS as a novel drug development target of CMB. A CS is formed after charnolophagy in the most vulnerable cell in response to DPCI as an immediate and early attempt of ICD to remain healthy. This discovery opens a brand-new era of charnolomics and DSST charnolopharmacotherapeutics including CB agonists/antagonists, charnolophagy agonists/antagonists, and CS exocytosis enhancers,

inhibitors, and stabilizers for the targeted, safe, and effective EBPT of NDDs (AD, PD, drug addiction, MDDs, and schizophrenia), CVDs, MDR malignancies, and numerous proinflammatory diseases beyond the scope of this book. Identification of CB contributes to understand the cellular, molecular, and genetic basis of conception and infertility, delayed eye conditioning response in fetal alcohol syndrome (FAS), drug addiction, PD, AD, HD, ALS, MS, aging, environmental neurotoxicity, CVDs, MDR malignancies, and numerous chronic inflammatory diseases. The book also describes origin, development, maturation, and degradation of CB and CS and their theranostic significance in health and disease.

It is important to emphasize that mitochondrial repair, rejuvenation, regeneration, and synthesis are constantly required to sustain normal MB and ICD. The accumulation of CBs in the most vulnerable cell triggers apoptotic cell death and eventually, morbidity and mortality due to release of highly toxic substances such as Cyt-C, iron, 8-hydroxy, 2 deoxy guanosine (as mitochondrial DNA oxidation product), 2,3, dihydroxy nonenal (as a byproduct of lipid peroxidation), glyceraldehyde phosphate dehydrogenase (GAPDH), acetaldehyde, H_2O_2, ammonia, lactic acid, Bax, caspase-3, and apoptosis-inducing factor (AIF) which induce further degeneration in chronic diseases. No other intracellular organelle and its metabolites are as toxic as compared to the mitochondrial metabolites in the structurally and functionally-labile CS. Hence, their disposal, particularly in chronic diseases, remains a significant challenge in MDR diseases. The condensation of free radical-induced degenerated mitochondrial membranes to form CB is an initial and early attempt to contain highly toxic Cyt-C, which is loosely and non-covalently attached to the inner mitochondrial membrane. The Cyt-C can be easily delocalized and scattered in the cell in response to any physico-chemical injury. Hence, CB formation is an immediate and early attempt to maintain ICD by condensing degenerated mitochondrial membranes in the most vulnerable cell during DPCI.

As CB is a nonfunctional intracellular inclusion, it is phagocytosed by a lysosome to form a charnolophagosome (CPS). The CPS is transformed to CS when the phagocytosed CB is hydrolyzed by the lysosomal enzymes. The CS is subsequently exocytosed by an energy (ATP)-driven process as a basic molecular mechanism of ICD as a secondary attempt to remain healthy. Thus, a physico-chemically-injured cell initially attempts to prevent CB formation and subsequently augments charnolophagy and CS exocytosis to remain free from toxins of mitochondrial metabolism to remain structurally and functionally-intact. Accumulation of CBs in the perinuclear regions can impair microRNAs-mediated signal transduction at the transcriptional and translational level to induce chronic diseases, and at the junction of the axon hillock, impairs the axoplasmic transport of ions, neurotransmitters, neurotropic factors (insulin like growth factor-1: IGF-1, and brain-derived neurotrophic factor: BDNF), enzymes, and mitochondria to cause initially synaptic silence during acute phase, followed by synaptic atrophy and synaptic degeneration during chronic phase of disease progression. Similarly, accumulation and destabilization of CB or CS at the junction of axon hillock releases toxic substances of mitochondrial metabolism to cause synaptic degeneration and early cognitive impairments in learning, intelligence, memory, and behavior. The CS destabilization and permeabilization can also induce atherosclerotic plaque rupture as noticed in coronary artery diseases and hemorrhagic stroke patients. Therefore, drugs may be developed to inhibit CB formation and

augment charnolophagy during acute phase and stabilize CS and augment its exocytosis during chronic phase in NDDs and CVDs, and vice versa for the safe and effective EBPT of MDR malignancies. A nonspecific induction of CB formation in hyper proliferating cells during cancer chemotherapy causes adverse effects including: alopecia, myelosuppression, GIT symptoms, cardiovascular toxicity, neurotoxicity, infertility and selective degeneration of highly proliferating cells and organs rich in mitochondria. Hence, drugs may be developed to induce cancer stem cell-specific CB formation to cure MDR malignancies, chronic inflammations, and infections.

Natural abundance and genetic susceptibility of mtDNA qualify CB as an early, unique, and sensitive universal biomarker of clinical significance. Indeed, a balanced diet and moderate exercise alleviate clinical symptoms of various chronic diseases by activating anti-inflammatory, antioxidant, and antiapoptotic metal (Zn^{2+})-binding proteins. Particularly, MTs prevent CB formation and its interconversion to CS, inflammasome, apoptosome, necroapoptosome, and metallosome, and are involved in the etiopathogenesis of NDDs, CVDs, and MDR malignancies. Recently, I reported that nutritional rehabilitation, physiological Zn^{2+}, and MTs confer theranostic potential as potent free radical scavengers and mitochondrial protective agents. Hence, a balanced diet rich in antioxidants and moderate exercise can alleviate clinical symptoms of pro-inflammatory and apoptotic events in chronic diseases. MTs as potent antioxidants and free radical scavengers induce charnolophagy, CS stabilization, and CS exocytosis as a basic molecular mechanism of ICD to prevent chronic diseases as highlighted in this book.

It is now well-established that translocation of MTs in the nucleus is implicated in storing, buffering, and sequestering Zn^{2+} ions. MTs-mediated release of Zn^{2+} regulates transcriptional activation of genes and triggers microRNA synthesis involved in DNA cell cycle, cell growth, proliferation, migration, differentiation, and development through the induction of AgNOR. The AgNOR induction in the nucleolus enhances ribosomal formation for protein synthesis on the rough endoplasmic reticulum (RER) depending on the extent of Zn^{2+}-induced transcriptional activation of microRNAs during induction/repression of mitochondrial and nuclear genes. Free radical-induced destabilization of CS releases toxic mitochondrial metabolites and inhibits protein synthesis involved in normal growth and development by inhibiting AgNOR and by preventing poly-ribosomal assembly on the RER membranes as observed in developing UN rat cerebellar Purkinje neurons and in chronic patients of cancer, drug addiction, AD, and aging as described in this book.

The origin, development, maturation, and degradation of CB facilitates further understanding the cellular, molecular, and genetic basis of ZIKV-induced microcephaly, FASD in new born infants, and Guillain Barre Syndrome (GBS) in adults as described in detail in my recently published books "ZIKV Disease; Prevention & Cure" and Fetal Alcohol Spectrum Disorders: Concepts, Mechanisms, and Cure, Nova Science Publishers, New York, U.S.A. This book elucidates the basic molecular mechanism of depression, drug addiction, and progressive NDDs such as: PD, AD, MS, HD, ALS, environmental neurotoxicity, infertility, drug addiction, alcoholism, aging, CVDs, and many other MDR diseases (including malignancies with special reference to free radicals-induced CMB and induction of DSST charnolopathies).

The mtDNA is a highly sensitive, GC-rich, intron-less, transmitted exclusively through the female germ line, double-stranded, circular molecule of 16569 bp and

contains 37 genes coding for two rRNAs, 22 tRNAs, and 13 polypeptides, which remains in a hostile microenvironment of free radicals, generated as a byproduct of oxidative phosphorylation in the electron transport chain. Hence, genetic and epigenetic modification occur more readily in the mtDNA as compared to nuclear DNA. The DNA methylating molecule, S-adenosyl methionine (SAM) is synthesized in the mitochondria by methionine and ATP in the presence of an enzyme, methyl transferase to cause mtDNA methylation at N-3 position of the cytosine residue to induce epigenetic changes. The mtDNA is oxidized to synthesize 8-OH, 2dG, which may be estimated from the serum, plasma, CSF, amniotic fluid, saliva, tear, toe nails, hair, and urine samples along with estimation of cytosine, methyl cytosine, and hydroxymethyl cytosine to clinically evaluate epigenetic changes as well as mtDNA oxidation simultaneously by microtiter colorimetric ELISA, immunoblotting, RT-PCR, cDNA microarray, antibody microarrays, flow cytometry, capillary electrophoresis, next generation sequencing, LC-MS analysis, and surface plasmon resonance (SPR) spectroscopy as described systematically in this book.

It is important to emphasize that soon after fertilization, CB formation occurs in the middle piece of the spermatocyte due to down-regulation of MB in the oocyte. The paternal CB in the oocyte is eliminated soon after the conception by energy (ATP)-driven lysosomal-dependent charnolophagy. Subsequently, CS is eliminated by ATP-dependent exocytosis as a basic molecular mechanism of ICD for the normal growth, proliferation, and development of an embryo. Mother Nature has provided between 22–75 spirally-arranged and condensely-packed mitochondria in the middle piece for sperm motility and translocation of paternal nuclear DNA to hybridize with the oocyte nuclear DNA during fertilization. However, a considerable amount of energy (ATP) is required for charnolophagy and CS exocytosis. Hence, an oocyte has as many as 200,000 to 600,000 mitochondria, which also participate in the normal growth and development of the fetus during the post-zygotic phase in addition to their involvement in paternal charnolophagy and CS exocytosis to prevent zygote death which can occur in severe malnutrition, nicotine addiction, binge drinking, severe microbial (bacteria, virus, fungus) infection, and in response to certain drugs and environmental neurotoxins.

As the number of mitochondria (usually > 1000) are more compared to the lysosomes in a cell, charnolophagy is compromised in severe malnutrition, microbial infections, aging, progressive NDDs, CVDs, and cancer. These deleterious events pose a significant challenge in ICD and may trigger denaturation/aggregation of intracellular proteins in chronic MDR diseases as illustrated systematically in this book. In addition, I have proposed the charnolophagy index as a sensitive biomarker to quantitatively assess ICD. CB formation occurs in chronic NDDs, CVDs, and drug addiction, where it induces early aging, morbidity, and mortality by augmenting the microRNA-induced apoptotic signaling cascade. However, CB formation is attenuated due to induction of genes (particularly MTs, cmyc, P^{53}, HSPs, and heat shock factor-α) involved in cellular immortalization in MDR malignancies in cancer stem cells. Hence, it is logical to assume that "*Life begins and ends with CB*", because "functionally-efficient mitochondrial bioenergetics sustains our health; whereas CMB triggers CB formation involved in charnolophagy as a basic molecular mechanism of ICD or apoptotic cell death due the release of highly toxic mitochondrial metabolites to induce acute or chronic diseases." Although, microRNAs are quite stable, the release

of toxic substances from free radical-induced destabilized CS inhibits their post-transcriptional activity, and significantly influences their normal function in a physico-chemically-injured cell. The CS destabilization is characterized by permeabilization and sequestration during the acute phase, and fragmentation during the chronic phase of the disease progression.

The basic molecular mechanism of ICD by charnolophagy is compromised due to DPCI and in chronic MDR diseases in the most vulnerable cells.

Free radicals are produced as a byproduct of oxidative phosphorylation during ATP synthesis in the electron transport chain in the mitochondria. Thus, any physico-chemical injury to a cell poses a tremendous physiological burden of energy (ATP) requirement by the mitochondria to maintain ICD. During DPCI, the mitochondria are destroyed by their own free radicals due to lipid peroxidation and mtDNA oxidation, accompanied with structural and functional breakdown of polyunsaturated fatty acids (PUFA: linoic acid, linolinic acid, and arachidonic acid) in the plasma membrane and Cyt-C release. In addition, free radicals induce proteases to cause proteolysis, lipases to cause lipolysis, and nucleases to cause DNA fragmentation resulting in apoptosis and/or necrosis.

A degenerating CS is potentially harmful, particularly when it starts releasing iron, Cyt-C, GAPDH, heme iron, ammonia, acetaldehyde, H_2O_2, 2.3 dihydroxy nonenal, 8-OH, 2dG, and peptides like apoptosis-inducing factor (AIF), caspase-3, Bax and Bid due to CPS induction and CS destabilization. CB formation also occurs in a normally aging cell due to CMB in response to free radical-induced down-regulation of the mitochondrial genome and microRNAs, triggering molecular pathogenesis. However, this natural process of mitochondrial degeneration is very slow and occurs primarily in very old age. We confirmed these findings in cultured mitochondrial genome knock out (RhO_{mgko}) human DArgic (SK-N-SH, and SH-SY5Y) neurons as *in vitro* experimental models of stroke, PD, AD, drug addiction, MDD, and aging. The charnolophagy index was increased as a function of increasing concentrations of 1-methyl, 4-phenyl, 1, 2, 3, 6 tetrahydropyridinium (MPP^+) treatment in the DAergic cell lines in culture. The charnolophagy index was proportional to the α-synuclein index, involved in neurodegenerative α-synucleinopathies.

Indeed! CB, charnolophagy, charnolophagy index, CPS, CS, and CS bodies as excellent drug discovery targets and biomarkers to develop novel charnolopharmacotherapeutics are now well established, published, and quoted in several international journals of high impact factor. Hence, further systematic studies along this direction will go a long way in the targeted, safe, and effective EBPT of various NDDs, CVDs, and MDR malignancies as elegantly described in this book.

CB Discovery

I was conducting basic research on developing normal (N) and undernourished (UN) rat cerebellar Purkinje neurons employing neurochemical, electrophysiological, neuromorphological, and neuropharmacological studies at the light and electron microscopic level as a Research Officer and Ph.D. student, in the Department of Neurology at the A.I.I.M.S., New Delhi (India) during the early eighties. At the ultrastructural level, I discovered peculiar, pleomorphic, quasi-crystalline, multi-lamellar, electron-dense membrane stacks of degenerated mitochondrial membranes

in the developing UN rat cerebellar Purkinje neuron dendrites and synaptic terminals possessing maximum number of highly susceptible mitochondria. Subsequently, I named these pleomorphic structures as "Charnoly bodies (CBs)" as a token of love, respect, and appreciation to my late mother "Charnoly".

This book describes the clinical significance of CB in accomplishing the EBPT of progressive NDDs, CVDs, and cancer. The book describes particularly free radical-induced CB formation, charnolophagy, and CS destabilization as biomarkers of microRNA down-regulation and novel DSST charnolopharmacotherapeutics for the targeted, safe, and effective EBPT of chronic MDR diseases, involving mitochondrial oxidative and ER stress, inflammation, and apoptosis.

The most unique feature of this book is that it presents a novel concept of DSST charnolosomics which can be accomplished with cDNA, antibody, and nanoparticle probes employing state-of the art biotechnologies (such as LC-MS, fluorescent multiplex ELISA, capillary electrophoresis, flow cytometry, next generation sequencing, magnetic resonance spectroscopy, and SPR spectroscopy) to determine the MB implicated in ICD for normal cellular function and homeostasis to remain healthy. Correlative and combinatorial bioinformatics of DSST charnolosomic microarrays in combination with conventional omics employing genomics, proteomics, metabolomics, metallomics, and lipidomics microarray analysis can provide precise information to accomplish the targeted, safe, and effective EBPT of progressive NDDs, CVDs, and chronic MDR malignancies for a better quality of life.

In earlier studies, I described the clinical significance of α-synuclein index (SI) in the differential diagnosis of progressive neurodegenerative α-synucleinopathies. This book introduces systematically-described the original discovery of CB as a universal biomarker of cellular injury and novel concepts of charnolophagy index and CS stability index (CSSI) for the EBPT of chronic MDR diseases, such as AD, PD, stroke, ALS, HD, MS, MDDS, schizophrenia, multiple drug addiction, CVDs, and cancer. More specifically, it describes the clinical significance of CB, charnolophagy, and CS-labelled NPs and radiotracers to quantitatively assess the MB, microRNAs, and ICD and to discover novel DSST charnolopharmacotherapeutics for the safe and effective EBPT of MDR diseases with currently limited therapeutic options.

This book is divided in three major parts: Part-I General introduction of CB which describes its basic cellular and molecular biology; Part-II Emerging biotechnology in CB research; and Part-III Clinical significance of DSST, CB, and CS in EBPT.

The primary objective of presenting this unique manuscript is to motivate, guide, and inspire young budding scientists to work hard and think rationally without any reservation and/or hesitation. Sometimes your guides, mentors, teachers, and professional colleagues may ignore your hard-earned original research work as happened with Alexander Fleming during his original discovery of Penicillin. So, we should not be scared that we are alone in this venture. Several others have already undergone through hardships and accomplished difficult tasks and several others are ready to experience similar social, physical, mental, economical, and psychological stresses, irrespective of their profession.

Any invention, innovation, and/or discovery passes through different phases of ignorance, straight-forward rejection, frustration, professional jealousy, laughter, serious consideration, and eventually national and international recognition as a function of time. Let me share with you the following nice words Professor Luis

Pasteur said on his 70th birthday which was being celebrated at the National level in France: "*Future will belong to those who have suffered for humanity*". Luis Pasteur suffered for humanity even though he did not receive a Nobel Prize like Professor Robert Koch, Madam Marie Currie, and Alexander Fleming.

CB is formed as an early pre-apoptotic biomarker in the most vulnerable cell due to free radical-induced CMB and is a universal biomarker of DPCI. CB, charnolophagy, and CS were also discovered in the cultured human DArgic (SK-N-SH and SH-SY5Y) neurons due to $\Delta\Psi$ collapse and mtDNA down-regulation in RhO_{mgko} neurons as a cellular model of aging.

Based on the original discovery of CB in the developing UN Purkinje neurons of the rat cerebellar cortex and in the intrauterine domoic acid exposed developing mice, a novel concept of charnolopharmacotherapeutics was introduced as described in my several manuscripts and books.

This book describes the clinical significance of CB as a novel biomarker of CMB in chronic diseases. The development of drugs based on CB-based CMB promises to have either minimum or no adverse effects and an increased therapeutic index with acceptable margin of safety. Hence, DSST-CB agonists/antagonists, charnolophagy agonists/antagonists, CPS and CS stabilizers/destabilizers, and CB sequestrates/desequestrates are introduced for the safe and effective treatment of progressive NDDs, CVDs, cancer, and infectious diseases (including: ZIKV, cytomegalovirus, and rubella virus) for the first time in this book.

This manuscript provides an original and unique approach to treat chronic intractable diseases and will serve as a "Text Book" for biomedical students (M.D., M.Sc., Ph.D., D.Sc.), nurses, and other healthcare professionals; and "Reference Book" for doctors, researchers, professors, and public. All universities, medical schools, and public libraries across the globe will be interested in going through the original, thought-provoking, motivating concepts, and basic molecular mechanisms of charnolopathies and CB-based charnolopharmacotherapeutics as described elegantly in this book.

This book confers original thought-provoking basic concepts and mechanisms to explore further in the multidisciplinary areas of EBPT. The book is not simply based on the conventional wisdom and existing literature evidence as it has a significant component of nonconventional wisdom and highlights lacunae in our existing knowledge regarding basic molecular mechanisms of diversified charnolopathies involved in chronic diseases and potential theranostic strategies of targeting CMB-based CB formation, charnolophagy, and CS exocytosis for ICD to remain healthy.

CB molecular pathogenesis occurs primarily in three major phases. Phase-1: synaptic silence involving mild cognitive impairment (MCI); Phase-2: synaptic atrophy involving early morbidity; and Phase-3: synaptic degeneration involving early mortality as noticed in cerebral palsy, Down's syndrome, progeria, AD, PD, ALS, MS, HD, schizophrenia, multiple drug addiction, and MDDs.

The book also explains how the nonspecific induction of CB formation causes alopecia, myelosuppression, and GIT abnormalities in MDR malignancies, and evaluates how drugs may be developed to prevent/inhibit CB formation and augment MB to enhance charnolophagy as a basic molecular mechanism of ICD. More specifically, it describes how novel drugs may be developed to prevent CS

destabilization, permeabilization, sequestration, and/or fragmentation. Although charnolostatic drugs for the treatment of chronic intractable diseases will be clinically-beneficial to control acute disease states, charnolocidals will be required for chronic MDR diseases particularly for the immunocompromised and aging patients.

While going through the interesting and thought-provoking concepts and mechanisms involved in disease progression/regression, the readers will enhance their basic knowledge regarding charnolophagy, CPS, CS, and ICD in relation to down-regulation of microRNA-mediated post-transcription of genes involved in DNA cell cycle, proliferation, migration, differentiation, and development to remain healthy. The book also provides the molecular mechanism of CB formation, charnolophagy, and CS as novel drug discovery targets in chronic inflammatory diseases (CIDs) including; depression, AD, PD, FASD, MDDs, schizophrenia, chronic drug addiction, infectious disease, CVDs, and MDR malignancies.

The concepts and mechanisms described are original, interesting, thought-provoking, and will motivate young scientists, doctors, and other health care professionals to explore further in this clinically-significant and challenging area to discover novel charnolopharmacotherapeutics for a better quality of life. Novel charnolopharmacotherapeutics and the crucial role of genomics, microRNA, and epigenomics in sustaining MB by CB prevention/inhibition, charnolophagy induction to maintain ICD, and CS stabilization are original and clinically-significant for the prevention and/or treatment of CIDs. Hence, drugs augmenting MB by inhibiting CB formation, augmenting charnolophagy during the acute phase, and inhibiting CB formation and CS destabilization during the chronic phase will be promising for the EBPT of CIDs, as elegantly described in this book.

It is well-established that antioxidants (glutathione, MTs, CoQ_{10}, melatonin, selegiline, polyphenols (resveratrol), flavonoids, sirtuin, rutin, lycopene, and catechin) inhibit CB formation as free radical scavengers. Although they can pass through the blood brain barrier readily, their reduced potency necessitates bulk consumption. Hence, ROS-scavenging antioxidant-loaded NPs may be developed to improve CNS delivery to enhance MB and charnolophagy in CIDs. MTs provide neuroprotection by regulating Zn^{2+}-mediated transcriptional regulation of genes involved in microRNA synthesis implicated in normal or abnormal growth, proliferation, differentiation, development, and invasion. MTs also inhibit MAOs activation, and TRPC and TSPOs delocalization to prevent CB formation and CS destabilization by augmenting (a) lysosome-sensitive CB (LSCB) formation during acute phase and (b) by preventing lysosome-resistant CB (LRCB) formation during the chronic phase of disease progression. Particularly, this book provides emerging concepts of MB, genomics, and epigenomics of CIDs with ROS scavenging antioxidant loaded NPs to accomplish EBPT of chronic MDR diseases.

It is well-known that brain regional induction of MAO-A and MAO-B-specific CBs induces the down-regulation of monoaminergic (NE-ergic, 5HT-ergic, and DA-ergic) neurotransmission to cause cognitive impairments in CIDs. This book presents MB-based CB prevention/inhibition, charnolophagy induction, and CS stabilization for executing normal microRNAs-mediated post-transcriptional regulation of disease-specific genes as potential therapeutic targets for the safe and effective treatment of CIDs. Particularly, malnutrition, FASD, ZIKV disease, AD, PD, CVDs, and MDR malignancies are described as DSST charnolopathies and the

development of novel charnolopharmacotherapeutics for their targeted, safe and effective cure.

This book augments the existing knowledge and wisdom regarding CIDs and their safe and effective clinical management by targeting CB-induced CMB. The CMB is characterized by down-regulation of mitochondrial membrane potential ($\Delta\Psi$ collapse), formation of megapores, influx of calcium ions due to TRPCs, MAOs, and TSPO delocalization, membrane fragmentation, aggregation, and condensation to trigger CB formation and induce charnolophagy, followed by CPS and CS destabilization involved in degenerative apoptosis and chronic intractable diseases.

More specifically, this book describes the original discovery of CB and its origin and life cycle, classification of CB, and maternal and paternal CB formation during conception, CB formation and its elimination during normal embryonic growth and development, classification of CB based on structure and genetic susceptibility; MAO-A and MAO-B-specific CB formation and its therapeutic significance; inducers of CB formation including (a) drugs, (b) environmental toxins, (c) microbial infections, and (d) life style; prevention of CB formation by antioxidants such as sirtuins, rutins, resveratrol, (e) diet and exercise; charnolophagy and its clinical significance. Furthermore, it illustrates CPS and its clinical significance; CB in health and disease (basic concepts and mechanisms); therapeutic potential of MTs as CB antagonists in obesity, hippocampal CB formation in MDDs, epilepsy, and AD; medio basal-hypothalamic CB formation in bulimia; transcriptional regulation of CB formation, genetics, and epigenetics of CB formation; CB as a universal biomarker of cell injury; CB formation in the hippocampus and medio-basal hypothalamus in dementia and obesity; CB as a novel therapeutic target of drug discovery; clinical significance of charnolophagy in personalized theranostics; early detection of CS biomarkers and their clinical significance; charnolosomics in EBPT; and DSST circulating CS biomarkers and their clinical significance in EBPT. In addition, cancer stem cell specific CS vs normal CS; cancer stem cell-specific CS biomarkers vs normal CS biomarkers; disease-specific cellular and circulating CS biomarkers; charnolopharmacotherapeutics as charnolophagy agonist/antagonists; and future prospect of CB-based research in novel drug discovery and EBPT are described in detail.

Although, it is generally believed that only maternal fetal alcohol abuse can induce diversified embryopathies in the developing fetus, I have now evidence to suggest that chronic ethanol abuse by both parents can induce deleterious consequences in the developing embryo. The original discovery of CB formation, charnolophagy, CPS formation, and CS exocytosis at the ultrastructural level provide scientific evidence to propose that both paternal as well as maternal mitochondria participate in the normal fertilization and subsequent growth and development of an embryo during the postzygotic phase.

Recently, I reported that intrauterine ethanol exposure causes apoptosis of the most vulnerable NPCs, derived from iPPCs via CB formation. Generally, two types of CBs are formed during fetal alcohol exposure (FAE): (a) lysosome-sensitive CB formation (LSCB) during the acute phase and (b) lysosome-resistant CB (LRCB) formation during the chronic phase. The LSCB is subjected to charnolophagy as an efficient basic molecular mechanism of ICD. Following charnolophagy, the lysosome becomes almost 2.5 times enlarged, electron-dense, and can be easily distinguished from the normal lysosomes. An abnormally enlarged lysosome, possessing phagocytosed CBs

is classified as CPS. When CB is hydrolyzed in the CPS, it is named as CS. The CS is a relatively more unstable and toxic intracellular organelle as compared to CPS and is eliminated by exocytosis. Intrauterine ethanol and/or nicotine exposure can inhibit or impair charnolophagy, induce CPS destabilization/sequestration, and inhibit CS exocytosis to trigger charnolopathies, involved in diversified embryopathies. Thus, microbial infections and drugs of abuse (nicotine and alcohol) compromise zygote detoxification and trigger charnolopathies, involved in diversified embryopathies including microcephaly and other congenital anomalies. These basic molecular events are highly crucial for the ICD, cell proliferation, differentiation, and normal development of the fetus during the intrauterine life.

Based on two types of monoamine oxidases (i.e., MAO-A, and MAO-B) on the outer mitochondrial membranes and their heterogeneous micro-distribution in the brain, I have proposed two types of CBs: that is, (i) MAO-A and (ii) MAO-B-specific CB formation in response to toxins, which can cause down-regulation of NE-ergic, 5HT-ergic, and DA-ergic neurotransmission, respectively, involved in sensorimotor and cognitive impairments, and chronic intractable diseases such as PD, AD, ALS, HD, MS, MDDs, drug addiction, and schizophrenia. This book promotes existing knowledge by introducing novel concepts, mechanisms, and the cure of diversified charnolopathies/embryopathies with DDST charnolopharmacotherapeutics. The deleterious consequences of DPCI are described right from the pre-conceptional stage. The DSST-MB of both spermatocytes and oocytes, and toxins-induced CB formation, compromised charnolophagy, CS destabilization, and microRNAs down-regulation are unique, and are described for the prevention and/or cure of chronic diseases by antioxidants and novel charnolopharmacotherapeutics.

Although, several concepts, mechanisms, and potential therapeutics have been introduced recently in the healthcare arena to overcome the deleterious consequences of chronic intractable diseases, I have now proposed a novel concept of charnolopharmacology, that is, based on DSST-CMB and charnolopathies in the most vulnerable developing and aging cells. Hence, drugs inhibiting CB formation and/or augmenting charnolophagy as a basic molecular mechanism of ICD during the acute phase, and stabilizing CPS, and preventing CB and CPS/CS sequestration during the chronic phase will have promising therapeutic potential in NDDs, CVDs, and cancer. The book is primarily focused on conferring novel preventive and theranostic strategies for the clinical management of chronic intractable diseases such as FAS, AD, PD, CVDs, and MDR malignancies with deleterious consequences and how to prevent or treat them by developing safe and effective DSST charnolopharmacotherapeutics for a better quality of life.

The number of mitochondria (usually > 1000) is more than lysosomes in a cell, hence charnolophagy as a primary molecular mechanism of ICD is compromised in severe malnutrition, aging, chronic and progressive NDDs, CVDs, and in MDR malignancies. The primary highlights of this book are as follows:

(i) Functionally-efficient MB keeps us healthy whereas free radical-induced CMB triggers CB formation involved in either charnolophagy or apoptotic cell death due the release of toxic substances from the degenerating mitochondria.

(ii) Mitochondrial repair, rejuvenation, and regeneration is constantly required to maintain intracellular bioenergetics for ICD to remain healthy.

(iii) CB formation is triggered due to CMB. The CB is a highly unstable, pre-apoptotic, pleomorphic, multi-lamellar (usually penta or heptalemellar), quasi-crystalline, electron-dense stack of primarily degenerated mitochondria, that is formed in a highly vulnerable cell (such as NPCs and CPCs, derived from iPPCs) due to free radical-induced oxidative and nitrative stress.

(iv) Accumulation of CBs triggers apoptotic cell death and eventually morbidity and mortality form degenerating mitochondrial membranes, proteins, and DNA.

(v) Degenerating mitochondria are potentially harmful particularly when they start releasing toxic substances such as Cyt-C, iron, acetaldehyde, acetone, H_2O_2, ammonia, heme iron, and other proteins like Bax, Bid, caspase-3, and AIF due to CB sequestration, inducing apoptosis to cause cell death as occurs in normal aging due to the down-regulation of the MB. These findings were confirmed in mitochondrial genome knock out (RhO_{mgko}) cells as an *in vitro* model of aging.

(vi) This book describes the origin, development, maturation, and degradation of CB and its clinical significance in NDDs, CVDs, and cancer in addition to chronic MDR infections. The appearance and disappearance of CB is a reversible process as it can be regulated by microRNA-mediated nuclear or mitochondrial gene manipulation. The CB formation is eliminated by nutritional rehabilitation as we discovered in developing UN rat Purkinje neurons. CB formation does not occur under normal physiological conditions and is eliminated efficiently by lysosomes, as an energy (ATP)-driven process called "charnolophagy" to represent CB autophagy as a basic molecular mechanism of ICD. The persistence of CB in any vulnerable cell is potentially harmful as it can lead to progressive NDDs, CVDs, and cancer.

(vii) Recently, I proposed that CB can be utilized as an early, sensitive, and universal biomarker of cell injury. I also proposed charnolostatic drugs for acute cell injury and charnolocidal drugs for the prevention and treatment of chronic NDDs and CVDs, and charnolomimetic drugs for the safe and effective treatment of cancer. Nonspecific induction of CB is involved in alopecia, GIT distress, and myelosuppression in MDR malignancies. Hence, drugs may be developed to prevent CB formation in the follicle for the hair growth and regeneration. Further investigations in this direction will go a long way in the safe and effective clinical management of chronic intractable MDR diseases (Sharma and Ebadi 2014a).

(viii) To make this book more interesting particularly for the young biomedical students and scientists, I have written in very simple and straight-forward language. In addition to biomedical students and paramedical professionals, the book will be of considerable interest for the general-public, accomplished and well-experienced biomedical scientists, doctors, professors, nurses, and researchers.

Rationale of Mitochondrial Vulnerability. (i) Mitochondria serve as constant source of energy (ATP) in a cell and maintain the intracellular homeostasis and detoxification. (ii) Free radicals are generated in the mitochondria as a byproduct of oxidative phosphorylation during ATP synthesis in the electron transport chain. (iii) Mitochondrial membranes are highly rich in PUFA (linoic acid linolinic acid,

and arachidonic acid), which render them highly vulnerable to free radical-induced lipid peroxidation. (iv) The mtDNA is GC-rich, which renders it highly susceptible to oxidation at guanosine and methylation at the cytosine moiety to induce genetic and epigenetic modifications. (v) The mtDNA is non-helical, intron-less, and remains in a hostile microenvironment of free radicals ($^{\cdot}OH$, $NO^{\cdot}$, $CO^{\cdot}$). (vi) Mitochondria contain highly toxic metabolites such as Cyt-C, GAPDH, 8-OH, 2dG, 2, 3 dihydroxy nonenal, acetaldehyde, acetone, ammonia, and H_2O_2. The release of these toxic substance can induce spontaneous apoptosis and/or necrosis to trigger chronic MDR diseases. Hence, CB formation is an immediate and early attempt of ICD to contain highly toxic mitochondrial metabolites in a physico-chemically-injured cell.

CB is an immediate early and most sensitive pre-apoptotic biomarker of CMB. DPCI and many drugs enhance CB formation in the most vulnerable cells such as NPCs, CPCs, EPCs, and OPCs, derived from iPPCs cells to induce microcephaly, craniofacial abnormalities, and other embryopathies in FASD victims. CB formation, charnolophagy, CPS, and CS can serve as early, pre-apoptotic biomarkers of CMB and can be detected at a much earlier stage of disease progression as intracellular neuronal inclusions in NDDs and other chronic diseases. Natural abundance of mitochondria and genetic and epigenetic susceptibility of mtDNA qualify CB as an early, unique, and sensitive universal biomarker of clinical significance.

The most important events in CB molecular pathogenesis are the efficient induction of energy-driven charnolophagy (CB autophagy) and CS exocytosis as a basic molecular mechanism of ICD. The CS is structurally and functionally highly labile intracellular organelle and is readily destabilized by free radical attack. The destabilized CS releases highly toxic mitochondrial metabolites through permeabilization, sequestration, and fragmentation depending on the frequency and intensity of free radical attack which disrupts microRNA-mediated transcriptional regulation of genes involved in normal cell growth, DNA cell cycle, proliferation, differentiation, and development. Glutathione and MTs provide structural and functional stability to CS by maintaining intracellular sanitation and normal post-transcriptional regulation of microRNAs. Hence, CB and microRNA-based biomarkers can be utilized to differentially diagnose and effectively treat preclinical stages of progressive NDDs (such as PD, AD, drug addiction, schizophrenia, and MDDs), CVDs, and MDR malignancies (Sharma 2016, 2017, 2018).

Free radicals are generated as a byproduct of mitochondrial oxidative phosphorylation in the electron transport chain; CB is the byproduct of primary free radical-induced CMB. Charnolophagy is a byproduct of ATP-dependent lysosomal autophagy of CB, whereas CS is a byproduct of phagocytosed CB when it is completely hydrolyzed by the lysosomal enzymes; CS body is the byproduct of secondary free radical-induced CS destabilization due to lipid peroxidation; Apoptotic body is the byproduct of CS body destabilization; Apoptosis is a byproduct of apoptotic body disintegration; NDDs and CVDs are byproducts of degenerative apoptosis; and MDR malignancy is a byproduct of inhibited apoptosis. Hence, DSST charnolopharmacotherapeutics can be developed to inhibit cancer stem cell specific CS destabilization involved in malignant transformation of nonproliferating cells. DPCI-induced cortisol release augments CB formation, whereas MTs, IGF-1, and BDNF inhibit hippocampal CB formation, augment charnolophagy, CS stabilization, and exocytosis (involved in post-transcriptional regulation of microRNAs) to prevent

early morbidity and mortality in neurodegenerative α-synucleinopathies such as PD, AD, HD, ALS, and MS, schizophrenia, drug addiction, and MDDs.

Compensation-effect doctrine states that accumulated mtDNA mutations in the cell must reach a certain set threshold before they have a negative effect on cellular function from mtDNA (Chen et al. 2013). However, accumulation of aberrant mtRNA transcribed from mtDNA mutations negatively influences cellular function through complex internal and external mitochondrial pathways, and might be a significant cause of aging and aging-associated diseases due to CB formation, impaired charnolophagy, and CS destabilization, leading to down-regulation of microRNA-mediated post-transcriptional regulation of genes involved in DNA cell cycle, cell proliferation, differentiation, development, and malignant transformation (Sharma 2017).

TEM analysis revealed that ER stress occurs primarily in the dendritic regions and in the growth cones, where active protein synthesis takes place; whereas the mitochondrial oxidative stress and CB formation occurs more frequently at the synaptic region of developing UN rat Purkinje neurons to induce initially synaptic silence, followed by synaptic atrophy, and eventually synaptic degeneration depending on the frequency and intensity of free radical-induced CS destabilization, involving permeabilization, sequestration, and fragmentation, respectively.

The mitochondrial and ER-stress inhibits AgNOR to disrupt nucleolar synthesis of ribosomes required for the protein synthesis (particularly mitofusion, involved in the synthesis and repair of mitochondria) during DPCI. E.R. stress was noticed primarily in the dendrites and growth cones, whereas the mitochondrial oxidative stress occurred primarily in the synaptic terminals during DPCI in the most vulnerable cell. Thus, CB formation in the synaptic region can induce synaptic silence, synaptic atrophy, and eventually synaptic degeneration, depending on the free radical-induced CS permeabilization, sequestration, and/or fragmentation to cause mild cognitive impairment (MCI) during the acute phase, and morbidity and mortality during the chronic phase, respectively in NDDs, CVDs, and MDR malignancies.

MTs provide neuroprotection by preventing and/or inhibiting CB formation during the acute phase, and by augmenting charnolophagy and stabilizing CS during the chronic phase by serving as potent free radical scavengers in MDR diseases. MTs also provide ubiquinone (CoQ_{10})-mediated neuroprotection by inhibiting CB formation, augmenting charnolophagy, preventing CS destabilization, and by augmenting CS exocytosis as a basic molecular mechanism of MB, ICD, and post-transcriptional regulation of microRNAs for health and prolongevity (Sharma 2016, 2017, 2018). Brain regional down-regulation of MTs-induced Zn^{2+} homeostasis in aging is involved in mitochondrial degeneration, CB formation, CS destabilization, and impaired CS exocytosis to trigger neurodegenerative apoptosis due to impaired microRNA-mediated post-transcriptional activity in early cognitive impairment, morbidity, and mortality (Sharma et al. 2013; Sharma 2016; Sharma 2016; Sharma 2017). Free radicals-induced CS destabilization triggers epigenetic modifications and disrupts microRNA-mediated post-transcriptional regulation of genes involved in DNA cell cycle, proliferation, differentiation, and development. Hyper-methylation of the promoter region of IGF-1 gene causes insulin-resistant (Type-2) diabetes; hyper methylation of the promoter region of VEGF gene causes stroke; hyper methylation of the promoter regions of nicotinic acetyl choline receptor (nAChR) gene is involved nicotinism and multiple drug addiction, whereas hyper methylation of the promoter

region of leptin gene in the medio-basal hypothalamic region causes bulimia and obesity.

Two main hypotheses have been proposed in the etiopathogenesis of AD. These are (a) amyloid-β (Aβ-1-42) hypothesis and (b) mitochondrial hypothesis. According to amyloid-β hypothesis, AD occurs due to abnormal accumulation of Aβ-1-42 in the senile plaques in the cortical ribbon. This hypothesis was confirmed by detecting Aβ-1-42 in the autopsy AD samples using Congo-Red and by immunohistochemical analyses using specific Aβ-1-42 antibody in the senile plaques of AD patients. The senile plaques can be detected *in vivo* by performing ^{18}F-PiB (^{18}F-Florbetapir) PET neuroimaging in AD patients. The Aβ-1-42 hypothesis was further confirmed by observing the progression of neurobehavioral symptoms with neurodegeneration and cognitive impairment proportional to the number of amyloid-β 1-42 containing senile plaques. It is now believed that the truncated form of Aβ-1-42 is particularly involved in the etiopathogenesis of AD. Nevertheless, several AD patients do not exhibit amyloid-β senile plaques in their brain yet exhibit progressive cholinergic and other neurodegeneration as a function of time due to free radical-induced CMB and lipid peroxidation of the mitochondrial membranes by structural and functional breakdown of PUFA. The MB can be evaluated *in vivo* by performing ^{18}FdG PET neuroimaging. AD patients exhibit distinct loss of glucose metabolism in the fronto-temporal regions, ventriculomegaly, and hippocampal atrophy accompanied with trans-callosal and cerebral atrophy due to the induction of CB molecular pathogenesis early in life. Hence, fluid biomarkers such as 8-OH, 2DG, lactate, glutamate, choline, and N-acetyl aspartate (NAA), acetate, ammonia, and H_2O_2 can be estimated as rudiments of CB formation. Platelets, lymphocytes, buccal cells, and skin cells can be cultured and used to examine $\Delta\Psi$ collapse using sensitive fluorescent indicator, dihydrofluorescein, JC-1, or rhodamine to assess MB and CB molecular pathogenesis at an earlier stage of disease progression as described in this book.

CB formation in the hippocampal region causes AD, in the NS-DAergic region causes PD, and in the medio-basal hypothalamic region causes bulimia and obesity. CB formation may also be utilized as an early and sensitive biomarker of neurodegeneration in MDDs. Nonspecific induction of CB formation in MDR malignancies, causes GIT distress, myelosuppression, alopecia, pulmonary fibrosis, cardiovascular and renal damage, and infertility (Sharma et al. 2013; Sharma 2014; Li et al. 2014). Hence, DSST novel CB antagonists may be developed as natural or synthetic antioxidants, anti-inflammatory, and anti-apoptotic agents to prevent CB formation and its inhibition during acute phase, charnolophagy agonists during the intermediate phase, and CB/CS sequestration inhibitors as intracellular detoxifiers during chronic phase as safe and effective theranostics in NDDs and CVDs, and vice versa for the EBPT of MDR malignancies.

It remains unknown whether epigenetic changes in the mtDNA and microRNAs can modify CB molecular pathogenesis involving charnolophagy and CS pharmacodynamics in chronic diseases. Hence, drugs may be developed to inhibit CB formation and augment microRNAs-mediated post-transcription in progressive NDDs and CVDs and vice versa for the EBPT of MDR malignancies. Hence, charnolophagy seems more appropriate to describe "CB autophagy and CS exocytosis" as a basic molecular mechanism of ICD. The clinical management of patients can be improved by utilizing DSST charnolopharmacotherapeutics involving CB prevention/inhibition,

charnolophagy induction, CS stabilization, and CS-exocytosis for normal microRNA-mediated post-transcriptional regulation of mitochondrial and nuclear genes involved in growth, proliferation, differentiation, and development. Hence, multimodality imaging employing novel NPs and RPs targeting DSST-CMB, CB, charnolophagy, and CS stabilization and exocytosis will be highly beneficial in pharmaceutical industries and research organizations for novel drug discovery.

It is envisaged that a combination of omics biotechnology along with microRNA profiling; "*Mouse Avatar*" and co-clinical trials of CMB, CB, charnolophagy, and DSST-CS-targeted charnolopharmacotherapeutics will revolutionize the drug development industry and EBPT of NDDs, CVDs, cancer, and other chronic inflammatory diseases.

Unfortunately, conventional chemotherapeutic drugs used for the treatment of cancer trigger generalized apoptosis of nonspecific hyper proliferating cells. Consequently, we encounter several undesirable adverse effects. Nonspecific induction of CB in the hyper-proliferating cells causes alopecia, myelosuppression, GIT symptoms, cardiovascular degeneration, neurotoxicity, renal impairments, and infertility during cancer chemotherapy. Hence, drugs may be developed to augment cancer stem cell-specific CB formation to eradicate MDR malignancies with minimum or no adverse effects. Nutritional rehabilitation, physiological Zn^{2+}, and MTs prevent CB formation by acting as potent free radical scavengers. Accumulation of CBs at the junction of axon hillock may impair axoplasmic transport of various ions, neurotransmitters, enzymes, neurotrophic factors (such as BDNF, IGF-1, and NGF-1), and mitochondria at the synaptic terminals to cause synaptic atrophy. The release of toxic substances from the destabilized CS due to increased permeabilization, sequestration, and fragmentation at the junction of axon hillock or in the synaptic terminals can induce initially synaptic silence followed by progressive NDDs, such as; PD, AD, ALS, HD, MS, stroke, depression, schizophrenia, and chronic drug addiction due to endogenous toxins-induced synaptic sequestration and synaptic degeneration. Hence, novel drugs may be developed to inhibit CB formation, induce charnolophagy, and prevent CS destabilization in NDDs and CVDs and vice versa for the personalized theranostics of MDR malignancies.

Soon after the conception, paternal CB must be phagocytosed by the maternal lysosomes in the oocyte by charnolophagy as a basic molecular mechanism of ICD; otherwise it could have deleterious effects on the zygote and may induce abortion, anencephaly, microcephaly, cyclopia, craniofacial anomalies, still birth, and abortion; as observed in FAS, chronic nicotine and toxins exposure, and microbial (ZIKV, cytomegalovirus, and rubella virus) infections. Many pharmacological agents used in routine clinical practice including anesthetics, antidepressants, antipsychotics, HMG-CO-A reductase inhibitors (statins), ACE inhibitors, and anti-epileptic drugs can also induce microcephaly due to free radical-induced CB formation and CS destabilization (involved in microRNAs-mediated post-transcriptional down-regulation of genes) in the NPCs and CSs derived from iPPCs in the embryo to cause developmental charnolopathies (also named as embryopathies).

CB formation is significantly influenced by inducers and microenvironment and is spatio-temporally and transcriptionally-regulated by microRNAs in healthy aging, whereas; it becomes uncontrolled in NDDs, CVDs, inflammatory diseases, cancer, and in microbial infections. Malnutrition, toxins (nicotine and ethanol) and

microbial (ZIKV, cytomegalovirus, rubella) infection induce CB formation and CS destabilization to cause developmental charnolopathies, involved in diversified embryopathies, including microcephaly. The CB, CPS, and CS are stored in the meconium during embryonic development under normal physiological conditions. In general, CB, CPS, and CS are subjected to hepatic metabolism through systemic circulation and after phase-1 (through cytochrome-P450) and phase-2 (through glucronidation, sulphatation, acetylation) metabolism for renal and fecal clearance.

CB can be classified depending on the type of MAO on the outer mitochondrial membrane. The neuronal mitochondria in the dorsal raphe and periaqueductal gray regions are rich in MAO-A, and are involved in the oxidation of NE and 5-HT at the synaptic terminals to modulate pain, perception, and depression; whereas mitochondria in the striatal neurons are rich in MAO-B. Thus, MAO-A-specific CB is formed in the dorsal raphe and PAG, whereas MAO-B-specific CB is formed in the striatum due to loss of monoaminergic neurotransmission as a function of disease and/or aging. The MAO-B specific CB formation can be prevented by selegiline, rasagiline, safinamide, and moclobemide to delay the requirement of L-DOPA therapy in PD. Selegiline induces MTs to provide neuroprotection in AD and PD by preventing free radical-induced CB formation and CS destabilization involved in apoptotic neurodegeneration. MTs as potent free radical scavengers also augment charnolophagy and CS exocytosis to boost MB, microRNA-modulated epigenetics, and maintain ICD for normal health and well-being. Premature degradation of CPS or CS membranes releases toxic substances such as: Cyt-C, iron, caspase-3, Bax, Bak, and AIF to cause craniofacial or other abnormalities depending on which iPPCs are involved and/or destroyed during FASD and multiple drug addiction. Similar pathological changes may occur during CB sequestration, when it is inefficiently phagocytosed and/or becomes lysosomal-resistant. A CPS is transformed to CS when the phagocytosed CB is completely hydrolyzed by the lysosomal (proteases, lipases, nucleases) enzymes. The CS is exocytosed by an energy (ATP)-driven process as a basic molecular mechanism of ICD to prevent chronic diseases and remain healthy.

Prolonged retention of CS in a cell results in the formation of CS bodies due to secondary or tertiary free radical attack during the chronic phase of disease progression. The CS bodies fuse with the plasma membrane to cause phosphatidyl serine externalization and the formation of apoptotic bodies by membrane blebbing. The apoptotic bodies eventually rupture to release toxic apoptogenic substances to further disseminate apoptosis in the neighboring cells. The exocytosed CS can also be endocytosed by the neighboring cells to cause MDR malignancies if a cancer stem cell-specific CS (CSscs) is endocytosed by the neighboring or remote nonproliferating cells.

Malignancies may develop by endocytosis of the circulating cancer stem cell-specific CS in a specific tissue of the human body. The CSscs is drug-resistant because it is rich in antiapoptotic proteins such as MTs, glutathione, BCl_2, HSP-70, and P^{53} which provide immortalization in a non-proliferative cell where it is endocytosed. Hence, drugs may be developed to prevent and/or eliminate CSscs formation, and/or inhibit induction of CS bodies involved in malignant transformation and chronic MDR diseases.

Accumulation of CB at the junction of axon hillock blocks the normal axoplasmic transport of various ions, neurotransmitters, hormones, neurotrophic factors (IGF-1,

BDNF), and mitochondria in the terminal end bulbs to cause synaptic atrophy; whereas the accumulation of structurally and functionally-destabilized CS causes the release of toxic substances of mitochondrial metabolism to cause synaptic degeneration, involved in early morbidity and mortality due to cognitive impairments in learning, intelligence, memory, and behavior (Sharma 2016, 2017). Hence, fusion imaging with ^{18}F or ^{11}C-labelled CB, charnolophagy, and/or CS biomarkers along with a specific PET-RPs for brain regional DAeric, cholinergic, 5-HT-ergic, GABAergic neurotransmission will provide a precise understanding and will establish the exact interaction between CMB, charnolophagy, CS stabilization, and brain regional neurotransmission, which is either compromised or impaired in NDDs and other chronic diseases due to down-regulation of microRNAs-mediated post-transcriptional regulation of genes involved in the DNA cell cycle, cell proliferation, differentiation, migration, and development. Further studies in this direction will provide the precise molecular mechanism of ICD and its exact significance in regulating brain regional neurotransmission in health and disease. The distinct advantage of antioxidants derived from functional foods is that these can pass through the blood brain barrier (BBB) without inducing adverse effects unlike presently-available antidepressants, antipsychotic, anti-histaminergic, cholinesterase, and NMDA receptor antagonists used in the treatment of AD and other NDDs.

Motivation to Discover CB

Once Luis Pasteur said "*luck always favors the prepared mind*" which is true to achieve success in any discipline and in every walk of life. Original scientific achievements are no exceptions. Let me describe some of my early life experiences which persuaded me to discover CB during my professional and scientific career as a biomedical student. There was increased prevalence of protein calorie malnutrition in India, South Africa, and China in young developing children during the early seventies when I started my career in biomedical sciences. There was no birth control and early childhood morbidity and mortality was quite prevalent. Relatives, friends, and the husband of a lady who lost her child would console by persuading her to reproduce another child within next year. In addition to ignorance and poverty, the child neglect, physical abuse, and psychological abuse was at maximum. Parents in remote villages were reluctant to send their children to school because they wanted them to work in their farms. The U.S. President John F. Kennedy was very kind to send milk powder and biscuits to poor children of Indian villages. Hot milk and biscuits were distributed to children in villages during lunch time as an incentive to attend the primary school. In addition to my early childhood memories, I observed deleterious signs and symptoms of children suffering from kwashiorkor and marasmus (types of malnutrition) which inspired, motivated, and encouraged me to discover something new for the welfare of humanity. These events inspired me to discover "Charnoly body" when I was a doctoral student at the All India Institute of Medical Sciences, New Delhi (India). Subsequently, I decided to continue my research on the effect of undernutrition in the developing brain. I was also interested in culturing neurons under glucose and serum-deprived conditions in a petri dish to evaluate the direct effect of various antiepileptic drugs *in vitro* and in N and UN rats because epilepsy and malnutrition were very common in those days in India. By trypsinization of the developing cerebellum

from the young developing pups, I could purify Purkinje neurons along with their structurally-intact synaptic terminals (Sharma and Ebadi 2014).

While serving as a Research Officer at the All India Institute of Medical Sciences, New Delhi (India), I met Dr. Baldev Singh who was the Emeritus Professor in the Department of Neurology and Physiology. Working along with intelligent doctors, neurologist, physiologist, and particularly with Dr. Baldev Singh was really challenging, thrilling, and an exciting experience, aiding my early professional and scientific development. He graciously agreed to guide me in my Ph.D. research project on the developing UN rat brain. I met Dr. GFX David who was the officer in-charge of the transmission and scanning electron microscope labs. Soon he became my very good friend and invited me to organize National Training Programs on Sophisticated Equipments as a Faculty Member. I processed brain samples obtained from the developing N and UN rats for T.E.M. analyses in addition to my regular electrophysiological experiments on these animals. One day, I was lucky to discover multilamellar electron dense membrane stacks in the developing Purkinje neurons of 15 days UN rats, which I named as CBs as a token of love, respect, and appreciation to my mother. I was quite convinced that these multi-lamellar electron membrane stacks (penta, hepta) are originating from the degenerating mitochondrial membranes during severe malnutrition due to free radical-induced lipid peroxidation. I wrote to my parents that I have discovered something new which I named as "Charnoly body" (abbreviation: CB).

I became curious and started counting the number of CBs in the UN Purkinje neurons. In the beginning my interest was restricted to only determining the number of mitochondria in the N and UN rat Purkinje neurons. But later, I realized that these electron dense membrane stacks (CBs) are formed by the degeneration of mitochondria due to nutritional stress. In severely UN animals, the mitochondria were depolarized, swollen, and degenerated to form penta or hepta lamellar structures. When I showed T.E.M. pictures of CB to my mother, she bowed before them and said "*Look how beautiful they are*". My first paper appeared in the Journal of Neurological Sciences where I described their unknown origin, without any clinical significance. My mentor, Dr. Baldev Singh encouraged me by saying, "*Although I do not know what is happening and neither, I am a neuromorphologist, all I can say is that there is something interesting going on in these developing UN neurons about which we do not know at this moment*".

In 1982, I was invited to Paris for the 3rd World Congress on Nuclear Medicine and Biology in the Invention and Innovation Session for my original discovery of an electro-microinjector for intra-neuronal microinjection to determine the influence of mitochondrial-targeted RPs and NPs, on CB formation or inhibition. Based on the original discovery of CB, I was awarded the Merck (German) Gold Medal in the Neurological Society of India Conference held in December 1984 at Varanasi (Kashi). In September 1985, I was invited to serve as a chairman in the 13th world congress of Neurology in Hamburg, where I presented this work in more detail. Subsequently, I was invited to Calgary for the 13th International Congress of Biometeorology on September 1993, where I presented my research on the CB life cycle, which was later published in the conference proceedings. In this lecture, I highlighted that malnutrition, environmental neurotoxins including Kainic acid (KA), Domoic acid

(DOM), and microbial (bacteria, virus, and fungal) infection induce apoptosis in the hippocampal CA-3 and dentate gyrus by CB formation to cause dementia, as noticed in AD. Subsequently, I was invited to present my original discovery on CB as a sensitive biomarker in Nanomedicine in the First International Conference on Translational Nanomedicine, and in the 12th International Congress on Drug discovery on July 25–27, 2013 in Boston. I presented CB as a universal biomarker and highlighted its clinical significance as a pre-apoptotic biomarker of cell injury for novel drug discovery. Recently, I was invited to deliver a speech on "CB as Novel Biomarker in Chronic Drug Addiction" in the 4th International Conference on Drug Addiction and Therapy in Orlando, FL, U.S.A. on August 3–5, 2015 and at the Harvard Medical School in September 22–25, 2016, I delivered a lecture on CB as a novel biomarker of nutritional stress in AD. I was again invited to deliver a lecture on "Antioxidant-Charnolosome Interaction in Health and Disease" at the Harvard Medical School in the 20th International Conference on Functional Foods in Health & Disease, September 22–25, 2017.

I had an opportunity to observe severely malnourished kwashiorkor and marasmus children in my village, which persuaded me to conduct basic research on developing UN brain. Particularly, clinical cases of severe malnutrition motivated me to conduct further research in this direction. These interesting cases are described below:

Case-1. This was a rich family of two truck drivers who had a single wife. No doubt they were rich, but illiterate and so was their wife. Usually their every evening was spent on heavy drinking after long routes of driving a heavy truck. Their wife was severely anemic, weak, and frail. During pregnancy, she was neglected and was treated like a sex object by both these truck drivers. Her condition deteriorated further during pregnancy particularly when she gave birth to a severely malnourished female child. The truck drivers did not like her giving birth to a female child, because a female child was considered a liability and burden to society in those days in their family. The young lady was psychologically demoralized and suffered from not only anemia and depression, but also lactation failure. Consequently, her female child was reduced to a skeleton and suffered from mixed symptoms of marasmus and kwashiorkor, characterized by loss of weight, stunted growth, folded dry skin and face like an old person, peripheral edema, and extremely thin abdominal skin. She could not even cry and suffered from severe diarrhea and dehydration. One day, her mother decided to dispose this severely malnourished child in a toilet sink when an old lady saw her committing this sin. She rushed to the scene and shouted at this young lady, "What are you doing?" The lady replied in a choking voice: "My husbands do not like female child in their family". I do not have milk in my breast and am suffering from severe depression and weakness. I cannot take care of my child who is almost dying in agony, and I can't see her suffering like this". The old lady gave her sincere advice like a big mother. "Look! Am I not a woman? Are you not a woman? If our parents would have done the same merciless job of killing us right after birth, we would not have been on this earth any more. It is a sin to do these types of heinous acts". Both the young lady and the old lady started weeping bitterly. In those days, I was staying with my friend who was our university photographer in a remote village about 3 Kilometer away from our university. He knew that I am conducting research on developing UN animals and perhaps he trusted my intellectual abilities. He brought me to this house where

this weak and frail female child was at the verge of her death. I notice that she was suffering from severe malnutrition and instructed her mother to prepare a feed under strict hygienic conditions. It was a simple formula of cow's milk, glucose, two to three lemon and multivitamin drops, supplemented with iron and folate. I also instructed her parents that they were also born by a female who was their mother to emphasize the significance of a female child. In addition, I gave example of almighty Goddess "Durga" and "Mrs. Indira Gandhi", who was the Prime Minister of India in those days. So, they agreed to purchase at least a small bottle of multivitamin drops for their miserably-dying child on the following day. Within 25 days, nutritional rehabilitation conferred a new life to this child. The edema and the folded skin dissappeared and hair started growing. There was no frequent diarrhea. The child regained coordination of muscular activity as shown in Fig. 1. There was a natural smile on her face as well as on the face of her parents. This experience made me believe that we are living on this earth only because of "*mitochondrial bioenergetics (MB)*" which is derived from the food we consume. By this time, I had learned from my biochemistry classes that mitochondria through oxidative phosphorylation synthesize ATP to provide energy. Particularly during vulnerable periods of brain development, protein calorie restriction has deleterious consequences on learning, intelligence, memory, and behavior due to the depletion of argyrophilic nucleolar organizer (AgNOR) involved in ribosome production and eventually protein (mitofusin) synthesis at the rough endoplasmic reticulum (RER) to synthesize and repair mitochondria and prevent CB formation implicated in progressive neurodegeneration as described elegantly in this book.

Usually body weight and chest to encephalic ratio (circumference) are determined to assess the severity of nutritional stress in developing malnourished children. On moral and ethical grounds, CB formation cannot be studied in the developing UN children. However, clinical manifestations of the deleterious effects of nutritional stress in the malnourished children motivated me to discover the physiological and pharmacological significance of MB in developing UN rats, which led to the discovery of CB formation as a universal pre-apoptotic biomarker of oxidative stress due to severe mitochondrial injury. Hence, CB formation in experimental animals and brain/body weight ratio in developing children may be used as novel biomarkers of nutritional stress and rehabilitation (or degeneration and regeneration) (Source:

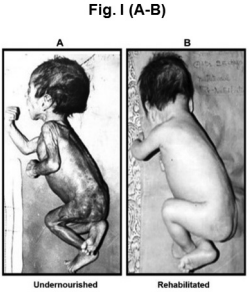

Fig. 1: Undernourished child (A) Clinical manifestations of 2.5-month old protein malnourished female child due to lactation failure in mother presenting folded skin, loss of muscle tone, neuromuscular degeneration, peripheral edema, loss of hair, and face like an old person. (B) The same child after 25 days of nutritional rehabilitation with cow's milk formula exhibiting alleviation of clinical symptoms, coordination of muscular activity, reappearance of muscle tone, and hair growth.

Author's original findings). From: Sharma, S. and M. Ebadi. 2014. Recent studies have shown that skeletal muscles from aging mice exhibit low levels of miR-434-3p and high levels of eIF5A1 involved in sarcopenia, as noticed in this child during severe malnutrition (Pardo et al. 2017).

Case-2. I noticed a male child suffering from marasmus due to his mother's unemployment, loneliness, lactation failure, and father's divorce in my village. The local doctor had already declared that this child will die in couple of days and refused to treat him. One day the doctor asked his mother if her malnourished child was still alive. His mother brought this dying child to me. I simply gave her strict instructions of sanitation and prepared a formula in cow's milk containing glucose, few drops of multivitamins, and lemon juice. Within 15 days, this child had a new life and was fully recovered within 3 weeks.

Brain Sparing Hypothesis. Since the brain weight was not affected significantly in protein calorie malnutrition (PCM) compared to body weight, some investigators proposed a brain sparing hypothesis, which mislead researchers in those days. However, later it was confirmed that, the developing brain, in fact, is most vulnerable to the deleterious consequences of malnutrition. This was based on neuromorphological studies at the light and E.M. level, neurochemical analysis of enzymes, neurotransmitters, and hormones. However, EEG findings were largely inconclusive unless the developing child was severely malnourished during the lactation phase. My own electrophysiological and morphological studies on developing N and UN rat Purkinje neurons confirmed that the brain sparing hypothesis is misleading and brain weight criteria was not appropriate to assess the nutritional status of the developing child. However, the ratio of the brain weight to body weight was significantly high in the UN rats as compared to their well-fed normal litter mates. The EEG of the UN rats exhibited considerable background inhibition with dominance of delta activity and lethargic behavior. Penicillin or kainic acid-induced seizure activity of prolonged duration was observed in UN animals. Although, the intensity of seizure discharge was reduced, they exhibited reduced seizure thresholds of prolonged duration in response to penicillin, KA, and DOM-induced seizures of hippocampal origin. Although, developing UN rats did not exhibit overt clinical symptoms of epilepsy; the seizure thresholds were significantly reduced in these animals. They also exhibited delayed neuronal recovery in response to antiepileptic treatment with intra-rectal sodium valproate (Shrama et al. 1987; Sharma et al. 1990). The latency of response was increased, whereas the duration, frequency, and amplitude of response were significantly reduced, and these animals were easily fatigued upon repeated peripheral electrical stimulation. Subsequently, it was confirmed that electrical fatigue is occurring in the developing UN animals due to significantly impaired MB and CB formation in the developing neurons (Sharma et al. 1986; Sharma et al. 1987). I published this research in the Indian Journal of Medical Research, Journal of Neurological Sciences, Journal of Neuroscience, Epilepsia, Journal of Neural Transmission, and in the Annals of New York Academy of Science.

I got interested in determining the number of mitochondria in the Purkinje neurons of developing N and UN rats employing E.M. examination. My presumption was that the UN animals had a reduced number of mitochondria as compared to N animals, or perhaps there could be only reduced functional activity of mitochondria.

A significant difference in the ^{14}C-glucose utilization between N and UN rat cerebellar vermis Purkinje neurons was observed from where electrical activity was recorded. At the ultrastructural level, the mitochondria appeared swollen and blotted with electron dense inclusions and increased Ca^{2+}. Their membranes were stacked together to form condensed multilamellar penta and heptalamellar structures. Instead of counting their number, I got interested in their subcellular and molecular pathology, which led to the discovery of charnolophagy, CPS, and CS and its exocytosis and endocytosis as a basic molecular mechanism of ICD. The number of these electron dense membrane stacks (CBs) increased as a function of nutritional stress. In fact, in 30 days UN rats, these multi-lamellar stacks were phagocytosed by lysosomes, which was named as "charnolophagy".

When I showed these findings to my doctoral committee members, they simply ignored and rejected these findings except for Dr. Baldev Singh and Dr. Subimal Roy who encouraged and motivated me to explore further along this direction. Particularly, Dr. GFX David encouraged me by saying: "*Mr. Sharma, if you have prepared a successful model of undernutrition and your electrophysiological results are accurate and making sense and the same animals you are subjecting to E.M. evaluation; you are bound to get neuromorphological differences as well. Moreover, these days researchers are demonstrating significant differences between N and UN animals even at the light microscopic level, then why you should not have differences at the E.M. level? In my opinion these observations are genuine and true. Unfortunately, we do not know their exact clinical significance at this moment*".

My guide, Dr. Baldev Singh introduced me to two eminent professors Dr. Parab Dastur from the Department of Pathology Bombay and Dr. Shastry from the National Institute of Science, Bangalore. Dr. Sastry only appreciated my observations and told that he was not an electron microscopist. But Dr. Parab Dastur said: "*Definitely these multilamallar stacks are not artifacts. Neither I can make any further comment on their origin and clinical significance*"; but he made a very important statement which will encourage and motivate young investigators. "*Observations are always true; interpretation may or may not be. What is more important is that you should: work hard, believe in yourself, and should have perseverance*".

These electron dense membrane stacks were not observed in the axons because axons are devoid of mitochondria. If that is so, then from where do we get mitochondria in the synaptic terminals? The synaptic terminals cannot synthesize mitochondria as they do not have regional protein synthesizing machinery. The mitochondria are synthesized in the cell body near the nucleus because out of 2000 proteins required for the mitochondriogenesis, only 13 are synthesized by the mtDNA for the electron transport chain and oxidative phosphorylation. The remaining proteins required to generate the electron transport chain of enzyme complexes (I–V) are synthesized by the nuclear DNA. The mitochondria are transported at the synaptic terminals by proximodistal axoplasmic transport through axons. The microtubules in the axons serve as molecular rail grids for the transport of mitochondria from the cell soma to the synaptic terminals.

Since we require continuous mitochondrial energy (ATP) during neurotransmitter release for interneuronal communication and cognitive and motor performance, we need a continuous supply of mitochondria, enzymes, neuro-transmitters, and neurotropic factors for the synthesis, storage, and release of the neurotransmitters,

physiologically-normal synaptic terminals for proper neurocybernatics and cognitive performance. However, CB formation can occur at the synaptic terminals to cause cognitive impairments associated with impairments in learning, intelligence, memory, and behavior, and early morbidity and mortality.

CB Definition

CB is a highly pleomorphic, unstable, pre-apoptotic, multi-lamellar (usually penta or heptalamellar), quasicrystalline, electron-dense membrane stack that is formed in the most vulnerable cell due to free radical-induced mitochondrial oxidative and nitrative stress of DPCI. CB is formed in response to any physicochemical injury in the most vulnerable cell (including NPCs, CPCs, derived from iPPCs in the developing embryo) due to free radical-induced CMB and down-regulation of the mitochondrial genome due to DPCI as a basic molecular mechanism of ICD during the acute phase for normal cellular function to remain healthy.

Basic Knowledge about CB

(i) Matter can neither be created nor destroyed, although it may transform from one form to the other.

(ii) Similar moleculars tend to associate, whereas dissimilar moleculars tend to dissociate.

(iii) Molecular association induces wound healing, whereas molecular dissociations results in wound progression depending on the inducers and microenvironment in its vicinity.

(iv) Mitochondrial bioenergetics is compromised as a function of increase in intracellular toxicity due to DPCI.

(v) CB formation, charnolophagy, and CS exocytosis are immediate and initial attempts to contain highly toxic metabolites of mitochondrial metabolism for ICD.

(vi) Novel charnolopharmacotherapeutics targeting CB inhibition, augmenting charnolophagy, CS stabilization, and exocytosis will be clinically beneficial for the safe and effective EBPT of NDDs, CVDs, and MDR malignancies.

A TEM picture illustrating CB molecular pathogenesis is presented in Fig. 2 (upper panel). Severe protein malnutrition triggers the formation of small sized mitochondria without cristae. These nonfunctional mitochondria aggregate to synthesize electron-dense membrane stacks (also named as CBs). The damaged mitochondria behave as destabilized CS and release toxic mitochondrial metabolites to cause plasma membrane perforations (arrow). The condensation of multilamellar electron-dense membrane stacks form penta or heptalamellar units to synthesize a mature CB, which is phagocytosed by lysosome and ATP-driven charnolophagy to form structurally and functionally labile CS (lower panel). The structural and functional destabilization of CS triggers non-DNA-dependent apoptotic cell death involved in MDR disease.

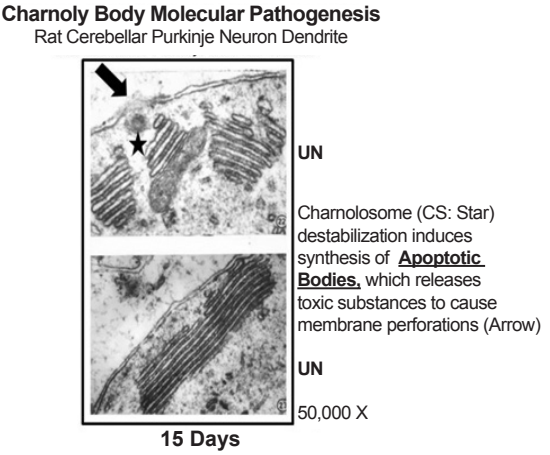

Fig. 2: Charnoly body molecular pathogenesis in 15 days UN rat cerebellar Purkinje neuron.

Charnolosome (CS)

A CS is an intracellular organelle which is formed by phagocytosis of "CB" by a lysosome (also named as CB autophagy or "charnolophagy"). A CS is eliminated by energy (ATP)-driven exocytosis as a basic molecular mechanism of ICD during the chronic phase of disease progression. The degenerated mitochondria condense to form pleomorphic, multilamellar, electron-dense membrane stacks (CBs) as noticed in Fig. 3 (Left panel). Charnolophagy (CB autophagy) is induced by free radical-induced lysosomal activation to synthesize CPS, which is transformed to CS when the phagocytosed CB is hydrolyzed by the lysosomal enzymes as a basic molecular mechanism of ICD (Fig. 3: right panel).

Charnolophagy Index. (charnolophagy/autophagy) is a novel biomarker to quantitatively assess the molecular pathogenesis of CB in health and disease.

Charnolosome Stability Index (CSSI). The CS stability index (CSSI) can be determined by taking a ratio of stable CS divided by the sum of stable CS, permeable

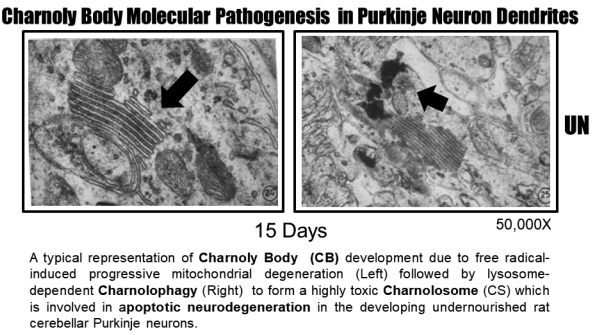

Fig. 3: A TEM picture illustrating CB formation in the Purkinje neuron of developing UN rat cerebellar cortex due to free radicals-induced mitochondrial oxidative and nitrative stress.